

**Bist, Kevin**

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**From:** Jeffrey Sharkey <jeffreysark@gmail.com>  
**Sent:** Monday, January 05, 2015 9:18 AM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated rule making committee request

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patricia

Hope you had a nice New Year's break.

I received notice of the negotiated rule making committee published today and wanted to submit my request to participate on the committee.

As I mentioned at the recent meeting, we created the Medical Marijuana Business Association of Florida last spring to provide a forum to gather information from various stakeholders both within and outside of Florida to share ideas on best practices for a coherent and effective regulatory structure and business model for medical marijuana.

We have gained tremendous knowledge and experienced over the last year through the passage of SB 1030, active participation in the DOH rule making process and hundreds of hours discussing with Florida-based nurseries, growers and medical cannabis stakeholders, as well as extensive research and discussions with regulators and participants in other state's about their experiences and their requirements for successful framework, that we believe could contribute to a regulatory structure in Florida that would meet the needs of patients, growers, public safety and local communities.

I think the list of participants you have outlined represents a strong stakeholder group. However, I believe we could offer an unbiased, timely and holistic perspective on what has worked in other states, what the most effective approach might be in Florida for dispensaries, patients and the Department.

As I mentioned, we have convened a series of meetings over the last 2 months with proponents of the medical cannabis industry as well as opponents. We are currently working on potential legislative corrections to SB 1030 with those interests as well as recommendations to the existing rule.

I would like to offer our services to participate on the negotiating committee on behalf of the Medical Marijuana Business Association of Florida. Our members include growers, clinical researchers, patients, investors and others from a variety of perspectives on the MMJ issue.

Let me know if you need any more information for our request. I know you will be getting a number of requests to participate but believe our perspective gleaned from in depth knowledge of Florida and other states, would add value to your discussions.

Thank you.

Jeff

**Dr. Jeffrey Sharkey**

President  
Medical Marijuana Business Association  
of Florida  
106 E. College Avenue, Suite 640  
Tallahassee, FL 32301  
850.224.1660 office

850.224.6785 fax  
850.443.3355 cell  
jeffreysark@gmail.com

www.capitolalliancegroup.com

**Bist, Kevin**

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**From:** david.roberts@akerman.com  
**Sent:** Monday, January 05, 2015 11:24 AM  
**To:** Nelson, Patricia A  
**Subject:** Notice of Negotiated Rulemaking Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Good morning Patricia and Happy New Year!

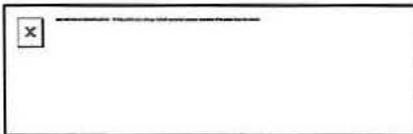
I saw the notice and I would like to inquire as to how will the members of the committee will be selected and appointed? Are you accepting recommendations for the various representative groups? Do they need to follow the criteria listed below the general list of committee members? That process appears to be for application with the committee if you "believe that your interests are not adequately represented by the committee members listed above". Is it the same process?

Please let me know.

Thank you,

Dave Roberts

**David J. Roberts**  
Public Policy Advisor  
Akerman LLP | Suite 1200 | 106 East College Avenue | Tallahassee, FL 32301  
Dir: 850.521.8009 | Main: 850.224.9634 | Cell: 850.443.4820 | Fax: 850.325.2548  
[david.roberts@akerman.com](mailto:david.roberts@akerman.com)



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**Bist, Kevin**

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**From:** Joel Ewusiak <joel@ewusiaklaw.com>  
**Sent:** Monday, January 05, 2015 1:32 PM  
**To:** Nelson, Patricia A  
**Subject:** Rulemaking Committee for Low THC Cannabis Law  
**Attachments:** 64-4.001 - 1.5.2015 Notice of Developmental Rule Making.doc

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Hi Patricia:

I've reviewed the attached Notice of Development of Rulemaking for the low THC cannabis law. How are the committee members being selected and if they are already selected, who are the committee members? The notice outlines a procedure that must be followed in order to apply to participate in the February 2015 meeting, but the procedure seems to apply to non-committee members only.

Thanks,

-Joel

Joel Ewusiak

Ewusiak Law, P.A.

100 Main St., Suite 205

Safety Harbor, FL 34695

P: 727.286.3559 | F: 727.286.3219 | [www.ewusiaklaw.com](http://www.ewusiaklaw.com)

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## NOTICE OF DEVELOPMENT OF RULEMAKING

### DEPARTMENT OF HEALTH

#### Office of Compassionate Use

RULE NOS.:	RULE TITLES:
64-4.001	Regulatory Structure Rule 1
64-4.002	Regulatory Structure Rule 2
64-4.003	Regulatory Structure Rule 3
64-4.004	Regulatory Structure Rule 4
64-4.005	Regulatory Structure Rule 5
64-4.006	Regulatory Structure Rule 6
64-4.007	Regulatory Structure Rule 7
64-4.008	Regulatory Structure Rule 8
64-4.009	Compassionate Use Registry

**PURPOSE AND EFFECT:** The Department of Health announces the convening of a negotiated rulemaking proceeding to address the regulatory structure for dispensing organizations of low-THC cannabis. The purpose of the negotiated rulemaking is to draft mutually acceptable proposed rules.

**SUBJECT TO BE ADDRESSED:** The subject and scope of the rules to be developed through negotiated rulemaking will be the regulatory structure for dispensing organizations of low-THC cannabis.

**RULEMAKING AUTHORITY:** 381.986 FS.

**LAW IMPLEMENTED:** 381.986 FS.

**NEGOTIATED RULEMAKING COMMITTEE:** The negotiated rulemaking committee members will be selected from the following representative groups:

1. A nursery that meets the criteria in Section 381.986(5)(b)1., Florida Statutes;
2. A qualified patient or patient representative;
3. A testing laboratory;
4. A member of the Florida Bar experienced in administrative law;
5. An individual with demonstrated experience in sound agricultural practices and necessary regulation;
6. A physician authorized to order low-THC Cannabis products for qualified patients;
7. An individual with demonstrated experience establishing or navigating regulatory structures for cannabis in other jurisdictions; and
8. Representatives of the Department of Health.

If you believe that your interests are not adequately represented by the committee members listed above, you may apply to participate within 30 days of the date of publication of this notice. Your application must contain the following information: your name, business address, and telephone number; the name of any organization you are representing; a description of the organization or the members of the organization; a description of how the proposed rulemaking proceedings will affect you or the parties that you represent; a statement identifying the reasons why you believe the representative groups listed above will not adequately represent your interests; and a statement that you are willing to negotiate in good faith and can attend the scheduled meeting. Please submit your application to Patricia Nelson, Department of Health, 4052 Bald Cypress Way, Bin A-02, Tallahassee, Florida 32399, email address: [Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov).

#### NEGOTIATED RULEMAKING COMMITTEE MEETING:

The committee will meet at the following date, time, and place to discuss rule development: February 4, 2015, 8:00 a.m. - 10:00 p.m. and February 5, 2015, 8:00 a.m. until concluded, Department of Health, Room 301, 4052 Bald Cypress Way, Tallahassee, FL 32399.

Pursuant to provisions of the Americans with Disabilities Act, any person requiring special accommodations to participate in this meeting is asked to advise the agency at least 72 hours before the meeting by contacting Sophia Flowers, Department of Health, (850)245-4005, [Sophia.Flowers@flhealth.gov](mailto:Sophia.Flowers@flhealth.gov). If you are hearing or speech impaired, please contact the agency using the Florida Relay Service, 1 (800)955-8771 (TDD) or 1 (800)955-9770 (Voice).

THE PERSON TO BE CONTACTED REGARDING THE PROPOSED RULE DEVELOPMENT AND A COPY OF THE PRELIMINARY DRAFT, IF AVAILABLE IS: Patricia Nelson, Department of Health, 4052 Bald Cypress Way, Bin A-02, Tallahassee, Florida 32399, Email address: Patricia.Nelson@flhealth.gov  
THE PRELIMINARY TEXT OF THE PROPOSED RULE DEVELOPMENT IS NOT AVAILABLE.

## Bist, Kevin

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**From:** Kerry Herndon <kherndon@kerrys.com>  
**Sent:** Monday, January 05, 2015 1:33 PM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated Rules Meeting  
**Attachments:** APP CRITERIA.pdf

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Hello Patricia Nelson,

I was a pleasure to participate in the rules workshop and meet you in person on December 30, 2014. I would like to submit my request to be the member representing growers or as a person with demonstrated agricultural ability. I have been a professional grower and nursery owner for forty two years. I have substantial operations in Homestead and Apopka, FL. I also own a plant tissue culture laboratory in Apopka. Because growers are so profoundly affected by the rules it would make some sense to have more than one grower on the panel.

I have been involved with the legislation from the beginning including testifying before the House Judiciary Committee by invitation. I was the only grower that I know of that showed up to testify during the legislative session. I have attended and participated in all but one rules making meeting and have submitted substantial written material post meeting for consideration. I have some definite ideas about how the system can be self funding over time and fair to growers.

I have a particular interest in the high quality production of CBD for the population in Florida that needs it. For several years I have personally funded research in partnership with the USDA to create an economic system transient protein expression in plants. The goal of this research was to provide relief to people suffering from rare genetic disorder that have no current options. This is extreme cutting edge biotechnology research. I came as quite a shock to me that many of the disorders my research is targeting are helped substantially with the extract from a fast growing plant. For me the production of Cannabis is just an extension of my original personal mission to help alleviate suffering. I have an M.B.A. from Florida International University. I have been awarded Environmental Agriculturist of the year by the State of Florida in 1999. I can supply a complete resume upon request.

Attached is a preliminary draft of a scoring system that embodies some of the characteristics that greenhouse operators should have. This is a first draft and subject to substantial change. It does not cover testing, extraction methods, or anything to do with dispensaries. It is a start.

I hope to be allowed to bring my knowledge and experience to the panel.

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**KERRY HERNDON PRESIDENT + CEO**  
TEL: 305.247.7096 EXT:231 | CELL: 786.229.2444 | EMAIL: [KHERNDON@KERRYS.COM](mailto:KHERNDON@KERRYS.COM)

21840 SW 258th STREET  
HOMESTEAD, FL 33031  
TF: 800.331.9127  
FAX: 305.247.3392

Application Scoring			Max Score	
			111	100%
<b>Size potential</b>		<b>Because there are only five potential producers it is important that the selected growers have the physical ability to produce in quantity for the demand.</b>	10	9%
	0-49,999 square feet	1 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	50K to 99,999	2 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	100K - 199,999	3 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	200K-299,999	4 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	300k-4399,999	6 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	400K-499,999	6 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	500K-749,999	8 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
	750K+	10 The square footage will be defined as the gross square footage available for growing medical Cannabis. Aisles, walkways, and packing areas are included in space calculation.		
<b>Greenhouse type 10 points</b>		<b>The product must be produced in enclosed structures with good pest prevention (bird, rodents, insects, etc).</b>	10	9%
	Shade houses	1 These are structures that are shade houses that uses plastic sheeting temporarily during cold seasons and grow without protection from the general climate other times of the year.		
	cold frame houses	2 These structures are defined as metal or wood framed buildings that have a solid barrier against rain but otherwise not climate control. The roof material can be any substance that allows sunlight to pass but blocks rain.		
	heated single houses	3 Single houses are free standing units that have a with that is defined by a single ground to ground span.		
	Gutter connected poly with heat	5 Gutter connected houses are greenhouses with more than one span connected together at the gutter to create a larger growing area under one roof.		

Application Scoring			Max Score	
			111	100%
	Gutter connected fiberglass panel roofs with heat	7	Greenhouses that are constructed with permanent fiberglass panels to protect plants from rain and incorporate a heating system to protect plants from cold and maintain growth during cold weather.	
	Double poly / poly carbonate / glass with heat	10	three distinct greenhouse system all representing a considerable investment in an improved infrastructure to enhance and protect crops grown in them.	
<b>Greenhouse enhanced production environment</b>			To provide maximum flexibility for producing a continuous secure supply for processing and distribution to patients.	20 18%
	Ability to maintain multiple growing areas and environments that can be controlled separately within one facility	3	Able to isolate different plant crops. This provides the best protection for continuous supply.	
	Automated shade system	1	shade systems that automatically react to changing light conditions.	
	Sealed floors	1	Floors that allow for pressure or steam cleaning for greater sanitation in the building.	
	Thrip screens	1	An incorporated system of fine mesh screens that prevent thrips and other insects from entering the greenhouse.	
	Rolling benches	1	A mobile bench system that allows for the efficient movement of growing crops through the growing cycle.	
	Overhead forced air heaters	1	Direct combustion to air exchange systems. Typically heat is distributed through poly tubes above crops.	
	boiler heat source	2	Boilers burn propane, oil, or natural gas to heat water and distributes heat through metal pipes through the greenhouse primarily under benches and radiate heat.	
	multiple boilers	3	More than one boiler for redundant crop protection.	
	overhead irrigation	1	Water delivered through a sprinkler system so crops do not require hand watering.	
	drip or flood irrigation	2	A system where water is delivered directly to the root zone of the plants and leaves remain dry.	
	backup power	2	Any system, primarily generators, that provides backup in the event of electric grid loss.	
	black out curtains	1	A curtain system that allows for the creation of a short day condition during a natural long day photo period.	
	Lights	1	Supplemental lighting to extend photo period beyond natural day length.	
<b>Other infrastructure extras</b>				3 3%
	Clean processing room	2	A room that can be maintain is a semi sterile state.	
	Clean drying room	1	A room that can be maintain is a semi sterile state.	

Application Scoring			Max Score	
			111	100%
<b>Certifications</b>			<b>22</b>	<b>20%</b>
		<b>Cumulative of different certifications and experience with same</b>		
Best management practices (nursery)	1	A Florida certification required by most Water Management districts assuring good water and nutrient management practices.		
BMP - 1 YR or more experience since certification	1			
Good Agricultural Practices (Federal Certification)	2	USDA program of requirements verified by independent auditors (including - in Florida - FDACS) initially and annually.		
GAP - 1 YR minimum experience since certification	2			
Good Handling Practices (Federal Certification)	3	USDA program of requirements verified by independent auditors (including - in Florida - FDACS) initially and annually.		
GHP - 1 YR minimum experience since certification	3			
USDA Certified organic	5	USDA program of requirements verified by independent auditors initially and annually.		
USDA Certified Organic - 1 YR minimum experience since certification	5			
<b>Process for Growing</b>			<b>5</b>	<b>5%</b>
Defined Process	1	Is there a defined process for production of the plants?		
Facilities Capability	2	Will the facilities support the process?		
Certification Integration	1	Do certifications support the process plans? (i.e., Has the applicant had the certifications for more than 1 year?)		
Clarity of Process	1	Is the process well explained so an independent group can verify adherence to the process?		
<b>Process for Drying</b>			<b>5</b>	<b>5%</b>
Defined Process	1	Is there a defined process for drying the plants?		
Facilities Capability	2	Will the facilities support the process?		
Certification Integration	1	Do certification support the process plans?		
Clarity of Process	1	Is the process well explained so an independent group can verify adherence to the process?		
<b>Process for Extraction</b>			<b>5</b>	<b>5%</b>
Defined Process	1	Is there a defined process for extracting CBD oil from the plants?		
Facilities Capability	2	Will the facilities support the process?		
Certification Integration	1	Do certification support the process plans?		
Clarity of Process	1	Is the process well explained so an independent group can verify adherence to the process?		

Application Scoring			Max Score	
			111	100%
<b>Process for packaging</b>			5	5%
Defined Process	1	Is there a defined process for packaging CBD oil from the plants?		
Facilities Capability	2	Will the facilities support the process		
Certification Integration	1	Do certification support the process plans?		
Clarity of Process	1	Is the process well explained so an independent group can verify adherence to the process?		
<b>Process for Trace and track</b>			6	5%
Defined Process	1	Is there a defined process for packaging CBD oil from the plants?		
Facilities Capability	2	Will the facilities support the process? Are computers and the correct software in use.		
Existing verified system	3	Does the business have a proven trace and track system already in use?		
<b>Business Plan</b>			10	9%
Completeness	3	Is the plan complete for two years?		
Comprehensive	2	Does the plan consider all costs including Capital Expenses?		
Detailed	2	Does the plan contain sufficient detail allowing a third party to understand it?		
Credible	3	Does it make sense to an independent reviewer?		
<b>Security System</b>			10	9%
Comprehensive plan	3	Does the plan incorporate all of the access points with adequate controls to prevent diversion of Cannabis.		
Adequacy	2	Are the measure adequate to prevent inside and outside forces from diversion of Cannabis products.		
Safety	2	Does the plan consider the safety of the work force handling the product?		
Technology	1	Does the plan utilize available technology to enhance the overall security of the facility.		
Location	2	Is the production facility reasonably located where it is possible to secure the perimeter.		
<b>new criteria</b>				0%
<b>Copy &amp; Paste this row ABOVE HERE to add more criteria groups</b>				0%

**Bist, Kevin**

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**From:** Robert Buck <email@attorneybuck.com>  
**Sent:** Monday, January 05, 2015 3:14 PM  
**To:** Nelson, Patricia A; Patricia.nelson@eog.myflorida.com  
**Cc:** zzzz Feedback, Compassionate Use  
**Subject:** Re: Tree-King Comments on SB1030 Rule Rewrite  
**Attachments:** Dr. William Clark 12-19-14.pdf

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Ms. Nelson,

It appears that the statement from Dr. William Clark did not attach to my prior email. I apologize for the mistake. Please find attached a duplicate copy.

Please consider Dr. Clark's experience in your search for an expert panel. We consider his knowledge in chemistry and out-of-state rule making to be unparalleled.

Regards

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Robert L. Buck, Esq., P.A.  
PO Box 15146., Brooksville, FL 34604  
352-584-2062 Phone  
352-686-7455 Fax  
[email@attorneybuck.com](mailto:email@attorneybuck.com)

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December 19, 2014

**RE: Implementation of GMPs (Good Manufacturing Practices) for medical cannabis under SB1030**

To Whom It May Concern,

Given the recent developments surrounding proposed modifications of the rules for SB1030 I would like to highlight the critical importance of having regulations, Good Manufacturing Practices, that govern the quality of the manufacture, production, development, and testing of medical cannabis.

Having nearly 20 years experience with natural products and dietary supplements, helping NSF International develop their dietary supplement certification program for quality and safety, and having served on the committee that developed the GMPs for the dietary supplement industry, I cannot stress enough how important it is to incorporate GMPs into any rules modification for SB1030.

In addition, after first hand observance of the medical cannabis business in the States of California, Nevada, and Colorado that lack this oversight, it is clear that it is necessary to implement these rules to ensure delivery of product that is not only of a consistent, high quality standard, but is also safe for consumers.

Thank you for your time and consideration.

Sincerely,

A handwritten signature in black ink that reads 'William D. Clark'.

William D. Clark, Ph.D.  
President & Founder  
Pure Naturals Certified  
Clearwater, FL  
Tel: (727) 489-9201  
[www.PureNaturalsCertified.com](http://www.PureNaturalsCertified.com)



PURE NATURALS CERTIFIED



### William D. Clark, Ph.D.

*Former Director Research & Development, PharmaPrint, Inc.*

*Developer of Centrum™ Herbals*

*Former Vice President of Product Development & Regulatory Affairs, Mannatech, Inc.*

For nearly 2 decades, Dr. William (Bill) Clark has been working with and developing natural products and nutraceuticals. A strong advocate of preventative care, Dr. Clark has directed research and development at the executive level, delivering monumental value to an entire industry. In fact, Dr. Clark has been instrumental to the establishment of nutraceuticals as a market force in the United States, Canada, Britain, Japan, New Zealand, & Australia. Dr. Clark's particular expertise spans the entire product continuum - from formula development to full commercial launch. Dr. Clark's efforts at PharmaPrint delivered six entirely new landmark products to the marketplace - the world renowned Centrum™ Herbals line. Dr. Clark was instrumental in the conception, formulation, and launch of Centrum™ Herbals (a Wyeth brand), resulting in over \$150 million of retail sales in its first year.

Some additional key industry achievements for Dr. Clark include helping NSF International complete initial development of their Dietary Supplement Certification Program. His contribution to the development of NSF's program was substantial. Dr. Clark also served on the Dietary Supplement Joint Committee that formed the new GMPs for the industry that have now been implemented into law by the FDA.

Dr. Clark's technical expertise includes not only research and development, product formulation, clinical studies, and product development, but also regulatory affairs and compliance, quality assurance and quality control, project management, and front line manufacturing and packaging of nutritional supplements.

Dr. Clark contributes an extraordinary magnitude of nutritional supplement experience, insight, business acumen, and industry relationships to the success of Pure Naturals Certified.

A few of his accomplishments include the following roles:

#### **President & Co-Founder**

*Pure Naturals Certified*

Dr. William Clark currently leads Pure Naturals Certified, a Contract Research Organization (CRO) that specializes in the research and development, product formulation & development, and clinical studies of nutraceuticals, natural products, and dietary supplements. Pure Naturals Certified is the only operating CRO with this targeted specialty.

#### **President & Co-Founder**

*Pacific Nutritional Research, LLC*

Dr. William Clark formerly led Pacific Nutritional Research, a Contract Research Organization (CRO) that performed clinical studies and provided marketing & technical services specifically for nutritional supplements to determine their benefits or other effects in the categories of weight management, general health and condition specific applications.

#### **President & CEO**

*Ultimate Synergy, LLC*

Dr. William Clark recently served as the CEO of Ultimate Synergy, a developer of high quality human and equine nutritional supplements. He was the developer of the HALO™ Leaf of Life and Natricence™ product lines. Ultimate Synergy's HALO Leaf of Life™ is the most potent antioxidant drink on the market, based on a proprietary Fresh Olive Leaf Complex (FOLC).

#### **Vice President, Product Development & Regulatory Affairs**

*Mannatech*

While with Mannatech, Dr. Clark directed the global development for new and reformulated products. He served to improve product and process development in conjunction with contract manufacturers, R&D, QA, and Operations. Dr. Clark oversaw the complete manufacturing and project management processes, ensuring efficiency, consistency, and regulatory compliance with all Good Manufacturing Practice (GMP) Regulations during each cycle of product development. Further, Dr. Clark identified key manufacturing and raw material sources, and ensured regulatory review, compliance, and approval of all labeling, training, and marketing materials.

#### **Director Research & Development**

*PharmaPrint*

Dr. Clark supervised and directed ongoing QA/QC release, method development, and stability studies for all PharmaPrint products. Dr. Clark personally supervised and directed multiple laboratories in methods transfer, development, and validation of analytical test procedures for both Centrum Herbals and PharmaPrint Combination Products in accordance with pharmaceutical Current Good Manufacturing Practices (CGMPs). Dr. Clark was integral in the formulation, development, and manufacturing of PharmaPrint products and the institution of core enterprise standard operating procedures.

#### **Education**

Ph.D., Chemistry

University of California Santa Cruz, CA, 1997

**M.S., Chemistry**  
University of California Santa Cruz, CA, 1995

**B.A., Chemistry**  
University of California Santa Cruz, CA, 1992

**Publications**

"Investigations of Halogenated Constituents Isolated from Marine Sponges Associated with Cyanobacterial Symbionts" by W. D. Clark  
Ph.D. Dissertation Thesis, June 1997.

"Cyclocinamide A, An Unusual Cytotoxic Halogenated Hexapeptide from the Marine Sponge *Psammocinia*", by W. D. Clark, T. H. Corbett, F. A. Valeriote, and P. Crews  
*Journal of the American Chemical Society*, **119** (39), 9285, 1997.

"A Novel Chlorinated Keride Amino Acid, Herbanide A, from the Marine Sponge *Dysidea herbacea*", by W. D. Clark and P. Crews  
*Tetrahedron Letters*, **36**, 1185, 1995.

**Bist, Kevin**

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**From:** Nelson, Patricia A  
**Sent:** Monday, January 05, 2015 5:17 PM  
**To:** jeffreysark@gmail.com  
**Subject:** RE: Low-THC Cannabis Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

I got your request. Right now, I am just collecting them. I haven't started reviewing them.

Patty

**From:** Jeffrey Sharkey [mailto:[jeffreysark@gmail.com](mailto:jeffreysark@gmail.com)]  
**Sent:** Monday, January 05, 2015 5:16 PM  
**To:** Nelson, Patricia A  
**Subject:** Re: Low-THC Cannabis Rulemaking

Patti

Thanks for sending. I sent you a request to participate on the committee earlier this morning. I know you get a lot of emails. I will try to call in the am.

Thanks

**Dr. Jeffrey Sharkey**  
**Managing Partner**  
**Capitol Alliance Group, Inc**  
**106 E. College Avenue, Suite 640**  
**Tallahassee, FL 32301**  
**850.224.1660 office**  
**850.224.6785 fax**  
**850.443.3355 cell**  
**[jeffreysark@gmail.com](mailto:jeffreysark@gmail.com)**

**[www.capitolalliancegroup.com](http://www.capitolalliancegroup.com)**

On Mon, Jan 5, 2015 at 5:11 PM, Nelson, Patricia A <[Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov)> wrote:

Dear Interested Parties,

Please see the attached Notice of Negotiated Rulemaking (on page 1 of the attached document) scheduled for February 4 and 5, 2015, in Tallahassee.

**Bist, Kevin**

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**From:** Jorge Chamizo <jorge@flapartners.com>  
**Sent:** Monday, January 05, 2015 5:17 PM  
**To:** Nelson, Patricia A  
**Subject:** Question regarding Notice of Negotiated Rulemaking  
**Attachments:** Jorge Chamizo.vcf

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Ms. Nelson:

Good afternoon. I was writing to inquire about the Noticed Negotiating Rulemaking Proceeding which appeared in today's FAR. My client is Knox Nursery, Inc, one of the qualified nurseries meeting the statutory criteria of section 381.986(5), F.S. Has the nursery committee member already been selected or is this something that will take place during the February 4<sup>th</sup> meeting?

Thank you for your time and courtesy.

Sincerely,

Jorge Chamizo



Sincerely,

Patty

Patricia Nelson

Director

Office of Compassionate Use

## **Bist, Kevin**

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**From:** Genester Wilson-King <drwilsonking@drwilsonking.com>  
**Sent:** Monday, January 05, 2015 8:13 PM  
**To:** Nelson, Patricia A  
**Subject:** Rules Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Good afternoon,

I would like to commend you in the establishment of this committee. It's a brilliant idea!

I am a physician who has expertise and certification in cannabinoid medicine. I am Board Certified in OB/GYN, and Bioidentical Hormone Therapy.

I have done many seminars on the uses of cannabis in medicine. I can send you a resume if you would like.

I would like to be on the committee, but I have a question. Would being on the committee exclude me from being able to participate in the cannabis industry in Florida?

Would it cause a conflict of interest?

I have a few suggestions for you, whether I am on the committee or not. I will send upon receipt of your response.

Thank you

--Genester Wilson-King MD, FACOG

**Victory Rejuvenation Center**

**1540 International Pkwy**

**Lake Mary, FL 32746**

Website: [www.victoryrejuvenationcenter.com](http://www.victoryrejuvenationcenter.com)

Email: [drwilsonking@drwilsonking.com](mailto:drwilsonking@drwilsonking.com)

Phone: 407-536-5125

Fax: 321-280-6977

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## **Bist, Kevin**

---

**From:** AHall22@aol.com  
**Sent:** Monday, January 05, 2015 11:26 PM  
**To:** Nelson, Patricia A  
**Cc:** AHall22@aol.com  
**Subject:** Notice of Development of Proposed Rules and Negotiated Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

January 5, 2015  
Patricia Nelson  
Department of Health  
4052 Bald Cypress Way, Bin A-02  
Tallahassee, FL 32399  
Re: Negotiated Rulemaking Committee Membership

Dear Ms. Nelson:

I am in receipt of the Florida Administrative Register, Vol. 41, No. 2, dated today. In it Section I mentions the composition of the proposed Rulemaking Committee. As one of the initial physicians authorized to order low-THC Cannabis products for qualified patients in the State of Florida, I took especial interest in the Notice.

I do believe that the composition as proposed will likely represent the interest of my practice and my patients. I am a neurological surgeon and interested in the proposed applications for clinical practice. I am also Volunteer Faculty at the Herbert Wertheim FIU College of Medicine and Nova Southeastern College of Medicine. I am also the President of the Miami-Dade Chapter of the National Medical Association (James W. Bridges Medical Society). The proposed rules will have significant effect on clinical practice in all counties of Florida and impact minority and non-minority communities alike.

If you have not yet chosen the physician for the panel, I would like to volunteer my interest in becoming a member of the Committee. I am available on the proposed dates of February 4 and 5, 2015.

Sincerely,

Anthony J. Hall, MDCM, FACS  
Icon Medical Centers  
426 SW 8th Street  
Miami, FL 33130  
Tel: 305-858-8845  
Fax: 305-858-8840  
Email: [ahall22@aol.com](mailto:ahall22@aol.com)

## **Bist, Kevin**

---

**From:** Brian A. Kahan <Bkahan@kahanlaw.com>  
**Sent:** Tuesday, January 06, 2015 9:11 AM  
**To:** Nelson, Patricia A  
**Cc:** linda.mcmullen@flhealth.gov  
**Subject:** RE: Low-THC Cannabis Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Dear Ms. Nelson:

Please allow me to introduce myself. I am an attorney and a pharmacist with my primary law practice focused on administrative law. I practiced as a pharmacist for 27 years before going to law school later in life. I have been practicing health care law for the last 12 years primarily defending health care practitioners before their respective regulatory agencies. I am an Affiliate Clinical Associate Professor for Pharmacy Health Care Administration at the University of Florida College of Pharmacy, Gainesville, FL and am an Adjunct Professor of Law at Nova Southeastern Shepard Broad Law Center, Ft. Lauderdale, FL.

From time to time, I have assisted the Bureau of Enforcement and the Prosecutorial Services Unit of the Florida Department of Health providing my unique perspective as a pharmacist and attorney. I routinely attend rulemaking workshops for the Florida Board of Pharmacy working closely with David Flynn, Senior Assistant Attorney General and counsel to the Florida Board of Pharmacy in the review and development of new pharmacy regulation.

I do not have any clients involved in the application process or considering becoming a dispensing organization. Moreover, I do not represent prospective patients or families advocating the use of medical marijuana. Although I do not have any constituency in the medical marijuana universe, as a pharmacist and an attorney, I recognize the importance of compassion especially as it relates to those afflicted with medical conditions that remain uncontrolled after exhausting the use of conventional medication therapies.

Pursuant to the "Notice of Development of Proposed Rules and Negotiated Rulemaking ("Notice:)", I would like to submit my name for membership on the Negotiated Rulemaking Committee as published in #4 of the Notice. I would like to offer myself as the candidate for the position as a member of the Florida Bar having experience in administrative law.

Unfortunately, I have a previous commitment causing a conflict in my schedule preventing me from attending the meeting on February 4-5, 2015. If I were to be chosen as the attorney member, I would have no problem accommodating all future meetings.

Should you have any additional questions or require the completion of an application for consideration as a member of the Negotiated Rulemaking Committee, please do not hesitate to contact me. Thank you for your assistance.

Respectfully,

Brian A. Kahan

**Brian A. Kahan, Esq.**  
[bkahan@kahanlaw.com](mailto:bkahan@kahanlaw.com)

***To assist with the efficient work flow, please make sure to copy [pharman@kahanlaw.com](mailto:pharman@kahanlaw.com) with all requests.***



**Kahan Heimberg, PLC  
2300 N.W. Corporate Blvd.  
Suite 123  
Boca Raton, FL 33431  
Office: (561) 392-9000  
Fax: (561) 405-6467**



Please consider the environment before printing this email.

---

**From:** Nelson, Patricia A [<mailto:Patricia.Nelson@flhealth.gov>]  
**Sent:** Monday, January 05, 2015 5:11 PM  
**To:** DL 64-4 Interested Parties; Dunn, Nathan P  
**Subject:** Low-THC Cannabis Rulemaking

Dear Interested Parties,

Please see the attached Notice of Negotiated Rulemaking (on page 1 of the attached document) scheduled for February 4 and 5, 2015, in Tallahassee.

Sincerely,  
Patty

Patricia Nelson  
Director  
Office of Compassionate Use

## Bist, Kevin

---

**From:** Jodi James <jjamesflorida@gmail.com>  
**Sent:** Tuesday, January 06, 2015 10:31 AM  
**To:** Nelson, Patricia A  
**Subject:** applying for the rulemaking group

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Hi Ms. Nelson,

I am the Executive Director of the Florida Cannabis Action Network. We are the longest continuous cannabis reform group in the state working for patient rights since 1998. Personally, I have worked on 16 of the 23 states to help pass medical cannabis laws.

I am interested in putting in my resume to be the patient advocate on your panel. My group is statewide, 28 year survivor with ALS, Cathy Jordan is the president of our organization and we have worked closely to educate the legislature for four years.

Our group takes an apartment during committee weeks through session, so we are here at the Capital right now.

Please allow me to interview with you. I was at the last meeting in Orlando and chose not to speak. My only interest in this business is to assure people have safe, affordable access.

The Canadian program promises products that are pure, precise and predictable. That is what I want for our patients.

Thanks for taking on this big job.

Jodi

Jodi James  
FLCAN.org, FLDecides.org  
P 321-253-3673  
C 321-890-7302

---

**From:** Nelson, Patricia A [<mailto:Patricia.Nelson@flhealth.gov>]  
**Sent:** Monday, January 5, 2015 5:11 PM  
**To:** DL 64-4 Interested Parties; Dunn, Nathan P  
**Subject:** Low-THC Cannabis Rulemaking

Dear Interested Parties,

Please see the attached Notice of Negotiated Rulemaking (on page 1 of the attached document) scheduled for February 4 and 5, 2015, in Tallahassee.

Sincerely,  
Patty

Patricia Nelson  
Director



## Bist, Kevin

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 1:07 PM  
**To:** joel@ewusiaklaw.com  
**Subject:** RE: Rulemaking Committee for Low THC Cannabis Law

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Joel,

The negotiating committee has not been selected. Any person interested in being on the committee should send a request to me by email. The request should include an explanation of why they are interested in being on the committee as well as what qualifications they believe would make them a good choice for the committee.

Patty

---

**From:** Joel Ewusiak [<mailto:joel@ewusiaklaw.com>]  
**Sent:** Monday, January 05, 2015 1:32 PM  
**To:** Nelson, Patricia A  
**Subject:** Rulemaking Committee for Low THC Cannabis Law

Hi Patricia:

I've reviewed the attached Notice of Development of Rulemaking for the low THC cannabis law. How are the committee members being selected and if they are already selected, who are the committee members? The notice outlines a procedure that must be followed in order to apply to participate in the February 2015 meeting, but the procedure seems to apply to non-committee members only.

Thanks,  
-Joel

Joel Ewusiak  
Ewusiak Law, P.A.  
100 Main St., Suite 205  
Safety Harbor, FL 34695  
P: 727.286.3559 | F: 727.286.3219 | [www.ewusiaklaw.com](http://www.ewusiaklaw.com)

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**Bist, Kevin**

---

**From:** Genester Wilson-King <drwilsonking@drwilsonking.com>  
**Sent:** Tuesday, January 06, 2015 1:16 PM  
**To:** Nelson, Patricia A  
**Subject:** Rules Committee  
**Attachments:** Genester Wilson-King FINAL RESUME.docx

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Good afternoon,

This is an addendum. I would like to clarify my question.

I am very interested in being on the rules committee. I am currently NOT on any application. However, I would like to know if being on the committee would PRECLUDE me from future participation in the pursuit of a license, being a medical director, etc.

I have attached my resume for your review.

Thank you and I will await your response.

--Genester Wilson-King MD, FACOG

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**Lake Mary, FL 32746**  
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Email: [drwilsonking@drwilsonking.com](mailto:drwilsonking@drwilsonking.com)  
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# GENESTER WILSON-KING, MD, FACOG

1540 International Pkwy Suite 2000, Lake Mary, FL 32746  
Home: 407-536-5125 - Cell: 352-636-5018 - drwilsonking@drwilsonking.com

---

## PROFESSIONAL SUMMARY

Highly-motivated, team-oriented and compassionate Board Certified Obstetrics and Gynecology Physician with 20+ years of direct obstetric and gynecologic private practice care experience.

Certified in Bioidentical Hormone Therapy and Preventive Medicine (Worldlink Medical).

Certified in Cannabinoid Medicine by the American Academy of Cannabinoid Medicine, and am an experienced cannabinoid clinician.

An active member of the Society of Cannabis Clinicians.

---

## SKILLS

- Community medicine competence
  - Dedicated to integrity
  - Obstetrics and gynecology expertise
  - Medical staff management
  - Medical research comprehension
  - Conscientious provider
  - Culturally sensitive
  - Conflict resolution ability
  - Strong interpersonal skills
  - Compassionate professional
  - Open-minded communicative physician
  - Calm under pressure
  - Recipient of Florida Hospital Waterman Distinguished Physician Award, (1994, 1996)
  - Florida Hospital Altamonte Physician Recognition Award (1999)
  - Has achieved a competent knowledge base in the field of Cannabinoid Medicine
  - Member of the Society of Cannabis Clinicians and the American Academy of Cannabinoid Medicine
- 

## WORK HISTORY

07/1986 to 06/1990 **Physician**

**Florida Community Health Centers – Fort Pierce, FL**

Provided Obstetrical and Gynecological care to the medically underserved in St. Lucy County and Okeechobee County.

Provided prenatal care and delivered up to 90 babies per month. Also provided gynecological care including routine, diagnosing and treating gynecological conditions and performing gynecological surgery.

07/1990 to 07/2010 **Physician in Private Practice**

**Self – Lake County, FL, FL**

Maintained the largest Obstetrical and Gynecological practice in Lake County. Name of practice is A Place for Women.

07/2010 to Current **Physician in Private Practice**

**Self – Lake Mary, FL**

I transitioned from a typical OB/GYN practice to a Functional Medicine (also called Preventive Medicine) practice and became Victory Rejuvenation Center, Inc.

While in Private practice in Lake County, I became known as the "Hormone Doctor". I now manage women with PMS, perimenopause and postmenopause, and men with andropause (male menopause).

I have additional certification and training in cannabinoid medicine.

---

## EDUCATION

- 1978 **Bachelor of Arts: Biology**  
**Swarthmore College - Swarthmore, 19081 PA**
- 1982 **M.D.: Medicine**  
**Jefferson Medical College/Sydney Kimmel Medical College - Philadelphia, PA**
- 1986 **M.D.: OB/GYN Residency**  
**Thomas Jefferson University Hospital - Philadelphia, PA**

---

## ACCOMPLISHMENTS

Elected Chairman Department of OB/GYN at every hospital I was a staff physician. That includes

1. Lawnwood Regional Medical Center, Ft. Pierce Florida, 1988-89
2. Florida Hospital Waterman, Eustis, Florida, 1993-1995
3. Florida Hospital Altamonte, Altamonte Springs, Florida 1998-2000

Speakers Bureau for Watson Pharmaceuticals from 1997-2001

Nationally known speaker for a variety of topics including oral contraceptives, premenstrual syndrome, perimenopause, menopause, andropause (male menopause), polycystic ovary syndrome, hormone deficiency and cannabis in medicine.

Fellow of the American College of Obstetricians and Gynecologists

Member of American Board of OB/GYN, Board Certified initially 1988, Recertified 1998, 2008, 2009, 2010, 2011, 2012, 2013, 2014

Certification in Bioidentical Hormone Therapy and Preventive Medicine, WorldLink Medical Salt Lake City, UT 2012

Speaker throughout the state of Florida for the use of Cannabis in Medicine

Member American Academy of Cannabinoid Medicine, and the Society of Cannabis Clinicians

Certified Cannabinoid Medicine Specialist (2015)

## Bist, Kevin

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 1:16 PM  
**To:** Jorge@flapartners.com  
**Subject:** RE: Question regarding Notice of Negotiated Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Jorge,

The negotiating committee has not been selected. Any person interested in being on the committee should send a request to me by email. The request should include an explanation of why they are interested in being on the committee as well as what qualifications they believe would make them a good choice for the committee.

Patty

---

**From:** Jorge Chamizo [<mailto:jorge@flapartners.com>]  
**Sent:** Monday, January 05, 2015 5:17 PM  
**To:** Nelson, Patricia A  
**Subject:** Question regarding Notice of Negotiated Rulemaking

Ms. Nelson:

Good afternoon. I was writing to inquire about the Noticed Negotiating Rulemaking Proceeding which appeared in today's FAR. My client is Knox Nursery, Inc, one of the qualified nurseries meeting the statutory criteria of section 381.986(5), F.S. Has the nursery committee member already been selected or is this something that will take place during the February 4<sup>th</sup> meeting?

Thank you for your time and courtesy.

Sincerely,

Jorge Chamizo



## Bist, Kevin

---

**From:** Jorge Chamizo <jorge@flapartners.com>  
**Sent:** Tuesday, January 06, 2015 1:17 PM  
**To:** Nelson, Patricia A  
**Subject:** RE: Question regarding Notice of Negotiated Rulemaking  
**Attachments:** Jorge Chamizo2.vcf

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Thank you Patty. That is most helpful. We will get you something by the end of the week. Thank you for getting back to me so quickly.



---

**From:** Nelson, Patricia A [<mailto:Patricia.Nelson@flhealth.gov>]  
**Sent:** Tuesday, January 06, 2015 1:16 PM  
**To:** Jorge Chamizo  
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Patty

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Thank you for your time and courtesy.

Sincerely,

Jorge Chamizo



## Bist, Kevin

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 1:28 PM  
**To:** 'Genester Wilson-King'  
**Subject:** RE: Rules Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Dr. Wilson-King,

You should consult an attorney for an analysis of your specific situation. I can say that no law or rule has ever prohibited participation in the rulemaking process because of participation in the regulated activity. In fact, it is usually the opposite. I do not, however, know your entire situation, and you should seek the opinion of an attorney retained by you. As an employee of the State of Florida, I cannot give you legal advice.

Sincerely,

Patricia Nelson  
Director  
Office of Compassionate Use

---

**From:** Genester Wilson-King [<mailto:drwilsonking@drwilsonking.com>]  
**Sent:** Tuesday, January 06, 2015 1:16 PM  
**To:** Nelson, Patricia A  
**Subject:** Rules Committee

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I have attached my resume for your review.

Thank you and I will await your response.

--Genester Wilson-King MD, FACOG

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**Bist, Kevin**

---

**From:** Genester Wilson-King <drwilsonking@drwilsonking.com>  
**Sent:** Tuesday, January 06, 2015 1:52 PM  
**To:** Nelson, Patricia A  
**Subject:** Re: Rules Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

I am not asking for legal advice. I am asking if that is a policy of the rules committee. I think you answered my question. There is not physician rule or regulation that is violated.  
I am just making sure that I do the right thing.:-)

Do you have a doctor on the committee already? If not, I am interested. I attached my resume to the previous email.

Thank you.

--Genester Wilson-King MD, FACOG

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On 1/6/15 1:27 PM, "Nelson, Patricia A" <[Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov)> wrote:

Dr. Wilson-King,

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participation in the rulemaking process because of participation in the regulated activity. In fact, it is usually the opposite. I do not, however, know your entire situation, and you should seek the opinion of an attorney retained by you. As an employee of the State of Florida, I cannot give you legal advice.

Sincerely,

Patricia Nelson  
Director  
Office of Compassionate Use

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**Bist, Kevin**

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 1:53 PM  
**To:** 'Genester Wilson-King'  
**Subject:** RE: Rules Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

We have not selected any committee members yet.

---

**From:** Genester Wilson-King [<mailto:drwilsonking@drwilsonking.com>]  
**Sent:** Tuesday, January 06, 2015 1:52 PM  
**To:** Nelson, Patricia A  
**Subject:** Re: Rules Committee

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I am just making sure that I do the right thing.-)

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Sincerely,

Patricia Nelson  
Director  
Office of Compassionate Use

**From:** Genester Wilson-King [<mailto:drwilsonking@drwilsonking.com>]  
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**To:** Nelson, Patricia A  
**Subject:** Rules Committee

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## Bist, Kevin

---

**From:** Joel Ewusiak <joel@ewusiaklaw.com>  
**Sent:** Tuesday, January 06, 2015 2:31 PM  
**To:** Nelson, Patricia A  
**Subject:** RE: Rulemaking Committee for Low THC Cannabis Law

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Thanks, Patty. I'll send you a request via email tomorrow. I think I would offer a fair and balanced approach to the rule-making process that is consistent with the law. I represent one of the few doctors currently authorized in Florida to order low-THC Cannabis (she has taken and passed the CLE course), as well as several Florida businesses and individuals wishing to apply for dispensary and cultivation licenses in the future. During last year's legislative session, I also extensively analyzed all of Florida's proposed medical marijuana laws, including the low THC Cannabis law that passed, so I am more than familiar with the issues at hand. By way of example only, please see:

<http://www.flamedicalmarijuanalawyer.com/news/2014/2/13/several-part-series-on-floridas-new-proposed-medical-marijuana-bill>.

Anyway, I will be in touch and thank you for your consideration.

-Joel

Joel Ewusiak

Ewusiak Law, P.A.

100 Main St., Suite 205

Safety Harbor, FL 34695

P: 727.286.3559 | F: 727.286.3219 | [www.ewusiaklaw.com](http://www.ewusiaklaw.com)

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---

**From:** Nelson, Patricia A [<mailto:Patricia.Nelson@flhealth.gov>]

**Sent:** Tuesday, January 6, 2015 1:07 PM

**To:** [joel@ewusiaklaw.com](mailto:joel@ewusiaklaw.com)

**Subject:** RE: Rulemaking Committee for Low THC Cannabis Law

Joel,

The negotiating committee has not been selected. Any person interested in being on the committee should send a request to me by email. The request should include an explanation of why they are interested in being on the committee as well as what qualifications they believe would make them a good choice for the committee.

Patty

---

**From:** Joel Ewusiak [<mailto:joel@ewusiaklaw.com>]

**Sent:** Monday, January 05, 2015 1:32 PM

**To:** Nelson, Patricia A

**Subject:** Rulemaking Committee for Low THC Cannabis Law

Hi Patricia:

I've reviewed the attached Notice of Development of Rulemaking for the low THC cannabis law. How are the committee members being selected and if they are already selected, who are the committee members? The notice outlines a procedure that must be followed in order to apply to participate in the February 2015 meeting, but the procedure seems to apply to non-committee members only.

Thanks,

-Joel

Joel Ewusiak  
Ewusiak Law, P.A.  
100 Main St., Suite 205  
Safety Harbor, FL 34695  
P: 727.286.3559 | F: 727.286.3219 | [www.ewusiaklaw.com](http://www.ewusiaklaw.com)

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**Bist, Kevin**

---

**From:** Scott <scott@skdgrp.com>  
**Sent:** Tuesday, January 06, 2015 3:00 PM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated Rule Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patty, this is a term I am not familiar with, but sounds like a good idea. How will you be selecting people to serve on the committee? I have a nursery owner in Alachua that could provide valuable input. Thanks. Scott Dick

Scott Dick  
SKD Consulting Group, Inc.  
210 South Monroe Street  
Tallahassee, FL 32301  
O: 850-421-9100 C: 850-545-4526



## Bist, Kevin

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 3:03 PM  
**To:** scott@skdgrp.com  
**Subject:** RE: Negotiated Rule Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Anyone who would like to serve on the committee can send an email to me (through you is fine) detailing their desire and qualifications.

---

**From:** Scott [mailto:scott@skdgrp.com]  
**Sent:** Tuesday, January 06, 2015 3:00 PM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated Rule Committee

Patty, this is a term I am not familiar with, but sounds like a good idea. How will you be selecting people to serve on the committee? I have a nursery owner in Alachua that could provide valuable input. Thanks. Scott Dick

Scott Dick  
SKD Consulting Group, Inc.  
210 South Monroe Street  
Tallahassee, FL 32301  
O: 850-421-9100 C: 850-545-4526



**Bist, Kevin**

---

**From:** Kirsten S. Yeager <kirsten@marijuanalegalconsultants.com>  
**Sent:** Tuesday, January 06, 2015 3:04 PM  
**To:** Nelson, Patricia A  
**Cc:** jfeiler@jeffreyfeiler.com  
**Subject:** Application for Rule-Making Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Dear Ms. Nelson:

Below please find my application information for the Rule-making Committee for SB 1030. I am very much interested in assisting with this process. If you have any questions, please do not hesitate to contact me at (305)670-7700.

Thank for considering me for this position.

Sincerely,  
Jeff Feiler

your name, business address, and telephone number; the name of any organization you are representing; a description of the organization or the members of the organization; a description of how the proposed rulemaking proceedings will affect you or the parties that you represent; a statement identifying the reasons why you believe the representative groups listed above will not adequately represent your interests; and a statement that you are willing to negotiate in good faith and can attend the scheduled meeting.

1.Name: Jeffrey E. Feiler

2. Business Address: Jeffrey E. Feiler Law Firm

7685 SW 104 Street, Suite 200

Miami, Fl. 33156

(305) 670-7700

Cell (305) 586-7492

3. Representing Grass Roots Ventures, Inc. and Clients interested in participating in a Dispensing Organization

4. Grass Roots Ventures, Inc. is a Florida Corporation with subsidiary companies Grass Roots Grows, Inc., Grass Roots Franchise, Inc., Asa Cannabis Genetics, Inc., Dispensing Organization Administrator, Inc., Grass Roots Foundation for a Cure, Inc. and individual Clients of Marijuana Legal Consultants (MLC).

5. The Rulemaking affects all aspects of the Companies' and Clients' interests including Dispensaries, Cultivation, Production, Extractions, Medications and Delivery systems.

6. I am applying for the Committee in accordance with number 7--An

individual with demonstrated experience in establishing and navigating regulatory structures for cannabis in other jurisdictions.

My experience is as follows; I am an Attorney admitted and practicing in Florida since 1982 (Office in Miami, Fl) and in Colorado since 2001 (Office in Boulder, Co.). My focus is Criminal Law. I was a Prosecutor from 1982-1985 with Janet Reno in Miami. I have been a Criminal Defense Attorney in private practice since 1986 (President of the Florida Association of Criminal Defense Attorneys 1999-2000 Miami). AV Rated by Martindale-Hubbell. I have been on the Boards of numerous philanthropic Foundations. I have been a Past President of Kiwanis International (Miami) and Past President of (BNI) Business Networking International (Miami) In 2009-2010 I spent one full year in Colorado monitoring and participating in the process of creating the Rules and Regulations which implemented the Colorado Constitutional Amendment through HB 1284, the Administrative Agency created by the Department of Revenue being the Marijuana Enforcement Division and the process through which Municipalities (Boulder County, City of Boulder, Longmont, Denver) made their rules. I assisted my family in establishing Dispensaries, Growing Operations and Infused Product Operations which are amongst the most well respected licensed entities in Colorado to this day. I have personally experienced in Colorado everything that is being formulated in Florida today. It is a fact that I am one of the most experienced Attorneys in Florida regarding the Cannabis industry.

7. Notwithstanding that I have personal future financial interests, represent Companies in which I have ownership with future financial interests and represent Clients with future financial interests, I will indeed negotiate in good faith and will be available to attend the meetings in Tallahassee. It is my belief that SB 1030 will at some point merge with eventual Legislation (whether or not via a Constitutional Amendment) and become part of a larger Statewide Cannabis industry. It is important that we get the implementation of SB 1030 correct from the start in order that Cannabis based medications will be formulated for the benefit of patients in this State and in the Nation.

## Bist, Kevin

---

**From:** Scott <[scott@skdgrp.com](mailto:scott@skdgrp.com)>  
**Sent:** Tuesday, January 06, 2015 3:05 PM  
**To:** Nelson, Patricia A  
**Subject:** RE: Negotiated Rule Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Thanks.

Scott Dick  
SKD Consulting Group, Inc.  
210 South Monroe Street  
Tallahassee, FL 32301  
O: 850-421-9100 C: 850-545-4526



---

**From:** Nelson, Patricia A [<mailto:Patricia.Nelson@flhealth.gov>]  
**Sent:** Tuesday, January 6, 2015 3:03 PM  
**To:** Scott  
**Subject:** RE: Negotiated Rule Committee

Anyone who would like to serve on the committee can send an email to me (through you is fine) detailing their desire and qualifications.

---

**From:** Scott [<mailto:scott@skdgrp.com>]  
**Sent:** Tuesday, January 06, 2015 3:00 PM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated Rule Committee

Patty, this is a term I am not familiar with, but sounds like a good idea. How will you be selecting people to serve on the committee? I have a nursery owner in Alachua that could provide valuable input. Thanks. Scott Dick

Scott Dick  
SKD Consulting Group, Inc.  
210 South Monroe Street  
Tallahassee, FL 32301  
O: 850-421-9100 C: 850-545-4526



## Bist, Kevin

---

**From:** arthur rosacker <treyrosacker@me.com>  
**Sent:** Tuesday, January 06, 2015 4:08 PM  
**To:** Nelson, Patricia A  
**Subject:** Growers position on compassionate use program

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Dear Patty,

It was a pleasure speaking to you today regarding the position for a grower on the compassionate marijuana panel.

I'm a fourth generation nurseryman, my grandfather came down in 1955 in Delray Beach Florida and started Floral Acres nursery with his brothers and my father. That nursery was 150 acres. We were the largest Grower on the East Coast for cut pom-poms. After South America put that program to rest we became the largest grower of certain potted plants. For example, over a million six-inch poinsettias, 500,000 six-inch chrysanthemums and 20 other flowering species plus 60 different foliage varieties. Today, I specialize still in Poinsettias, Hydrangeas, Mandavillias, Bougainvillea's and different types of Potted ferns.

I have been the head grower/manager for over 30 years and the owner for the past 20 years. I received my degree at the University of Florida in horticulture and continued my studies at the University of Riverside in tissue culture.

I would like to be on the panel because I have a friend who has a child that is three years old with epilepsy and I would like to make a difference!

My company is also one of the 80+ nurseries that have been approved to be a compassionate use marijuana grower/nursery. My zone is in the south east region.

Thank you for your consideration,

Arthur (Trey) Rosacker

Go Noles!  
Sent from my iPad

## Bist, Kevin

---

**From:** Nelson, Patricia A  
**Sent:** Tuesday, January 06, 2015 4:08 PM  
**To:** 'arthur rosacker'  
**Subject:** RE: Growers position on compassionate use program

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Got it!

-----Original Message-----

**From:** arthur rosacker [<mailto:treyrosacker@me.com>]  
**Sent:** Tuesday, January 06, 2015 4:08 PM  
**To:** Nelson, Patricia A  
**Subject:** Growers position on compassionate use program

Dear Patty,

It was a pleasure speaking to you today regarding the position for a grower on the compassionate marijuana panel.

I'm a fourth generation nurseryman, my grandfather came down in 1955 in Delray Beach Florida and started Floral Acres nursery with his brothers and my father. That nursery was 150 acres. We were the largest Grower on the East Coast for cut pom-poms. After South America put that program to rest we became the largest grower of certain potted plants. For example, over a million six-inch poinsettias, 500,000 six-inch chrysanthemums and 20 other flowering species plus 60 different foliage varieties. Today, I specialize still in Poinsettias, Hydrangeas, Mandavillias, Bougainvillea's and different types of Potted ferns.

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Thank you for your consideration,

Arthur (Trey) Rosacker

Go Noles!  
Sent from my iPad

## Bist, Kevin

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**From:** Watson.Strategies <watson.strategies@comcast.net>  
**Sent:** Tuesday, January 06, 2015 7:47 PM  
**To:** Nelson, Patricia A  
**Cc:** Watson.strategies@comcast.net  
**Subject:** negotiated rulemaking committee suggestion

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patty:

I think this guy would be great for the "testing laboratory" position.  
Said hey to the Surgeon General today at the capital.  
Hope you are well.

Ron Watson, President  
Watson Strategies  
(850) 567-1202



On Jan 6, 2015, at 6:21 PM, George Fernandez <[george@moderncanna.com](mailto:george@moderncanna.com)> wrote:

Good afternoon Ron,

I reviewed your email and would like to inform you that I am interested in being the rule making committee representative for testing laboratories. As you know, I have been following Senate Bill 1030 and the drafting of Regulations very closely. I attended the Rule Development Workshops in 2014 and gave several recommendations to the DOH. I am currently the Vice President of Phoslab Environmental Services, Inc. (PES), located in Lakeland, FL. We are regarded as a full-service, analytical laboratory that provides testing services to multiple business segments, including environmental, developmental, industrial, pharmaceutical, agricultural, and phosphate industries. We are certified and accredited by the Department of Health, recognized for accreditation by the National Environmental Laboratory Accreditation Program, and approved by the State Surgeon General. We were recently inspected by the Department of Health in July 2014 and received minimal deficiencies.

We already have Quality Control Manuals, Safety Manuals, Chemical Hygiene Plans, Internal Audit Guidelines, Corrective Action Plans, and Standard Operating Procedures for every laboratory function needed to perform low-THC cannabis testing. These Standard Operating Procedures include proficiency testing, accuracy, comparability, sample tracking, sample acceptance, sample receipt protocol, sample custody, sample storage, sample disposal, calibration procedures, equipment maintenance, and reporting.

Additionally, I have consulted with medical cannabis laboratories that have been operating in California, Colorado, and Washington, and met with a number of well-established dispensary owners in the Denver area to learn everything about the specifics of cannabis testing.

PES has been operating in central Florida since 1965. I formed Modern Canna Science, LLC. (MCS) to allow for easier identification and recognition among operating organizations within this new industry. We have the following instrumentation at our testing facilities:

- High Performance Liquid Chromatography (HPLC) to provide precise cannabinoid profiles of cannabis plants and derivatives. This is the only accepted quantitative method for potency analysis, HPLC testing provides Tetrahydrocannabinol and Cannabidiol concentrations of a sample, reported as a percentage by weight.
- Gas Chromatography (GC) and Liquid Chromatography (LC) to detect and quantify residual solvents, chemical additives, pesticides, mycotoxins, etc.
- Inductively Coupled Plasma (ICP) to detect and quantify heavy metals down to parts per billion.
- 3.5X-180X 144-LED Zoom Stereo Microscope with 3MP Digital Camera for microbial analysis.

I think this is an incredible opportunity for Florida to really set the bar high in this industry in terms of quality control. It would be very exciting to be on the ground level.

Should you have any questions or would like to discuss these comments in further detail, please don't hesitate to contact me.

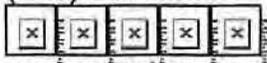
Best regards,

--

**George Fernandez**  
Chief Executive Officer



3615 Century Blvd., Unit 2  
Lakeland, FL 33811  
(863) 797-9963



[www.moderncanna.com](http://www.moderncanna.com)

## NOTICE OF DEVELOPMENT OF RULEMAKING

### DEPARTMENT OF HEALTH

#### Office of Compassionate Use

##### RULE NOS.: RULE TITLES:

64-4.001 Regulatory Structure Rule 1  
64-4.002 Regulatory Structure Rule 2  
64-4.003 Regulatory Structure Rule 3  
64-4.004 Regulatory Structure Rule 4  
64-4.005 Regulatory Structure Rule 5  
64-4.006 Regulatory Structure Rule 6  
64-4.007 Regulatory Structure Rule 7  
64-4.008 Regulatory Structure Rule 8  
64-4.009 Compassionate Use Registry

**PURPOSE AND EFFECT:** The Department of Health announces the convening of a negotiated rulemaking proceeding to address the regulatory structure for dispensing organizations of low-THC cannabis. The purpose of the negotiated rulemaking is to draft mutually acceptable proposed rules.

**SUBJECT TO BE ADDRESSED:** The subject and scope of the rules to be developed through negotiated rulemaking will be the regulatory structure for dispensing organizations of low-THC cannabis.

**RULEMAKING AUTHORITY:** 381.986 FS.

**LAW IMPLEMENTED:** 381.986 FS.

**NEGOTIATED RULEMAKING COMMITTEE:** The negotiated rulemaking committee members will be selected from the following representative groups:

1. A nursery that meets the criteria in Section 381.986(5)(b)1., Florida Statutes;
2. A qualified patient or patient representative;
3. A testing laboratory;
4. A member of the Florida Bar experienced in administrative law;
5. An individual with demonstrated experience in sound agricultural practices and necessary regulation;
6. A physician authorized to order low-THC Cannabis products for qualified patients;
7. An individual with demonstrated experience establishing or navigating regulatory structures for cannabis in other jurisdictions; and
8. Representatives of the Department of Health.

If you believe that your interests are not adequately represented by the committee members listed above, you may apply to participate within 30 days of the date of publication of this notice. Your application must contain the following information: your name, business address, and telephone number; the name of any organization you are representing; a description of the organization or the members of the organization; a description of how the proposed rulemaking proceedings will affect you or the parties that you represent; a statement identifying the reasons why you believe the representative groups listed above will not adequately represent your interests; and a statement that you are willing to negotiate in good faith and can attend the scheduled meeting. Please submit your application to Patricia Nelson, Department of Health, 4052

Bald Cypress Way, Bin A-02, Tallahassee, Florida 32399, email address: [Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov).

**NEGOTIATED RULEMAKING COMMITTEE MEETING:**

The committee will meet at the following date, time, and place to discuss rule development: February 4, 2015, 8:00 a.m. - 10:00 p.m. and February 5, 2015, 8:00 a.m. until concluded, Department of Health, Room 301, 4052 Bald Cypress Way, Tallahassee, FL 32399.

Pursuant to provisions of the Americans with Disabilities Act, any person requiring special accommodations to participate in this meeting is asked to advise the agency at least 72 hours before the meeting by contacting Sophia Flowers, Department of Health, [\(850\)245-4005](tel:(850)245-4005), [Sophia.Flowers@flhealth.gov](mailto:Sophia.Flowers@flhealth.gov). If you are hearing or speech impaired, please contact the agency using the Florida Relay Service, [1 \(800\)955-8771](tel:1(800)955-8771) (TDD) or [1 \(800\)955-9770](tel:1(800)955-9770) (Voice).

**THE PERSON TO BE CONTACTED REGARDING THE PROPOSED RULE DEVELOPMENT AND A COPY OF THE PRELIMINARY DRAFT, IF AVAILABLE IS:**  
Patricia Nelson, Department of Health, 4052 Bald Cypress Way, Bin A-02, Tallahassee, Florida 32399, Email address: [Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov)

**THE PRELIMINARY TEXT OF THE PROPOSED RULE DEVELOPMENT IS NOT AVAILABLE.**

## Bist, Kevin

---

**From:** anthony ardizzone <tvanursery@yahoo.com>  
**Sent:** Wednesday, January 07, 2015 6:52 AM  
**To:** Nelson, Patricia A  
**Subject:** Committee Participant

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patricia Nelson,

Anthony Ardizzone  
Ed Miller & Son Quallified Nursery under SB 1030

I would like to be part of the committee as a qualified Nursery, however, I do believe there should be more than one nursery on the committee, different perspectives are always a good thing. The past statements I made in the rule making process over the last several months show that I have been consistent in recommending what is fair to all. I will be open minded to all suggestions, and have many of my own. I can bring knowledge of the CBD industry, knowledge of rules in place for other states, as well as over 30 years of business experience in the landscape nursery industry which, will help us to succeed in this rule making process. I have been working on a set of rules since the challenge was initiated, knowing the outcome would delay getting this out to those who need it desperately. The rules must put the patient first, and allow nurseries to compete on a level field without the special interests of each competitor. Costs are a serious factor also, taking into account the end result to the patient. Rules and procedures can drive the price to a point that would not be affordable to most.

SB1030 gives us the outline, if we stick to it I belive we can do this quickly, and avoid challenges. With the right participation, understanding, and knowledge of the industry we will create rules for, we can get this done!

Look forward to working with you  
Any questions you may have for me I am always available by cell phone @ 772-201-3065

Anthony Ardizzone  
Ed Miller & Son Nursery  
Martin County FL Southeast Region

[Sent from Yahoo Mail on Android](#)

**Bist, Kevin**

---

**From:** anneliese clark <annelieseclark@gmail.com>  
**Sent:** Wednesday, January 07, 2015 11:06 AM  
**To:** Nelson, Patricia A  
**Subject:** Re: Low-THC Cannabis Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patty,  
I am very interested in committee position #2:  
2. *A qualified patient or patient representative;*

I was not clear as to the selection process of the actual committee members, so I am reaching out to you personally. I feel I would be an ideal candidate. I have attended every DOH Rule Workshop and spoke at the Senate Criminal Justice subcommittee. Spending my own money, I went to CO in September and toured 3 types of grow operations (hydro, indoor and outdoor); 8 dispensaries to better educate myself about what "successful programs" look like. In October, I spent 2 weeks in CA touring labs, dispensary, extraction facility and attended educational classes taught by a PhD plant scientist and a 20 year caregiver. I am not employed or representing any of the future applicants to SB1030 which allows me to remain objective without bias. I spent the summer months traveling around the state, speaking to groups about the importance of having medicinal cannabis as an option as well as the importance to regulated, structured safe access. I am a member of several "think tank" groups surrounding the science and delivery methods of the oils - tracking what works and what does not.

Personally - [REDACTED]  
these "other avenues" exist in our state, a structured, regulated program with education and safe, tested access is a must. [REDACTED]

Florida under these "other avenues". I do not favor CBD only legislation [REDACTED]  
cannabinoids are necessary, however I am strongly committed to seeing a medical marijuana program get up and running *correctly* in our state. I am hopeful and confident that our legislators will do the right thing in regards to expansion of the program.

A little background: My daughter is [REDACTED]. She has [REDACTED]  
[REDACTED] In 2013, [REDACTED],  
[REDACTED] I reached out to the Realm of Caring about moving to CO. The waiting list was over 10 months long. This made no sense to me and I knew my daughter would not make it another 10 months especially if we had to move to a strange place. I took matters into my own hands and threw myself into researching every aspect of this as medicine. [REDACTED]  
[REDACTED]

Last week I participated in a project to help raise awareness - legislatively, nationally. I am including the link.

<https://www.youtube.com/watch?v=l55kd5PyEuk>

A few references: Bill Wohlsifer, Ron Watson, Rutledge and Encina,

Thank you for your consideration  
Anneliese Clark  
904-813-5228

On Mon, Jan 5, 2015 at 5:11 PM, Nelson, Patricia A <[Patricia.Nelson@flhealth.gov](mailto:Patricia.Nelson@flhealth.gov)> wrote:

Dear Interested Parties,

Please see the attached Notice of Negotiated Rulemaking (on page 1 of the attached document) scheduled for February 4 and 5, 2015, in Tallahassee.

Sincerely,

Patty

Patricia Nelson

Director

Office of Compassionate Use

--

*Anneliese Clark*

904-813-5228

## Bist, Kevin

---

**From:** Paredes, Marco  
**Sent:** Wednesday, January 07, 2015 11:33 AM  
**To:** Nelson, Patricia A  
**Cc:** Bist, Kevin; Joos, Thomas  
**Subject:** FW: February 4th Rulemaking

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

FYI – Miami Children’s Hospital has interest in being a testing lab.

--

Marco T. Paredes, Jr.

Director | Office of Legislative Planning | Florida Department of Health | 2585 Merchants Row Blvd., Bin A-01 | Tallahassee, Florida 32399-1708 | Direct 850.245.4351 | Cell 850.728.5474 | [marco.paredes@flhealth.gov](mailto:marco.paredes@flhealth.gov)

---

**From:** Lani.Ferro@mch.com [mailto:Lani.Ferro@mch.com]  
**Sent:** Wednesday, January 07, 2015 11:08 AM  
**To:** Paredes, Marco  
**Cc:** MCantens@corcoranfirm.com; Brian@bhandassociates.com  
**Subject:** RE: February 4th Rulemaking

Thank you! We took a close look and think we would also qualify to participate as the non-university hospital with experience in low-THC trials or a physician authorized to order low-THC Cannabis products for qualified patients. Look forward to seeing you in Tally soon!

Lani

---

**From:** Paredes, Marco [mailto:Marco.Paredes@flhealth.gov]  
**Sent:** Wednesday, January 07, 2015 10:49 AM  
**To:** Lani Ferro  
**Cc:** MCantens@corcoranfirm.com; Brian@bhandassociates.com  
**Subject:** RE: February 4th Rulemaking

Good morning Lani,

Happy New Year to you as well. Sorry for the late response but it has been a packed week thus far. Regarding your question, to my knowledge we have not selected a testing laboratory. I am meeting with Patty this afternoon and will follow up and let you know. Thanks.

Marco

--

Marco T. Paredes, Jr.

Director | Office of Legislative Planning | Florida Department of Health | 2585 Merchants Row Blvd., Bin A-01 | Tallahassee, Florida 32399-1708 | Direct 850.245.4351 | Cell 850.728.5474 | [marco.paredes@flhealth.gov](mailto:marco.paredes@flhealth.gov)

---

**From:** [Lani.Ferro@mch.com](mailto:Lani.Ferro@mch.com) [<mailto:Lani.Ferro@mch.com>]  
**Sent:** Tuesday, January 06, 2015 2:47 PM  
**To:** Paredes, Marco  
**Cc:** [MCantens@corcoranfirm.com](mailto:MCantens@corcoranfirm.com); [Brian@bhandassociates.com](mailto:Brian@bhandassociates.com)  
**Subject:** February 4th Rulemaking

Hi, Marco!

Happy New Year!

I hope all is well! I was wondering, has DOH selected a testing laboratory? Our team recently spoke with Patricia regarding our clinical trial and protocol and I'm wondering if there is an opportunity to be involved. Please let me know.

Regards,

Lani

Lani Ferro, MPA, MBA  
Director, Governmental Affairs  
Miami Children's Hospital  
3100 SW 62nd Avenue  
Miami, FL 33155  
Office: 305.663.8435  
Mobile: 305.878.9590  
[lani.ferro@mch.com](mailto:lani.ferro@mch.com)

---

**From:** LobbyTools [<mailto:noreply@lobbytools.com>]  
**Sent:** Monday, January 05, 2015 6:37 PM  
**To:** Lani Ferro  
**Subject:** Legislative IQ: Evening Edition for Monday (1/5/15)

x

The Legislative Intelligence Company

January 5, 2015

**DOH schedules negotiated rulemaking workshop**

The Florida Department of Health announced it will create a negotiated rulemaking committee to implement a medicinal marijuana law. A committee of seven representing various parties with an interest in the state's Charlotte's Web law and DOH representatives will meet Feb. 4 in Tallahassee.

**Office of Insurance Regulation releases report on PIP reform**

The Florida Office of Insurance Regulation released its statutorily mandated Insurance Data Call Report Jan. 5, 2015, which found that preliminary data indicated that reforms enacted by the Florida Legislature in 2012 have had a significant positive impact on the trend of increasing PIP insurance rates due to fraudulent claims.

### **Same-sex marriage legalized in Florida**

District Judge Robert Hinkle settled a complicated legal dispute by declaring that same-sex marriage would become legal statewide following the Jan. 6 expiration of a stay on his original ruling striking down Florida's gay marriage ban.

### **Florida Department of Corrections investigates allegations of public records forgeries**

The Florida Department of Corrections has launched a criminal investigation into allegedly forged documents provided to reporters by a DOC spokesperson in connection with The Miami Herald's reporting on the death of DCI inmate Darren Rainey.

### **Fla. leads ACA enrollment**

There are 1.9 million new enrollees under the Affordable Care Act during the law's second enrollment period and Florida by far led the nation in that enrollment.

### **DEO requests \$5 million to advertise Florida's business climate**

The Department of Economic Opportunity is seeking \$5 million to advertise Florida as "The Perfect Climate for Business" in order to draw more industries and employers to the state, despite the Legislature's refusal to appropriate money to the project during the last two legislative sessions.

### **Families flock to Florida Prepaid**

Thanks to a 2014 bill cutting the projected costs of tuition, families are joining in droves a program allowing them to pre-pay for college.

### **Consumer Sentiment continues hitting post-recession highs**

Consumer Sentiment in Florida hit yet another post-recession high, standing at 87.4 -- the highest level of confidence measured by the monthly survey since February 2007.

### **Judge declares Fla. illegally deprived kids of health care**

A federal judge declared Florida's healthcare system for needy and disabled children to be in violation of several federal laws, handing a stunning victory to doctors and children's advocates who have fought for almost a decade to force the state to pay pediatricians enough money to ensure impoverished children can receive adequate care.

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## Bist, Kevin

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**Sent:** Wednesday, January 07, 2015 11:35 AM  
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**Subject:** RE: February 4th Rulemaking

**Follow Up Flag:** Follow up  
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On the negotiating committee?

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The Legislative Intelligence Company

January 5, 2015

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**Bist, Kevin**

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**From:** Darrick McGhee <darrick@teamjb.com>  
**Sent:** Wednesday, January 07, 2015 11:45 AM  
**To:** Nelson, Patricia A  
**Subject:** Holley letter to DOH.docx  
**Attachments:** Holley letter to DOH.docx; ATT00001.htm

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Good Afternoon Patty,  
Thank you for taking the time to speak with me is morning. Pursuant to our conversation and your request, attached, please find a letter from Ms. Holley expressing her interest in being considered for the Rule Task Force and why. Thank you again for taking the time.

## Education

- University of South Alabama, Bachelors in the Science of Nursing

## Work History

- Lee Memorial Children's Hospital, Fort Myers (May 2004 – Sept 2005)
  - Pediatric Floor Nurse
- Sacred Heart Children's Hospital, Pensacola (Sept 2005 – March 2007)
  - Infant/Toddler, School Age/Adolescent, PICU, and NICU
- Child Neurology Center / Clinical Research Group, Gulf Breeze (2005 – 2012)
  - Assisted with BOTOX administration
  - Clinical Research Nurse certified
  - Conducted pharmaceutical clinical trial for epilepsy, migraines, DMD, etc
- Epilepsy Society of Northwest Florida, Pensacola (Sept 2012 – Jan 2014)
  - Executive Director

But more important than my education or work history, I am the mother of a child with intractable epilepsy. My oldest daughter, RayAnn, is 11 years old and diagnosed with cerebral palsy and intractable epilepsy. I have done my research on high CBD cannabis oil and I believe it could change RayAnn's quality of life. I have had the opportunity to visit Charlotte Figi and the other patients using Charlotte's Web in CO, as well as, visit to Stanley Brother's greenhouses and lab. I would like to share what I have learned about high CBD oil with the DOH and help make this program the best it can be. My daughter's life depends on it!

Thank you for your consideration.

Holley Moseley

holleybythesea41@yahoo.com

**Bist, Kevin**

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**From:** Zachary Davis <daviszlaw1@gmail.com>  
**Sent:** Wednesday, January 07, 2015 1:55 PM  
**To:** Nelson, Patricia A  
**Subject:** Question

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Hello Ms./Mrs. Nelson,

Hope this email finds you well. Do you know whether it will be **considered** a conflict of interest if someone who is applying (nurseryman) or someone on his team (consultant) is part of the committee and subsequently receives the license?

Thank You,

Zachary Davis

## Bist, Kevin

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**From:** Brian A. Newman <brian@penningtonlaw.com>  
**Sent:** Wednesday, January 07, 2015 2:57 PM  
**To:** Nelson, Patricia A  
**Subject:** Negotiated Rulemaking Committee

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Hi Patty,

I am interested in serving on the Negotiated Rulemaking Committee for low-THC cannabis rules in the position of an attorney experienced in Administrative Law. I have not and do not represent any client with regard to this subject matter of the proposed rules.

Please let me know if you need any more information from me.

Kind regards,

**Brian A. Newman**  
Attorney At Law



215 S. Monroe Street, Suite 200  
Tallahassee, FL 32301  
P.O. Box 10095 (32302-2095)  
Tallahassee, FL 32301  
Office - (850) 222-3533  
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## Bist, Kevin

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**From:** Lois Adams <nloisadams@hhcs.com>  
**Sent:** Wednesday, January 07, 2015 4:49 PM  
**To:** Nelson, Patricia A  
**Subject:** APPLICATION FOR POSITION ON THE MEDICAL MARIJUANA RULES PANEL  
**Attachments:** Lois Word CV 2014.docx; PR 08-19-2010 good neighbor updated 9-1-2010 - Copy 2.pub; 2010 Lifetime Achievement Heroes - Lois.pdf; PR 05-12-2010 update 6-14-2010.docx; NAPW Press Release\_2011-2012.pdf; PR 4.30.10.doc; 2014 Release.docx; Industry Experts Proof for Cambridge.doc; FL House of Rep\_Ltr Recommendation.pdf; NECC\_2012 Continuum.pdf; 2014-11734\_PI.pdf .

**Importance:** High

**Follow Up Flag:** Follow up

**Flag Status:** Flagged

Dear Ms. Nelson:

You were referred to me by DeDe in Dr. John Armstrong's office. I would like to apply for a position on your Medical Marijuana Rules Panel. As a consultant pharmacist who has been active in health care in the State of Florida for many years, one who worked closely with a Board Certified Pain Specialist for approximately 18 years, and who was the Director of Inventory and Narcotic Controls for Orlando HealthCare Systems, I feel that I bring certain information and experience in this specialty arena. In addition, I opened and operated the Center for Memory Disorders which treated patients with neurological disorders including Alzheimer's Disease and dementia. In addition to the clinical skills, I bring business acumen to the panel and have been inducted in the University of Central Florida College of Business Hall of Fame.

Enclosed is my CV and other information to help with this possible appointment.

Thank you so much for taking my call. Please let me know if you need further information.

All the best,  
Lois Adams

*N. Lois Adams*

**Ms. N. Lois Adams, B. Pharm., MBA, CRPh**  
**President & CEO**  
**HHCS Health Group of Companies, LLC**  
**Freedom Pharmacy & Cystic Fibrosis Pharmacy**  
Phone: 407-898-4427 ext. 1001  
Fax: 407-897-2108  
**Web: [www.hhcs.com](http://www.hhcs.com)**



**[www.facebook.com/ourfreedompharmacy](http://www.facebook.com/ourfreedompharmacy)**

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e-mail do not necessarily represent the views or policies of HHCS Health Group of Companies, Freedom Pharmacy, Cystic Fibrosis Pharmacy or its employees.

**N. Lois Adams, B. Pharm. M.B.A. CRPh**

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**308 Palmway Lane  
Orlando, Florida 32828**

**CURRICULUM VITAE**

**HHCS HEALTH GROUP**

Freedom Pharmacy (HHCS Pharmacy, Inc. d/b/a)

Cystic Fibrosis Pharmacy, Inc.

Medical Management Enterprises, Inc.

Professional Pharmacy Network, LLC

**Corporate Headquarters:**

3901 East Colonial Dr.

Orlando, Florida 32803

(407) 898-4427

(888) 307-4427 Fax: (407) 898-6833

[www.ourfreedompharmacy.com](http://www.ourfreedompharmacy.com)

[www.cfpharmacy.com](http://www.cfpharmacy.com)

Email: [nloisadams@hhcs.com](mailto:nloisadams@hhcs.com)

**EDUCATION:**

American Academy of Anti-Aging; 2012- Present; Fellowship and Masters Degree in Anti-Aging, Regenerative and Functional Medicine.

Nova University, Ft. Lauderdale, Florida; 1990 - 1994. Doctoral program in Business Administration.

University of Central Florida; Orlando, Florida, 1979-1985. Masters Degree in Business Administration

University of Central Florida; Orlando, Florida, 1983. Post-Graduate Training. Biochemistry; Genetics.

University of Central Florida; Orlando, Florida, 1971. Postbaccalaureate. Non-degree.

Rollins College; Winter Park, Florida. 1970-1971. Postgraduate Training. Non-degree.

University of Florida; Gainesville, Florida. 1957-1962. Bachelor of Pharmacy.

Continuing Education Programs, Seminars, Consultant Seminars, Correspondence Courses

**INTERNSHIP:** 1960-1962.

**LICENSURES:**

Registered Pharmacist; State of Fla, 1962

Consultant Pharmacist: State of Fla, 1982

Certified Asthma Pharmacist - 2003

Certified OTC Advisor; Self Care Pharmaceutical Care - 2004

Certified Diabetes Manager - 2002

Certified Immunization Pharmacist - 2001

Certified Compounding Pharmacist - 1995

Certified Geriatric and Long Term Care Pharmacist - 2006

**2009 - Present**

President/CEO

**FLORIDA AMBULATORY INFUSION CENTERS, INC.;** Management of for-profit free-standing infusion suite providing comprehensive infusion services.

**2002 - 2013**

President/CEO/COB

**CENTER FOR MEMORY DISORDERS, INC.;** Management of not-for-profit medical clinic specializing in the treatment of memory and neurological disorders.

1984 - Present

President/CEO

**FREEDOM PHARMACY AND WELLNESS CENTERS;** Management of for-profit pharmacy specializing in the treatment of hard to manage, catastrophic illnesses. Participating pharmacy along with HHCS Research Institute, Inc. in Phase II and III clinical drug trials. Provision of IV infusions to home health agencies and ambulatory infusion centers; Specialty Compounding; Global outreach.

President/CEO -

**CYSTIC FIBROSIS PHARMACY, INC.** Management of for-profit pharmacy for the cystic fibrosis patient and their family. Working with major teaching medical centers as a source for information, education, and the provision of cost-effective therapy for the treatment of cystic fibrosis.

1996 - Present

President/CEO -

**MEDICAL MANAGEMENT ENTERPRISES, INC.;** Providing professional pharmacy case management; Recognized expert witness testimony in the field of pharmacy for the Technical Advisory Service for Attorneys (TASA). Consultant to Senior Resource Alliance.

1997 - Present

President/CEO

**INTERNATIONAL PHARMACY CORPORATION;** Engaged in the acquisition and sale of pharmaceuticals and related medical supplies in the international marketplace.

1993 - Present

President/CEO -

**MEDI-PAWS, INC.;** Management of specialty pharmacy providing veterinary medications and supplies.

1989 - Present

President/CEO -

**HHCS RESEARCH INSTITUTE, INC.** Management of private non-profit research institute; secure and conduct clinical drug trials; further independent research in areas of cancer, HIV disease, cystic fibrosis, inflammatory bowel disease, immunology and other complex illnesses; manufacture of specialty pharmaceutical products. Provide educational programs.

1985 - Present

President/CEO -

**PHYSICIANS WHOLESALE SUPPLY (HHCS, Inc.).** Management of physician's office wholesale distributor and medical retail sales. Design for medical offices and facilities, and hospital and retail pharmacies. Inventory

control systems; narcotic control systems. Cost analysis, containment and effectiveness studies and consults. Expert witness court testimony.

**1978 - Present**

President/CEO -

**MEDICAL CARE CONSULTANTS II, INC.** Financial and medical care management for health care industry. Systems analysis and design for medical offices and facilities, and hospital and retail pharmacies. Inventory control systems; narcotic control systems. Cost analysis, containment and effectiveness studies and consults. Expert witness court testimony.

**1985 - 1998**

President/CEO -

**HOME HEALTH CARE SERVICES, INC. (SouthMed Health Care div.).** Management of largest, privately held, multiple-location home health agency in Florida. Provided skilled nursing, home infusion therapy, pharmacy, respiratory and other health care services in the home setting; manufactured and compounded specialty pharmaceutical products; provided durable medical equipment and other supplies.

**1994-1998**

President/CEO-

**PROFESSIONAL STRATEGIES GROUP;** Management of private consulting firm providing professional marketing and strategic planning to business and industry.

**1982-1985**

Director for Inventory and Narcotic Control;

**ORLANDO REGIONAL MEDICAL CENTER,** Orlando, Florida . Dept of Pharmacy Services; Coordinate all pharmacy inventories in all hospital divisions, supervise purchasing of all pharmaceuticals develop and monitor all narcotic control systems, supervise all costs and revenues; supervise infusion pump services; supervise data management of department.

Chairman of the Formulary Committee; Member of hospital Pharmacy, Nutrition, and Therapeutics Committees; Member of hospital Investigational Drug and Device Committee. Wrote monthly column for physicians calendar.

Director of Narcotic Control. Developed new narcotic control systems for pharmacy, medical, and nursing staffs; insured compliance by hospital with all state and federal controlled substance laws; performed on-going audits, investigations, and in-services to medical and nursing staff on controlled substances regulations; developed seminars on controlled substances for professional staff in conjunction with the Drug Enforcement Agency and the Department of Professional Regulation. Developed instructional programs for continuing education in the proper use, pharmacological actions, and potential abuse of controlled substances for the professional staff. Maximized

pharmaceutical inventory turnover by establishing optimal inventory levels for all pharmaceuticals and supplies both in the pharmacy and nursing units of all hospital divisions; supervised the timely buying of pharmaceuticals by the pharmacy buyer. Reviewed all invoices prior to payment for accuracy to assure cost containment, and maximize discounts. Determined best contracts, annual bids, and special deals for drug purchases.

Completed Preceptorship in Oncology from M.D. Anderson Medical Center.

- 1981-1982** Staff and Clinical Pharmacist; **ORLANDO REGIONAL MEDICAL CENTER.**
- 1980-1981** Community Pharmacist, **LIGGETT DRUGS**, Orlando, Florida.
- 1966** Owner - **HOGUE PHARMACAL COMPANY.**
- 1966-1978** Owner - Officer, **COMPLETE BREATHING CARE, INC.**; Home office in Longwood, Florida.
- 1964-1965** Community pharmacist, **MOSES REXALL DRUGS**, Orlando, Florida.
- 1963-1964** Chief of Pharmacy Services, **PINELLAS COUNTY WELFARE DEPARTMENT.** St. Petersburg, Florida.
- 1962-1963** Chief of Pharmacy Services, **MONROE REGIONAL MEDICAL CENTER,** Ocala, Florida

#### **PROFESSIONAL ASSOCIATIONS:**

American Pharmacy Association; Member; Policy Review Committee 2011-present; House of Delegates (2012-Present)

American Society of Consultant Pharmacists

American Pharmacists Association Policy Committee (2011 - Present)

Florida Academy of Family Practice Foundation; Board of Trustees (1997 - 1999); Corporate Advisory Committee, Chairman (1997-1999); Charter Member;

Florida Pharmacy Association (Home Health Care Section, Chairman 1988-1990; Regulatory Affairs Chairman - 1990 - 1996; House of Delegates, 1982-1996); Legislative Committee Chairperson (1995 - 1996), Organizational Committee, Professional Affairs Committee (1992-1994); Governmental Affairs Committee (2011-present).

Central Florida Pharmacy Association (President, 1983-1984; 1985-1986); (Treasurer 1982-1983; 1984-1985); (Chairman, Grievance Committee, 1983-1986). Industrial Relations Committee. Regional Legislative Relations Committee. Regional Legislative Liaison. Chairman-Education Affairs Council, 1988-1989; Chairman and founder of Home Health Care Section of Academy of Pharmacy Practice (1988- 1994).

Central Florida Society of Hospital Pharmacy. (Chairman-Legislative Committee (1983-1984). PHARMPAC- Political Action Committee (Member, 1983- 1986).

Health Planning Council of West Central Florida (Districts 5 & 6); Co-Chairperson, Chronic Disease Committee.

Florida Independent Pharmacy Network; Vice Chair -Political Action Committee; (2012-Present); Vice President (2013-President)

#### CIVIC ACTIVITIES:

Specialty Pharmacy Continuum - McMahon Publication Advisory Board - 2011 to Present; Contributing author. 2011 to Present

University of Florida - National Advisory Board member; College of Pharmacy

American Diabetes Association - Board Member

Anthem College, Orlando, Fla- Professional Advisory Committee member 2007-Present

National Association of Community Pharmacists - Legislative Committee Member

American College of Clinical Pharmacy - Member

Florida Independent Pharmacy Network - Founding Member; Director

State of Florida, Agency for Health Care Administration - **Drug Utilization Review Committee** - 2002 - 2007

University of Florida College of Pharmacy - Clinical Assistant Professor; Preceptor, Pharmacy Internship Program. 2002 - Present; National Advisory Board 2011-Present

University of Massachusetts, College of Pharmacy, Preceptor, Pharmacy Internship Program. 2002 - Present

Nova Southeastern University, College of Pharmacy, Preceptor, Pharmacy Internship Program. 2002 - Present

**"Keeping Families Healthy"** Program; Faculty Advisor; Joint administration by: Univ. of Fla. Colleges of Medicine, Pharmacy and Nursing. 2002 - Present

Senior Resource Alliance (in Partnership with) - **Medication Management for Seniors Program** 2001 - Present

Republican Congressional Committee; Business Advisory Council - Honorary Chairman, 2002 - Present. State of Florida Delegation (present); Wash., DC.

Florida Presidential Business Commission

Transplant Recipients International Organization, Inc.; Member, Board of Directors (1999-2010).

University of Central Florida, College of Business; Dean's Executive Council, 2000. Board Member 2000 - Present;

East Orlando Rotary - Board of Directors

Leukemia Society; Central Florida Chapter; Secretary, 1994; Board of Directors 1991-1996; Chairperson, Patient Aid Committee, 1993 - 2000).

Orlando Philharmonic Orchestra; Board of Directors (1999 - 2001)

March of Dimes; Member, Board of Directors (1995 - 1998).

Orange County Republican Executive Committee - Precinct Chairperson 1995- Present.

Seminole County Republican Executive Committee - Precinct Chairperson.

National Foundation for Ileitis and Colitis (NFIC); Chapter President; Scientific Advisory Committee; Founded Support Group.

American Cancer Society (Board of Directors 1988-1993); Chairperson - Public Issues Committee (1998); Professional Affairs Committee.

College of Health and Public Affairs, University of Central Florida; Advisory Board 1988-1998

Town and Gown; University of Central Florida. 1996 - present.

Long-term Care Ombudsman Council, District 7, State of Florida (Appointed by Governor) 1987-1991. Consultant Pharmacist.

AREA AGENCY ON AGING, Coalition of Aging, Central Florida (1986-1998).  
Orlando Area Chamber of Commerce (Goals 2000 Committee - Long-term Care).  
American Marketing Association - Health Care Section Member  
Central Florida Cystic Fibrosis Support Group - consultant pharmacist  
B.A.S.E. Camp, Bd. of Directors, Secretary (1992 - present).  
Conway United Methodist Church, Orlando; Finance Committee, Secretary (1995 -1996).  
Professional Compounding Centers of America September 1996-Present  
Central Florida Candlelighters Support Group (for children with cancer)  
Orange County Public Schools Volunteer Services (ADDitions Program; Career Resource Volunteer).  
Central Florida Women's Resource Center  
Florida Executive Women.  
Winter Park Rotary Club (2013- present)

#### LECTURES:

Cystic Fibrosis Research, Inc. - "Things You Always Wanted to Know About Your Medications, But No One Would Tell You"  
Central Calif. Children's Hospital - "Things You Always Wanted to Know About Your Medications, But No One Would Tell You"  
Central Fla. CF Support Group - (1993 - Present) - Annual Presentations on CF Pharmacy Issues  
Univ. of Florida College of Pharmacy - "Dealing with CF in the World of Pharmacy"  
Children's Hospital, Fresno, CA - "Ask the Pharmacist About Generics & Alternative Meds"  
West Coast Area CF Education Day - "Pharmacy Questions You Never Get Answers For"  
Second Wind Transplant Organization - "What's New in Transplant Pharmacy"  
State of Florida - Dept. of Children & Families - "Mental Health Pharmacy - A New Age"  
(Many appearances on radio & television panels on the subject of cystic fibrosis, other chronic disease management, chemical dependence/abuse and other pharmacy related issues.)

#### HONORS:

University of Central Florida College of Business Administration 50<sup>th</sup> Anniversary Award for "CREATIVITY"  
Featured Member, Calendar Series, Who's Who Publishers (2012)  
Woman of the Year Award, National Association of Professional Women (2012)  
Woman of Outstanding Leadership Award, International Women's Leadership Assoc. (2012)  
Republican Leadership Award (2011)  
Featured Member, Elite Radio Network (2011)  
"Heroes of Hope Lifetime Achievement Award" Orlando Business Journal (2010)  
National U.S. Pharmacist of the Year " Good Neighbor Pharmacies of America (2010)  
Industry Expert, "Top 101 Industry Experts," Cambridge Who's Who (2010)  
Princeton Premier Professional Business Leaders - Esteemed Member (2009, 2008)  
Who's Who - Pharmacy Services Award and Lifetime Membership (2009)  
Who's Who - Professional of the Year (2009)  
"Wyeth - Bowl of Hygeia Award" - Fla. Pharmacy Association (2006)  
"5<sup>th</sup> Annual HHC Pharmacy Recognition Award" ( for Alzheimer's Work) - US Pharmacist & Esai Pharmaceuticals (2006)  
"Women of Magic" Award for Central Florida; *Orlando Sentinel* (2005)  
Innovative Pharmacy Practice Award, Florida Pharmacy Association (2004)

Florida Independent Pharmacy Network Award (2004)  
Elan Corporation, PLC Award (2004)  
R. Q. Richards Public Relations Award, Fla. Pharmacy Association (2004)  
"Women Who Mean Business " - Metro Orlando Economic Development Commission (2004)  
Hall of Fame Inductee - University of Central Florida College of Business (2004)  
Businessman of the Year - National Republican Congressional Committee (2003)  
Who's Who of Top Executives and Successful Businesses - Lifetime Member  
Who's Who of Top International Executives - Featured Member (1997)  
Who's Who and Why of Successful Florida Women - Featured Member  
Kappa Epsilon Professional Fraternity - Recipient of Audrey McCann Woman Pharmacist of the Year Award (1990)

**OTHER ACTIVITIES:** Pharmaceutical Consultant to U.S. Congressman Bill McCollum  
PROJECT HOPE

# 2010 Lifetime Achievement Heroes

Orlando Business Journal - by Melanie Stawicki Azam

Date: Thursday, November 18, 2010, 1:46pm EST

**Lois Adams** has spent the last 48 years working to provide the newest medicines, disease management and clinical trials to local patients. The pharmacist counsels her patients on wellness, has lectured on disease and has been honored for her advocacy on behalf of the chronically ill.



Adams is founder, president and CEO of the [HHCS Health Group](#) and its related companies, many of which she started during the past 20 years to help people with HIV, cystic fibrosis or memory disorders.

In 2010, Adams was named national Pharmacist of the Year as part of the Good Neighbor Pharmacy Recognition Awards. Adams also became the first woman in Florida to win the Wyeth-Bowl of Hygeia Award in 2006. In addition, she won an Innovative Pharmacy Practice Award in 2004 and was named

Businesswoman of the year by the National Republican Congressional Committee in 2003.

## **PERSONAL**

### Career goal as a child:

To win a Nobel Prize in a scientific area and to become a missionary.

### Little-known fact:

I enjoy writing comedy. I would like to have appeared on a program such as *Saturday Night Live*.

### Industry change I'd like to see:

The break-up of the medical and pharmaceutical monopolies.

### Favorite career memory:

The day we dedicated the Center for Memory Disorders, established in honor of my mother, Helen L. Littlefield.

### Last book read:

*The Language of Life: DNA and the Revolution in Personalized Medicine* by Francis Collins.

### Favorite website:

UpToDate.com, a medical website that reviews all journal articles written on any medical subject.

### What I'd teach a room full of fifth-graders:

Set goals of what you would like to be.



National Association of  
Professional Women  
THE POWER TO BE YOU

PRESS RELEASE  
— For Immediate Release —

**N. Lois Adams**  
**2011/2012 NAPW Professional Woman of the Year**  
*Making a Difference in the Healthcare Industry*



**N. Lois Adams**  
**B. Pharm, M.B.A., C.RPH**

President and Chief Executive  
Officer

**Company:**  
HHCS Health Group of Companies

N. Lois Adams, B. Pharm, M.B.A., C. RPH, President and Chief Executive Officer of HHCS Health Group of Companies, is being honored as a 2011/2012 Professional Woman of the Year in Healthcare by National Association of Professional Women. The prestigious distinction is awarded by the 400,000-strong membership of NAPW who join together to develop innovative business and social relationships.

N. Lois Adams' strong desire to help people inspired her to pursue a career in the healthcare field. In her nearly 50 years in the industry, she has never wavered in her commitment to assist those who need it most. In 1984, Ms. Adams founded HHCS Health Group of Companies, which expertly integrates health services and pharmaceuticals, providing a variety of community healthcare options to residents of Central Florida and beyond.

At HHCS, Ms. Adams works tirelessly to see that people have effective, convenient, and affordable healthcare choices. The organization boasts a number of independent pharmacies, medical clinics, medical management companies, and veterinary supply companies. HHCS recently announced the latest addition to its group of outstanding healthcare facilities with the opening of the Florida Ambulatory Infusion Center (FAIC), a state-of-the-art facility that offers infusion, injection, and comprehensive IV maintenance services in a comfortable, convenient, and cost-effective setting.

Prior to HHCS, Ms. Adams worked in the pharmaceutical management industry where she garnered the experience, skills, and knowledge that have made her an indisputable leader in the healthcare management and pharmaceutical fields. At HHCS, Ms. Adams is responsible for overseeing the operations of the group, clinical testing, mentoring, and teaching. She is also the Founder of the nonprofit Center for Memory Disorders, which is dedicated to helping those who suffer from illnesses such as Alzheimer's and dementia.

Those who know her often refer to Ms. Adams as 'Mother Teresa with a briefcase.' It is a well-deserved title as Ms. Adams continues to forge ahead, making a difference in the healthcare management and pharmaceutical industries.

**Awards & Accomplishments:**

Ms. Adams is a member of Cambridge Who's Who, The American Pharmacy Association, and the American College of Clinical Pharmacy. She earned a Master of Business Administration in Management from The University of Central Florida and a Bachelor of Pharmacy from The University of Florida.

**Keywords:**

healthcare options, pharmaceuticals, Central Florida, community healthcare options, HHCS Health Group of Companies, Florida Ambulatory Infusion Center

**Links:**

NAPW: <http://www.napw.com/profile/10866600/NLois-Adams/>



**Contact: Daniel Spitale**  
HHCS Health Group of Companies  
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Fax: 407-897-2108  
3901 E. Colonial Dr.  
Orlando, FL 32803  
Email: [spitale@hhcs.com](mailto:spitale@hhcs.com)



**GOOD  
NEIGHBOR  
PHARMACY®**



# Press Release

## Good Neighbor Pharmacy® Announces National Award Winner in Orlando

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*Lois Adams, B. Pharm., CPh, MBA of Freedom Pharmacy & Wellness Center named Pharmacist of the Year*

VALLEY FORGE, Pa. (August, 2010) – Good Neighbor Pharmacy Recognition Awards were presented to customers at their National Healthcare Conferences held in various locations across the country. The awards recognize the achievements of Good Neighbor Pharmacy stores in several categories, including the Pharmacist of the Year.

All award winners were selected from the Good Neighbor Pharmacy network of more than 3,600 independent pharmacies nationwide. This year's recognition award for the national Pharmacist of the Year is: **Lois Adams, B. Pharm, C. Ph, M.B.A., Freedom Pharmacy & Wellness Center in Orlando, FL.**

The Pharmacist of the Year award is bestowed to a pharmacist who is supremely dedicated to improving the quality of life of the people and towns he or she serves. Since 1984, Adams has served as President and CEO of Freedom Pharmacy, with the vision and mission to affect positive change for patients and the community at large. "We have been privileged to witness her dedication to pharmacy, her advocacy on behalf of so many causes and her remarkable achievements first hand," noted Joe Short, regional vice president, AmerisourceBergen.

As a dedicated pharmacist, Adams invites patients to make appointments with her outside of regular business hours and she hosts free glucose screenings for diabetes and provides free colorectal cancer screening kits. Adams is also an advocate for medication therapy management (MTM), which assists patients who may have issues in over-medicating, especially those with memory disorders. In addition to advocating for MTM, Adams has been actively involved in several causes such as Breast Cancer Awareness, the American Diabetes Association, American Cancer Society and the Leukemia Society.

Her impact in the pharmaceutical industry and her community does not go unnoticed. She became the first woman in Florida to win the "Wyeth - Bowl of Hygeia Award" in 2006; awarded an "Innovative Pharmacy Practice Award" in 2004; and named the "Businessman of the Year" by the National Republican Congressional Committee in 2003. Adams also served as a pharmaceutical consultant to U.S. Congressman Bill McCollum for Project Hope and is a member of the University of Central Florida Business Hall of Fame.

**About Good Neighbor Pharmacy**

For Immediate Release

**Contact: Daniel Spitale**  
HHCS Health Group of Companies  
Phone: 407-898-4427  
Fax: 407-897-2108  
3901 E. Colonial Dr.  
Orlando, FL 32803  
Email: [spitaled@hhcs.com](mailto:spitaled@hhcs.com)



# Press Release

## Freedom Pharmacy & Cystic Fibrosis Pharmacy Awarded Accreditation With ACHC

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**Orlando, FL, May 11, 2010:**

The HHCS Health Group of Companies proudly announces that Cystic Fibrosis Pharmacy, and Freedom Pharmacy and Wellness Center have been awarded a new three-year accreditation status, each with a perfect score of 100%, for the areas of specialty pharmacy, respiratory pharmacy and durable medical equipment services by the Accreditation Commission for Health Care, Inc. (ACHC).

ACHC, a private, not-for-profit corporation which is certified to ISO 9001:2008 standards, was developed by home care and community-based providers to help companies improve business operations and quality of patient care. Referring to the value of accreditation, ACHC President Tom Cesar indicated, "The survey process leads an organization to examine its policies and practices continually to clarify its strengths and improve its weaknesses."

The company was graded on the following areas:

Business Operations and Administration, Fiscal Management, Human Resource Management, Consumer Services/Records, QI/ Performance Management, Product Safety, Pharmacy Scope of Services and Home Medical Equipment Scope of Service

Accreditation is a voluntary activity where healthcare organizations submit to peer review of their internal policies, processes and patient care delivery against national standards. By attaining accreditation, Freedom Pharmacy and Cystic Fibrosis Pharmacy has demonstrated its commitment to maintain a higher level of competency and strive for excellence in its products, services and customer satisfaction.

Founded by N. Lois Adams in 1984, both Freedom Pharmacy and the Cystic Fibrosis Pharmacy stand as pharmacy exemplars with international reputations and multiple, award-winning pharmacists, providing special services throughout the United States, and as far away as South America and Europe.

**For Immediate Release**

**Contact: Daniel Spitale**

HHCS Health Group of Companies  
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**HHCS** *Health Group*  
of Companies



# Press Release

## HHCS Health Group of Companies Gives Two Scholarships to UF Pharmacy Students

**Orlando, FL, April 29, 2010:**

N. Lois Adams, President and CEO of the HHCS Health Group of Orlando, (Freedom Pharmacy and Wellness Center, the Cystic Fibrosis Pharmacy, Florida Ambulatory Infusion Centers and the Center for Memory Disorders) today awarded a \$1,000 scholarship for Pharmacy Entrepreneurship to Mr. Robert Bushey, of the University of Florida College of Pharmacy, the student whose overall background and essay best exemplifies a true understanding of what a pharmacist must do as a business person to succeed in an ever-changing health care environment.

Mr. Bushey has shown not only by his winning essay, but also by his participation in many scholastic and community activities that he truly has a grasp on the qualities that leaders must have.

A Second scholarship for \$500 was awarded to Mr. Ryan Milton, also of the University of Florida College of Pharmacy. What sets Mr. Milton apart is that he is also a cystic fibrosis patient.

Ms. Adams wished to show Mr. Milton how proud she was of his efforts to acquire a Pharm.D degree despite the many obstacles he has needed to overcome because of this disease.

Mr. Milton plans to use his education to find a treatment or a cure for the very disease that he has fought all his life.

**Bist, Kevin**

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**From:** Donna Blanton <dblanton@radeylaw.com>  
**Sent:** Wednesday, January 07, 2015 5:08 PM  
**To:** Nelson, Patricia A  
**Subject:** No conflict on low-THC cannabis rule development

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

I have confirmed that we are not involved with the issues on behalf of any client. Thanks for thinking of me -- I look forward to the opportunity.

Sent from my iPad

**Bist, Kevin**

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**From:** Nelson, Patricia A  
**Sent:** Wednesday, January 07, 2015 5:48 PM  
**To:** 'Donna Blanton'  
**Subject:** RE: No conflict on low-THC cannabis rule development

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Thank you for letting me know. I will keep you posted.

-----Original Message-----

**From:** Donna Blanton [<mailto:dblanton@radeylaw.com>]  
**Sent:** Wednesday, January 07, 2015 5:08 PM  
**To:** Nelson, Patricia A  
**Subject:** No conflict on low-THC cannabis rule development

I have confirmed that we are not involved with the issues on behalf of any client. Thanks for thinking of me -- I look forward to the opportunity.

Sent from my iPad

## Bist, Kevin

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**From:** ANNE <bestofalltherest@comcast.net>  
**Sent:** Wednesday, January 07, 2015 6:22 PM  
**To:** Nelson, Patricia A  
**Cc:** Bist, Kevin  
**Subject:** Hello from Anne Morgan, M.D  
**Attachments:** 6630507.pdf; ICRS2014.PROGRAMME.pdf; C.V.\_MMJ-2-1-2-2-4-2-2.doc

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Patty,

It was a pleasure to meet you at the Orlando workshop on Dec. 30, which I found very informative.

I am writing to ask your consideration to become a member of The Negotiated Rulemaking Committee.

I feel that I have a lot to bring to the table, both in medical management experience, my willingness to be open and honest about marijuana as a medicine, agree to **negotiate** in good faith and my goal of doing whatever I can as a physician to make sure that "We do it right in Florida."

At this early point in the history of cannabinoid medicine, very few physicians in Florida have the degree of interest and the background in cannabis education that I now have. I **sincerely believe that my interests as a clinician and scientist is not represented on the Committee at present.**

I am a Family Practitioner with more than 25 years of experience and no blemishes on my background. For quite a few years, I did pro bono work on the Peer Review Board for the Board Of Medicine.

A year ago, I became intrigued with the potential for cannabis as medicine, and have since spent a great deal of time getting up to speed.

I have applied for Board Certification with the American Academy Of Cannabinoid Medicine. In that effort, I have already passed the oral and written exam and will need to practice two years of cannabinoid medicine in order to become Board Certified in the field.

I attended the Patients Out of Time conference in Portland, Oregon last April, 2014.

The conference will be coming to the West Palm Beach Convention Center this Memorial Weekend and I will be playing a supporting role.

I expect to take the FMA CME course, completing that aspect by my application.

When at the Portland Conference, I met several scientists who invited me to become a member of the International Cannabinoid Research Society. I accepted their invitation and attended their 24th Annual Conference in Baveno, Italy last June/July. I learned that there is so much pertinent information that has not reached me or my colleagues in practice, especially in Florida.

I am taking this opportunity to send you **the** PDF file of the ICRS course syllabus. Several of the **authors** have agreed to act as "resource" persons when I asked them **last** June/July in advance of the upcoming Amendment 2, knowing that we would need help in Florida. I will do whatever I can to reach out to them in an effort to obtain as much medical/clinical information that we need to apply this law safely, efficiently, properly and fairly.

As a result, I was able to persuade ( along with some others) the founders of Patients Out Of Time to bring their next conference to West Palm Beach this coming May.

I have asked Greg Gerdeman, Ph.D, if he would agree to be a resource person for the DOH and he has graciously agreed. He is a world renowned expert in his field and we are very fortunate to have him at Eckerd College.

I am taking this opportunity to send you a copy of my long-form C.V., since most Medical Management positions want to know exactly what departments and programs an applicant has created and developed.

I do state that I am a member of the Palm Beach County Medical Society "Medical Marijuana Task Force" which does not give endorsements of medical cannabis, but wishes to work to keep this properly, safely and efficiently managed in the State of Florida. A letter to that effect will be sent to you directly very soon.

Please feel free to call me if I can be of any assistance: 954-592-0700.

I wish you all the best in your endeavor,

I will see you at the meeting on February 4th.

Respectfully submitted,

Anne Lynn Morgan, M.D.

860 US Highway 1

Suite 203 A

North Palm Beach, Fl 33408

# USPTO PATENT FULL-TEXT AND IMAGE DATABASE



( 1 of 1 )

United States Patent  
Hampson , et al.

6,630,507  
October 7, 2003

\*\*Please see images for: ( Certificate of Correction ) \*\*

Cannabinoids as antioxidants and neuroprotectants

## Abstract

Cannabinoids have been found to have antioxidant properties, unrelated to NMDA receptor antagonism. This new found property makes cannabinoids useful in the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, inflammatory and autoimmune diseases. The cannabinoids are found to have particular application as neuroprotectants, for example in limiting neurological damage following ischemic insults, such as stroke and trauma, or in the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and HIV dementia. Nonpsychoactive cannabinoids, such as cannabidoil, are particularly advantageous to use because they avoid toxicity that is encountered with psychoactive cannabinoids at high doses useful in the method of the present invention. A particular disclosed class of cannabinoids useful as neuroprotective antioxidants is formula (I) wherein the R group is independently selected from the group consisting of H, CH.sub.3, and COCH.sub.3. ##STR1##

**Inventors:** Hampson; Aidan J. (Irvine, CA), Axelrod; Julius (Rockville, MD), Grimaldi; Maurizio (Bethesda, MD)  
**Assignee:** The United States of America as represented by the Department of Health and Human Services (Washington, DC)  
**Family ID:** 26767641  
**Appl. No.:** 09/674,028  
**Filed:** February 2, 2001  
**PCT Filed:** April 21, 1999  
**PCT No.:** PCT/US99/08769  
**PCT Pub. No.:** WO99/53917  
**PCT Pub. Date:** October 28, 1999

Current U.S. Class:  
Current CPC Class:

514/454  
A61K 31/35 (20130101)

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*Primary Examiner:* Weddington; Kevin E.

*Attorney, Agent or Firm:* Klarquist Sparkman, LLP

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*Parent Case Text*

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## *Claims*

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We claim:

1. A method of treating diseases caused by oxidative stress, comprising administering a therapeutically effective amount of a cannabinoid that has substantially no binding to the NMDA receptor to a subject who has a disease caused by oxidative stress.

2. The method of claim 1, wherein the cannabinoid is nonpsychoactive.

3. The method of claim 2, wherein the cannabinoid has a volume of distribution of 10 L/kg or more.

4. The method of claim 1, wherein the cannabinoid is not an antagonist at the NMDA receptor.

5. The method of claim 1, wherein the cannabinoid is: ##STR22##

where R is H, substituted or unsubstituted alkyl, carboxyl, alkoxy, aryl, aryloxy, arylalkyl, halo or amino.

6. The method of claim 5, wherein R is H, substituted or unsubstituted alkyl, carboxyl or alkoxy.

7. The method of claim 2, wherein the cannabinoid is: ##STR23##

where A is cyclohexyl, substituted or unsubstituted aryl, or ##STR24## but not a pinene; R.sub.1 is H, substituted or unsubstituted alkyl, or substituted or unsubstituted carboxyl; R.sub.2 is H, lower substituted or unsubstituted alkyl, or alkoxy; R.sub.3 is of H, lower substituted or unsubstituted alkyl, or substituted or unsubstituted carboxyl; R.sub.4 is H, hydroxyl, or lower substituted or unsubstituted alkyl; and R.sub.5 is H, hydroxyl, or lower substituted or unsubstituted alkyl.

8. The method of claim 7, wherein R.sub.1 is lower alkyl, COOH or COCH.sub.3 ; R.sub.2 is unsubstituted C.sub.1 -C.sub.5 alkyl, hydroxyl, methoxy or ethoxy; R.sub.3 is H, unsubstituted C.sub.1 -C.sub.3 alkyl, or COCH.sub.3 ; R.sub.4 is hydroxyl, pentyl, heptyl, or diethylheptyl; and R.sub.5 is hydroxyl or methyl.

9. The method of claim 1, wherein the cannabinoid is: ##STR25##

where R.sub.1, R.sub.2 and R.sub.3 are independently H, CH.sub.3, or COCH.sub.3.

10. The method of claim 9, wherein the cannabinoid is: ##STR26##

where: a) R.sub.1 =R.sub.2 =R.sub.3 =H; b) R.sub.1 =R.sub.3 =H, R.sub.2 =CH.sub.3 ; c) R.sub.1 =R.sub.2 =CH.sub.3, R.sub.3 =H; d) R.sub.1 =R.sub.2 =COCH.sub.3, R.sub.3 =H; or e) R.sub.1 =H, R.sub.2 =R.sub.3 =COCH.sub.3.

11. The method of claim 2, wherein the cannabinoid is: ##STR27##

where R.sub.19 is H, lower alkyl, lower alcohol, or carboxyl; R.sub.20 is H or OH; and R.sub.21 -R.sub.25

are independently H or OH.

12. The method of claim 11, wherein R.sub.19 is H, CH.sub.3, CH.sub.2 OH, or COOH, and R.sub.20 - R.sub.24 are independently H or OH.

13. The method of claim 2, wherein the cannabinoid is: ##STR28##

where R.sub.19 and R.sub.20 are H, and R.sub.26 is alkyl.

14. The method of claim 10, wherein the cannabinoid is cannabidiol.

15. A method of treating an ischemic or neurodegenerative disease in the central nervous system of a subject, comprising administering to the subject a therapeutically effective amount of a cannabinoid, where the cannabinoid is ##STR29##

where R is H, substituted or unsubstituted alkyl, carboxyl, alkoxy, aryl, aryloxy, arylalkyl, halo or amino.

16. The method of claim 15, wherein the cannabinoid is not a psychoactive cannabinoid.

17. The method of claim 15 where the ischemic or neurodegenerative disease is an ischemic infarct, Alzheimer's disease, Parkinson's disease, and human immunodeficiency virus dementia, Down's syndrome, or heart disease.

18. A method of treating a disease with a cannabinoid that has substantially no binding to the NMDA receptor, comprising determining whether the disease is caused by oxidative stress, and if the disease is caused by oxidative stress, administering the cannabinoid in a therapeutically effective antioxidant amount.

19. The method of claim 18, wherein the cannabinoid has a volume of distribution of at least 1.5 L/kg and substantially no activity at the cannabinoid receptor.

20. The method of claim 19, wherein the cannabinoid has a volume of distribution of at least 10 L/kg.

21. The method of claim 1, wherein the cannabinoid selectively inhibits an enzyme activity of 5- and 15-lipoxygenase more than an enzyme activity of 12-lipoxygenase.

22. A method of treating a neurodegenerative or ischemic disease in the central nervous system of a subject, comprising administering to the subject a therapeutically effective amount of a compound selected from any of the compounds of claims 9 through 13.

23. The method of claim 22 where the compound is cannabidiol.

24. The method of claim 22, wherein the ischemic or neurodegenerative disease is an ischemic infarct, Alzheimer's disease, Parkinson's disease, and human immunodeficiency virus dementia, Down's syndrome, or heart disease.

25. The method of claim 24 wherein the disease is an ischemic infarct.

26. The method of claim 1, wherein the cannabinoid is not an antagonist at the AMPA receptor.

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*Description*

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## FIELD OF THE INVENTION

The present invention concerns pharmaceutical compounds and compositions that are useful as tissue protectants, such as neuroprotectants and cardioprotectants. The compounds and compositions may be used, for example, in the treatment of acute ischemic neurological insults or chronic neurodegenerative diseases.

## BACKGROUND OF THE INVENTION

Permanent injury to the central nervous system (CNS) occurs in a variety of medical conditions, and has been the subject of intense scientific scrutiny in recent years. It is known that the brain has high metabolic requirements, and that it can suffer permanent neurologic damage if deprived of sufficient oxygen (hypoxia) for even a few minutes. In the absence of oxygen (anoxia), mitochondrial production of ATP cannot meet the metabolic requirements of the brain, and tissue damage occurs. This process is exacerbated by neuronal release of the neurotransmitter glutamate, which stimulates NMDA (N-methyl-D-aspartate), AMPA (.alpha.-amino-3-hydroxy-5-methyl-4-isoxazole propionate) and kainate receptors. Activation of these receptors initiates calcium influx into the neurons, and production of reactive oxygen species, which are potent toxins that damage important cellular structures such as membranes, DNA and enzymes.

The brain has many redundant blood supplies, which means that its tissue is seldom completely deprived of oxygen, even during acute ischemic events caused by thromboembolic events or trauma. A combination of the injury of hypoxia with the added insult of glutamate toxicity is therefore believed to be ultimately responsible for cellular death. Hence if the additive insult of glutamate toxicity can be alleviated, neurological damage could also be lessened. Anti-oxidants and anti-inflammatory agents have been proposed to reduce damage, but they often have poor access to structures such as the brain (which are protected by the blood brain barrier).

Given the importance of the NMDA, AMPA and kainate receptors in the mechanism of injury, research efforts have focused on using antagonists to these receptors to interfere with the receptor mediated calcium influx that ultimately leads to cellular death and tissue necrosis. In vitro studies using cultured neurons have demonstrated that glutamate receptor antagonists reduce neurotoxicity, but NMDA and AMPA/kainate receptor antagonists have different effects. Antagonists to NMDAR prevent neurotoxicity if present during the glutamate exposure period, but are less effective if added after glutamate is removed. In contrast, AMPA/kainate receptor antagonists are not as effective as NMDA antagonists during the glutamate exposure period, but are more effective following glutamate exposure.

Some of the research on these antagonists has focused on cannabinoids, a subset of which have been found to be NMDA receptor antagonists. U.S. Pat. No. 5,538,993 (3S,4S-delta-6-tetrahydrocannabinol-7-oic acids), U.S. Pat. No. 5,521,215 (stereospecific (+) THC enantiomers), and U.S. Pat. No. 5,284,867 (dimethylheptyl benzopyrans) have reported that these cannabinoids are effective NMDA receptor blockers. U.S. Pat. No. 5,434,295 discloses that the 1,1 dimethylheptyl (DMH) homolog of [3R,4R]-7-hydroxy-.DELTA..sup.6 THC (known as HU-210) is a superpotent cannabinoid receptor agonist with cannabinomimetic activity two orders of magnitude greater than the natural .DELTA..sup.9 THC. The HU-210 dimethylheptyl cannabinoid, has severe side effects, including fatigue, thirst, headache, and hypotension. *J. Pharmacol. Sci.* 60:1433-1457 (1971). Subjects who received this synthetic cannabinoid with a dimethylheptyl group experienced marked psychomotor retardation, and were unwilling or incapable of assuming an erect position.

In contrast to HU-210, the (-)(3R,4R) THC-DMH enantiomer (known as HU-211) displays low affinity to the cannabinoid receptors, but retains NMDA receptor antagonist neuroprotective activity. ##STR2##

THC (tetrahydrocannabinol) is another of the cannabinoids that has been shown to be neuroprotective in cell cultures, but this protection was believed to be mediated by interaction at the cannabinoid receptor, and so would be accompanied by undesired psychotropic side effects. ##STR3##

Although it has been unclear whether cannabimimetic activity plays a role in neuroprotection against glutamate induced neurological injury, the teaching in this field has clearly been that a cannabinoid must at least be an antagonist at the NMDA receptor to have neuroprotective effect. Hence cannabidiol (2-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol or CBD), a cannabinoid devoid of psychoactive effect (Pharm. Rev. 38:21-43, 1986), has not been considered useful as a neuroprotectant. Cannabidiol has been studied as an antiepileptic (Carlini et al., J. Clin. Pharmacol. 21:417S-427S, 1981; Karler et al., J. Clin. Pharmacol. 21:437S-448S, 1981, Consroe et al., J. Clin. Pharmacol. 21:428S-436S, 1981), and has been found to lower intraocular pressure (Colasanti et al., Exp. Eye Res. 39:251-259, 1984 and Gen. Pharmac. 15:479-484, 1984). ##STR4##

No signs of toxicity or serious side effects have been observed following chronic administration of cannabidiol to healthy volunteers (Cunha et al., Pharmacology 21:175-185, 1980), even in large acute doses of 700 mg/day (Consroe et al., Pharmacol. Biochem. Behav. 40:701-708, 1991) but cannabidiol is inactive at the NMDA receptor. Hence in spite of its potential use in treating glaucoma and seizures, cannabidiol has not been considered a neuroprotective agent that could be used to prevent glutamate induced damage in the central nervous system.

## SUMMARY OF THE INVENTION

It is an object of this invention to provide a new class of antioxidant drugs, that have particular application as neuroprotectants, although they are generally useful in the treatment of many oxidation associated diseases.

Yet another object of the invention is to provide a subset of such drugs that can be substantially free of psychoactive or psychotoxic effects, are substantially non-toxic even at very high doses, and have good tissue penetration, for example crossing the blood brain barrier.

It has surprisingly been found that cannabidiol and other cannabinoids can function as neuroprotectants, even though they lack NMDA receptor antagonist activity. This discovery was made possible because of the inventor's recognition of a previously unanticipated antioxidant property of the cannabinoids in general (and cannabidiol in particular) that functions completely independently of antagonism at the NMDA, AMPA and kainate receptors. Hence the present invention includes methods of preventing or treating diseases caused by oxidative stress, such as neuronal hypoxia, by administering a prophylactic or therapeutically effective amount of a cannabinoid to a subject who has a disease caused by oxidative stress.

The cannabinoid may be a cannabinoid other than THC, HU-210, or other potent cannabinoid receptor agonists. The cannabinoid may also be other than HU-211 or any other NMDA receptor antagonist that has previously been reported. A potent cannabinoid receptor agonist is one that has an EC<sub>50</sub> at the cannabinoid receptor of 50 nM or less, but in more particular embodiments 190 nM or 250 nM or less. In disclosed embodiments the cannabinoid is not psychoactive, and is not psychotoxic even at high doses. In some particularly disclosed embodiments, the cannabinoid is selected from the group: ##STR5##

where A is aryl, and particularly ##STR6##

but not a pinene such as: ##STR7##

and the R.sub.1 -R.sub.5 groups are each independently selected from the groups of hydrogen, lower substituted or unsubstituted alkyl, substituted or unsubstituted carboxyl, substituted or unsubstituted alkoxy, substituted or unsubstituted alcohol, and substituted or unsubstituted ethers, and R.sub.6 -R.sub.7 are H or methyl. In particular embodiments, there are no nitrogens in the rings, and/or no amino substitutions on the rings.

In other embodiments, the cannabinoid is one of the following: ##STR8##

where there can be 0 to 3 double bonds on the A ring, as indicated by the optional double bonds indicated by dashed lines on the A ring. The C ring is aromatic, and the B ring can be a pyran. Particular embodiments are dibenzo pyrans and cyclohexenyl benzenediols. Particular embodiments of the cannabinoids of the present invention may also be highly lipid soluble, and in particular embodiments can be dissolved in an aqueous solution only sparingly (for example 10 mg/ml or less). The octanol/water partition ratio at neutral pH in useful embodiments is 5000 or greater, for example 6000 or greater. This high lipid solubility enhances penetration of the drug into the CNS, as reflected by its volume of distribution (V.sub.d) of 1.5 L/kg or more, for example 3.5 L/kg, 7 L/kg, or ideally 10 L/kg or more, for example at least 20 L/kg. Particular embodiments may also be highly water soluble derivatives that are able to penetrate the CNS, for example carboxyl derivatives.

R.sub.7-18 are independently selected from the group of H, substituted or unsubstituted alkyl, especially lower alkyl, for example unsubstituted C.sub.1 -C.sub.3 alkyl, hydroxyl, alkoxy, especially lower alkoxy such as methoxy or ethoxy, substituted or unsubstituted alcohol, and unsubstituted or substituted carboxyl, for example COOH or COCH.sub.3. In other embodiments R.sub.7-18 can also be substituted or unsubstituted amino, and halogen.

The cannabinoid has substantially no binding to the NMDAr (for example an IC.sub.50 greater than or equal to 5 .mu.M or 10 .mu.M), has substantially no psychoactive activity mediated by the cannabinoid receptor (for example an IC.sub.50 at the cannabinoid receptor of greater than or equal to 300 nM, for example greater than 1 .mu.M and a K.sub.i greater than 250 nM, especially 500-1000 nM, for example greater than 1000 nM), and antioxidant activity, as demonstratable by the Fenton reaction or cyclic voltametry.

In other particular embodiments, the cannabinoids are one of the following: ##STR9##

where R.sub.19 is substituted or unsubstituted alkyl, such as lower alkyl (for example methyl), lower alcohol (such as methyl alcohol) or carboxyl (such as carboxylic acid) and oxygen (as in .dbd.O); R.sub.20 is hydrogen or hydroxy; R.sub.21 is hydrogen, hydroxy, or methoxy; R.sub.22 is hydrogen or hydroxy; R.sub.23 is hydrogen or hydroxy; R.sub.24 is hydrogen or hydroxy; R.sub.25 is hydrogen or hydroxy; and R.sub.26 is substituted or unsubstituted alkyl (for example n-methyl alkyl), substituted or unsubstituted alcohol, or substituted or unsubstituted carboxy.

In yet other embodiments of the invention, the cannabinoids are ##STR10##

wherein numbering conventions for each of the ring positions are shown, and R.sub.27, R.sub.28 and R.sub.29 are independently selected from the group consisting of H, unsubstituted lower alkyl such as CH.sub.3, and carboxyl such as COCH.sub.3. Particular examples of nonpsychoactive cannabinoids that fall within this definition are cannabidiol and ##STR11##

and other structural analogs of cannabidiol.

In more particular embodiments, the cannabinoid is used to prevent or treat an ischemic or neurodegenerative

disease in the central nervous system of a subject, by administering to the subject a therapeutically effective amount of a cannabinoid to protect against oxidative injury to the central nervous system. The cannabinoid may be any of the compounds set forth above, or more specifically ##STR12##

wherein R.sub.27, R.sub.28 and R.sub.29 are independently selected from the group consisting of H, lower alkyl such as CH.sub.3, and carboxyl such as COCH.sub.3, and particularly wherein a) R.sub.27 =R.sub.28 =R.sub.29 =H b) R.sub.27 =R.sub.29 =H; R.sub.28 =CH.sub.3 c) R.sub.27 =R.sub.28 =CH.sub.3 ; R.sub.29 =H d) R.sub.27 =R.sub.28 =COCH.sub.3 ; R.sub.29 =H e) R.sub.27 =H; R.sub.28 =R.sub.29 =COCH.sub.3

When R.sub.27 =R.sub.28 =R.sub.29 =H, then the compound is cannabidiol. When R.sub.27 =R.sub.29 =H and R.sub.28 =CH.sub.3, the compound is CBD monomethyl ether. When R.sub.27 =R.sub.28 =CH.sub.3 and R.sub.29 =H, the compound is CBD dimethyl ether. When R.sub.27 =R.sub.28 =COCH.sub.3 and R.sub.29 =H, the compound is CBD diacetate. When R.sub.27 =H and R.sub.28 =R.sub.29 =COCH.sub.3, the compound is CBD monoacetate. The ischemic or neurodegenerative disease may be, for example, an ischemic infarct, Alzheimer's disease, Parkinson's disease, Down's syndrome, human immunodeficiency virus (HIV) dementia, myocardial infarction, or treatment and prevention of intraoperative or perioperative hypoxic insults that can leave persistent neurological deficits following open heart surgery requiring heart/lung bypass machines, such as coronary artery bypass grafts (CABG).

The invention also includes an assay for selecting a cannabinoid to use in treating a neurological disease by determining whether the cannabinoid is an antioxidant. Once it has been determined that the cannabinoid is an antioxidant, an antioxidant effective amount of the cannabinoid is administered to treat the neurological disease, such as a vascular ischemic event in the central nervous system, for example the type caused by a neurovascular thromboembolism. Similarly, the method of the present invention includes determining whether a disease is caused by oxidative stress, and if the disease is caused by oxidative stress, administering the cannabinoid in a therapeutically effective antioxidant amount.

The invention also includes identifying and administering antioxidant and neuroprotective compounds (such as cannabidiol) which selectively inhibit the enzyme activity of both 5- and 15-lipoxygenase more than the enzyme activity of 12-lipoxygenase. In addition, such compounds possess low NMDA antagonist activity and low cannabinoid receptor activity. Assays for selecting compounds with the desired effect on lipoxygenase enzymes, and methods for using identified compounds to treat neurological or ischemic diseases are also provided. Such diseases may include a vascular ischemic event in the central nervous system, for example a thromboembolism in the brain, or a vascular ischemic event in the myocardium. Useful administration of the compounds involves administration both during and after an ischemic injury.

These and other objects of the invention will be understood more clearly by reference to the following detailed description and drawings.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A is a graph showing NMDA induced cellular damage in a neuron (as measured by LDH release) in cells that were exposed to glutamate for 10 minutes, which demonstrates that increasing concentrations of cannabidiol in the cell culture protects against cellular damage.

FIG. 1B is a graph similar to FIG. 1A, but showing that AMPA/kainate receptor mediated damage (induced by glutamate and the AMPA/kainate receptor potentiating agents cyclothiazide or concanavalin A) is also reduced in a concentration dependent manner by the presence of cannabidiol in the culture medium.

FIG. 2A is a bar graph showing cellular damage (as measured by LDH release) in the presence of glutamate

alone (100  $\mu$ M Glu), and in the presence of glutamate and 5  $\mu$ M cannabidiol (CBD) or 5  $\mu$ M THC, and demonstrates that CBD and THC were similarly protective.

FIG. 2B is a bar graph similar to FIG. 2A, but showing the cellular damage assessed in the presence of the cannabinoid receptor antagonist SR 141716A (SR), which was not found to alter the neuroprotective effect of CBD (5  $\mu$ M) or THC (5  $\mu$ M), indicating the effect is not a typical cannabinoid effect mediated by the cannabinoid receptor.

FIG. 3 is a graph showing the reduction oxidation potentials determined by cyclic voltametry for some natural and synthetic cannabinoids, the antioxidant BHT, and the non-cannabinoid anandamide (arachidonyl ethanolamide) which is a ligand for the cannabinoid receptor. The voltage at which initial peaks occur is an indication of antioxidant activity.

FIG. 4 is a graph that demonstrates the antioxidant properties of BHT, CBD and THC, by plotting the fluorescence of a fluorescent dye against concentrations of these substances, where declining fluorescence is an indication of greater antioxidant activity.

FIG. 5A is a graph illustrating decreased t-butyl peroxide induced toxicity (as measured by LDH release) in the presence of increasing concentrations of cannabidiol, demonstrating that cannabidiol is an effective antioxidant in living cells.

FIG. 5B is a bar graph comparing the antioxidant activity of several antioxidants against glutamate induced toxicity in neurons, showing that CBD has superior antioxidant activity.

FIG. 6A is a graph showing the effect of CBD (as measured by the change in absorbance at 234 nm) on the enzymatic activity of two lipoxygenase enzymes, rabbit 15-LO and porcine 12-LO, which demonstrates that CBD inhibits 15-LO, but not 12-LO enzyme.

FIG. 6B is a graph demonstrating that inhibitory effect of CBD on 15-LO is competitive.

FIG. 7A is a graph similar to FIG. 6A, but was performed in whole cells rather than purified enzyme preparations, and shows the effect of CBD (as measured by the change in absorbance at 236 nm) on the enzymatic activity of 5-LO from cultured rat basophilic leukemia cells (RBL-2H3), which demonstrates that CBD inhibits 5-LO.

FIG. 7B is a graph showing the effect of CBD (as measured by the change in absorbance at 236 nm) on the formation of 12-HETE (the product of 12-LO) by human leukocytes (12-LO type 1).

FIG. 7C is a graph similar to FIG. 7B, showing the effect of CBD (as measured by the change in absorbance at 236 nm) on the formation of 12-HETE by human platelets (12-LO type 2).

FIG. 8 is a bar graph demonstrating that 12-HETE can protect cortical neurons from NMDA toxicity most effectively when administered during and post ischemia.

## DETAILED DESCRIPTION OF SOME SPECIFIC EMBODIMENTS

This invention provides antioxidant compounds and compositions, such as pharmaceutical compositions, that include cannabinoids that act as free radical scavengers for use in prophylaxis and treatment of disease. The invention also includes methods for using the antioxidants in prevention and treatment of pathological conditions such as ischemia (tissue hypoxia), and in subjects who have been exposed to oxidant inducing

agents such as cancer chemotherapy, toxins, radiation, or other sources of oxidative stress. The compositions and methods described herein are also used for preventing oxidative damage in transplanted organs, for inhibiting reoxygenation injury following reperfusion of ischemic tissues (for example in heart disease), and for any other condition that is mediated by oxidative or free radical mechanisms of injury. In particular embodiments of the invention, the compounds and compositions are used in the treatment of ischemic cardiovascular and neurovascular conditions, and neurodegenerative diseases. However the present invention can also be used as an antioxidant treatment in non-neurological diseases.

Molecular oxygen is essential for aerobic organisms, where it participates in many biochemical reactions, including its role as the terminal electron acceptor in oxidative phosphorylation. However excessive concentrations of various forms of reactive oxygen species and other free radicals can have serious adverse biological consequences, including the peroxidation of membrane lipids, hydroxylation of nucleic acid bases, and the oxidation of sulfhydryl groups and other protein moieties. Biological antioxidants include tocopherols and tocotrienols, carotenoids, quinones, bilirubin, ascorbic acid, uric acid, and metal binding proteins. However these endogenous antioxidant systems are often overwhelmed by pathological processes that allow permanent oxidative damage to occur to tissue.

Free radicals are atoms, ions or molecules that contain an unpaired electron, are usually unstable, and exhibit short half-lives. Reactive oxygen species (ROS) is a collective term, designating the oxygen radicals (e.g.  $\cdot\text{O}_2^-$  - superoxide radical), which by sequential univalent reduction produces hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\cdot\text{OH}$ ). The hydroxyl radical sets off chain reactions and can interact with nucleic acids. Other ROS include nitric oxide ( $\text{NO}\cdot$ ) and peroxy nitrite ( $\text{NOO}\cdot$ ), and other peroxy ( $\text{RO}_2\cdot$ ) and alkoxy ( $\text{RO}\cdot$ ) radicals. Increased production of these poisonous metabolites in certain pathological conditions is believed to cause cellular damage through the action of the highly reactive molecules on proteins, lipids and DNA. In particular, ROS are believed to accumulate when tissues are subjected to ischemia, particularly when followed by reperfusion.

The pharmaceutical compositions of the present invention have potent antioxidant and/or free radical scavenging properties, that prevent or reduce oxidative damage in biological systems, such as occurs in ischemic/reperfusion injury, or in chronic neurodegenerative diseases such as Alzheimer's disease, HIV dementia, and many other oxidation associated diseases.

## DEFINITIONS

"Oxidative associated diseases" refers to pathological conditions that result at least in part from the production of or exposure to free radicals, particularly oxyradicals, or reactive oxygen species. It is evident to those of skill in the art that most pathological conditions are multifactorial, and that assigning or identifying the predominant causal factors for any particular condition is frequently difficult. For these reasons, the term "free radical associated disease" encompasses pathological states that are recognized as conditions in which free radicals or ROS contribute to the pathology of the disease, or wherein administration of a free radical inhibitor (e.g. desferrioxamine), scavenger (e.g. tocopherol, glutathione) or catalyst (e.g. superoxide dismutase, catalase) is shown to produce detectable benefit by decreasing symptoms, increasing survival, or providing other detectable clinical benefits in treating or preventing the pathological state.

Oxidative associated diseases include, without limitation, free radical associated diseases, such as ischemia, ischemic reperfusion injury, inflammatory diseases, systemic lupus erythematosus, myocardial ischemia or infarction, cerebrovascular accidents (such as a thromboembolic or hemorrhagic stroke) that can lead to ischemia or an infarct in the brain, operative ischemia, traumatic hemorrhage (for example a hypovolemic stroke that can lead to CNS hypoxia or anoxia), spinal cord trauma, Down's syndrome, Crohn's disease, autoimmune diseases (e.g. rheumatoid arthritis or diabetes), cataract formation, uveitis, emphysema, gastric

ulcers, oxygen toxicity, neoplasia, undesired cellular apoptosis, radiation sickness, and others. The present invention is believed to be particularly beneficial in the treatment of oxidative associated diseases of the CNS, because of the ability of the cannabinoids to cross the blood brain barrier and exert their antioxidant effects in the brain. In particular embodiments, the pharmaceutical composition of the present invention is used for preventing, arresting, or treating neurological damage in Parkinson's disease, Alzheimer's disease and HIV dementia; autoimmune neurodegeneration of the type that can occur in encephalitis, and hypoxic or anoxic neuronal damage that can result from apnea, respiratory arrest or cardiac arrest, and anoxia caused by drowning, brain surgery or trauma (such as concussion or spinal cord shock).

As used herein, an "antioxidant" is a substance that, when present in a mixture containing an oxidizable substrate biological molecule, significantly delays or prevents oxidation of the substrate biological molecule. Antioxidants can act by scavenging biologically important reactive free radicals or other reactive oxygen species ( $\cdot\text{O}_2$ ,  $\text{H}_2\text{O}_2$ ,  $\cdot\text{OH}$ ,  $\text{HOCl}$ , ferryl, peroxy, peroxyxynitrite, and alkoxy), or by preventing their formation, or by catalytically converting the free radical or other reactive oxygen species to a less reactive species. Relative antioxidant activity can be measured by cyclic voltametry studies of the type disclosed in Example 5 (and FIG. 3), where the voltage (x-axis) is an index of relative antioxidant activity. The voltage at which the first peak occurs is an indication of the voltage at which an electron is donated, which in turn is an index of antioxidant activity.

"Therapeutically effective antioxidant doses" can be determined by various methods, including generating an empirical dose-response curve, predicting potency and efficacy of a congener by using quantitative structure activity relationships (QSAR) methods or molecular modeling, and other methods used in the pharmaceutical sciences. Since oxidative damage is generally cumulative, there is no minimum threshold level (or dose) with respect to efficacy. However, minimum doses for producing a detectable therapeutic or prophylactic effect for particular disease states can be established.

As used herein, a "cannabinoid" is a chemical compound (such as cannabiol, THC or cannabidiol) that is found in the plant species *Cannabis saliva* (marijuana), and metabolites and synthetic analogues thereof that may or may not have psychoactive properties. Cannabinoids therefore include (without limitation) compounds (such as THC) that have high affinity for the cannabinoid receptor (for example  $K_{d} < 250$  nM), and compounds that do not have significant affinity for the cannabinoid receptor (such as cannabidiol, CBD). Cannabinoids also include compounds that have a characteristic dibenzopyran ring structure (of the type seen in THC) and cannabinoids which do not possess a pyran ring (such as cannabidiol). Hence a partial list of cannabinoids includes THC, CBD, dimethyl heptylpentyl cannabidiol (DMHP-CBD), 6,12-dihydro-6-hydroxy-cannabidiol (described in U.S. Pat. No. 5,227,537, incorporated by reference); (3S,4R)-7-hydroxy- $\Delta^6$ -tetrahydrocannabinol homologs and derivatives described in U.S. Pat. No. 4,876,276, incorporated by reference; (+)-4-[4-DMH-2,6-diacetoxy-phenyl]-2-carboxy-6,6-dimethylbicyclo[3.1.1]hept-2-en, and other 4-phenylpinene derivatives disclosed in U.S. Pat. No. 5,434,295, which is incorporated by reference; and cannabidiol (-)(CBD) analogs such as (-)CBD-monomethylether, (-)CBD dimethyl ether; (-)CBD diacetate; (-)3'-acetyl-CBD monoacetate; and  $\pm$ -AF11, all of which are disclosed in Consroe et al., *J. Clin. Pharmacol.* 21:428S-436S, 1981, which is also incorporated by reference. Many other cannabinoids are similarly disclosed in Agurell et al., *Pharmacol. Rev.* 38:31-43, 1986, which is also incorporated by reference.

As referred to herein, the term "psychoactivity" means "cannabinoid receptor mediated psychoactivity." Such effects include, euphoria, lightheadedness, reduced motor coordination, and memory impairment. Psychoactivity is not meant to include non-cannabinoid receptor mediated effects such as the anxiolytic effect of CBD.

The "lipoxygenase enzyme activity" refers to the relative level of lipoxygenase enzyme activity for a

particular lipoxgenase, such as 5-, 15- or 12-lipoxygenase, as measured in Example 8. A compound would be said to "selectively inhibit a lipoxgenase enzyme" if the concentration of inhibitor required to reduce enzyme activity by 50% was at least about 5 times less than the amount required to reduce activity of a second lipoxgenase enzyme by the same degree (under the same conditions, i.e. temperature, substrate concentration, etc.)

An "antagonist" is a compound that binds and occupies a receptor without activating it. In the presence of a sufficient concentration of antagonist, an agonist cannot activate its receptor. Therefore, antagonists may decrease the neurotoxicity mediated by NMDA (as described in Example 3) or AMPA and Kainate (as described in Example 4).

An "agonist" is a compound that activates a receptor. When the receptor is activated for a longer than normal period of time, this may cause neurotoxicity, as in the case of NMDA, AMPA and kainate receptors (see Examples 3 and 4).

The term "alkyl" refers to a cyclic, branched, or straight chain alkyl group containing only carbon and hydrogen, and unless otherwise mentioned contains one to twelve carbon atoms. This term is further exemplified by groups such as methyl, ethyl, n-propyl, isobutyl, t-butyl, pentyl, pivalyl, heptyl, adamantyl, and cyclopentyl. Alkyl groups can either be unsubstituted or substituted with one or more substituents, e.g. halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "lower alkyl" refers to a cyclic, branched or straight chain monovalent alkyl radical of one to seven carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), cyclopropylmethyl, i-amyl, n-amyl, hexyl and heptyl. Lower alkyl groups can also be unsubstituted or substituted, where a specific example of a substituted alkyl is 1,1-dimethyl heptyl.

"Hydroxyl" refers to --OH.

"Alcohol" refers to R--OH, wherein R is alkyl, especially lower alkyl (for example in methyl, ethyl or propyl alcohol). An alcohol may be either linear or branched, such as isopropyl alcohol.

"Carboxyl" refers to the radical --COOH, and substituted carboxyl refers to --COR where R is alkyl, lower alkyl or a carboxylic acid or ester.

The term "aryl" or "Ar" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g. phenyl) or multiple condensed rings (e.g. naphthyl or anthryl), which can optionally be unsubstituted or substituted with, e.g., halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "alkoxy" refers to a substituted or unsubstituted alkoxy, where an alkoxy has the structure --O--R, where R is substituted or unsubstituted alkyl. In an unsubstituted alkoxy, the R is an unsubstituted alkyl. The term "substituted alkoxy" refers to a group having the structure --O--R, where R is alkyl which is substituted with a non-interfering substituent. The term "arylalkoxy" refers to a group having the structure --O--R--Ar, where R is alkyl and Ar is an aromatic substituent. Arylalkoxys are a subset of substituted alkoxy groups. Examples of useful substituted alkoxy groups are: benzyloxy, naphthyloxy, and chlorobenzyloxy.

The term "aryloxy" refers to a group having the structure --O--Ar, where Ar is an aromatic group. A particular aryloxy group is phenoxy.

The term "heterocycle" refers to a monovalent saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g. morpholino, pyridyl or furyl) or multiple condensed rings (e.g. indoliziny or benzo[b]thienyl) and having at least one heteroatom, defined as N, O, P, or S, within the ring, which can optionally be unsubstituted or substituted with, e.g. halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

"Arylalkyl" refers to the groups --R--Ar and --R--HetAr, where Ar is an aryl group. HetAr is a heteroaryl group, and R is a straight-chain or branched chain aliphatic group. Example of arylalkyl groups include benzyl and furfuryl. Arylalkyl groups can optionally be unsubstituted or substituted with, e.g., halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "halo" or "halide" refers to fluoro, bromo, chloro and iodo substituents.

The term "amino" refers to a chemical functionality --NR'R" where R' and R" are independently hydrogen, alkyl, or aryl. The term "quaternary amine" refers to the positively charged group --N<sup>+</sup>.R'R", where R'R" and R" are independently selected and are alkyl or aryl. A particular amino group is --NH<sub>2</sub>.

A "pharmaceutical agent" or "drug" refers to a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

All chemical compounds include both the (+) and (-) stereoisomers, as well as either the (+) or (-) stereoisomer.

Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (1985) and The Condensed Chemical Dictionary (1981).

The following examples show that both nonpsychoactive cannabidiol, and psychoactive cannabinoids such as THC, can protect neurons from glutamate induced death, by a mechanism independent of cannabinoid receptors. Cannabinoids are also shown to be potent antioxidants capable of preventing ROS toxicity in neurons.

## EXAMPLE 1

### Preparation of Cannabinoids and Neuronal Cultures

Cannabidiol, THC and reactants other than those specifically listed below were purchased from Sigma Chemical, Co. (St. Louis, Mo.). Cyclothiazide, glutamatergic ligands and MK-801 were obtained from Tocris Cookson (UK). Dihydrorhodamine was supplied by Molecular Probes (Eugene, Oreg.). T-butyl hydroperoxide, tetraethylammonium chloride, ferric citrate and sodium dithionite were all purchased from Aldrich (WI). All culture media were Gibco/BRL (MD) products.

Solutions of cannabinoids, cyclothiazide and other lipophiles were prepared by evaporating a 10 mM ethanolic solution (under a stream of nitrogen) in a siliconized microcentrifuge tube. Dimethyl sulfoxide (DMSO, less than 0.05% of final volume) was added to ethanol to prevent the lipophile completely drying

onto the tube wall. After evaporation, 1 ml of culture media was added and the drug was dispersed using a high power sonic probe. Special attention was used to ensure the solution did not overheat or generate foam. Following dispersal, all solutions were made up to their final volume in siliconized glass tubes by mixing with an appropriate quantity of culture media.

Primary neuronal cultures were prepared according to the method of Ventra et al. (J. Neurochem. 66:1752-1761, 1996). Fetuses were extracted by Cesarean section from a 17 day pregnant Wistar rat, and the feral brains were placed into phosphate buffered saline. The cortices were then dissected out, cut into small pieces and incubated with papain for nine minutes at 37.degree. C. After this time the tissue was dissociated by passage through a fire polished Pasteur pipette, and the resultant cell suspension separated by centrifugation over a gradient consisting of 10 mg/ml bovine serum albumin and 10 mg/ml ovomucoid (a trypsin inhibitor) in Earls buffered salt solution. The pellet was then re-suspended in high glucose, phenol red free Dulbecco's modified Eagles medium containing 10% fetal bovine serum, 2 mM glutamine, 100 IU penicillin, and 100 .mu.g/ml streptomycin (DMEM). Cells were counted, tested for vitality using the trypan blue exclusion test and seeded onto poly-D-lysine coated 24 multiwell plates. After 96 hours, 10 .mu.M fluoro-deoxyuridine and 10 .mu.M uridine were added to block glial cell growth. This protocol resulted in a highly neuron-enriched culture.

## EXAMPLE 2

### Preparation of Astrocytes and Conditioned Media

Astrocyte conditioned DMEM was used throughout the AMPA/kainate toxicity procedure and following glutamate exposure in the NMDAR mediated toxicity protocol. Media was conditioned by 24 hour treatment over a confluent layer of type I astrocytes, prepared from two day old Wistar rat pups. Cortices were dissected, cut into small pieces, and enzymatically digested with 0.25% trypsin. Tissue was then dissociated by passage through a fire polished Pasteur pipette and the cell suspension plated into untreated 75 cm.sup.2 T-flasks. After 24 hours the media was replaced and unattached cells removed. Once astrocytes achieved confluence, cells were divided into four flasks. Media for experiments was conditioned by a 24 hour exposure to these astrocytes, after which time it was frozen at -20.degree. C. until use. Astrocyte cultures were used to condition DMEM for no longer than two months.

## EXAMPLE 3

### NMDA Mediated Toxicity Studies

Glutamate neurotoxicity can be mediated by NMDA, AMPA or kainate receptors. To examine NMDAR mediated toxicity, cultured neurons (cultured for 14-18 days) were exposed to 250 .mu.M glutamate for 10 minutes in a magnesium free saline solution. The saline was composed of 125 mM NaCl, 25 mM glucose, 10 mM HEPES (pH 7.4), 5 mM KCl, 1.8 mM calcium chloride and 5% bovine serum albumin. Following exposure, cells were washed twice with saline, and incubated for 18 hours in conditioned DMEM. The level of lactate dehydrogenase (LDH) in the media was used as an index of cell injury.

Toxicity was completely prevented by addition of the NMDAR antagonist, MK-801 (500 nM, data not shown). However, FIG. 1A shows that cannabidiol also prevented neurotoxicity (maximum protection 88.+-.9%) with an EC.sub.50 of 2-4 .mu.M (specifically about 3.5 .mu.M).

## EXAMPLE 4

### AMPA and Kainate Receptor Mediated Toxicity Studies

Unlike NMDA receptors, which are regulated by magnesium ions, AMPA/kainate receptors rapidly desensitize following ligand binding. To examine AMPA and kainate receptor mediated toxicity, neurons were cultured for 7-13 days, then exposed to 100  $\mu\text{M}$  glutamate and 50  $\mu\text{M}$  cyclothiazide (used to prevent AMPA receptor desensitization). Cells were incubated with glutamate in the presence of 500 nM MK-801 (an NMDAR antagonist) for 18-20 hours prior to analysis. Specific AMPA and kainate receptor ligands were also used to separately examine the effects of cannabinoids on AMPA and kainate receptor mediated events. Fluorowillardiine (1.5  $\mu\text{M}$ ) was the AMPA agonist and 4-methyl glutamate (10  $\mu\text{M}$ ) was the kainate agonist used to investigate receptor mediated toxicity. When specifically examining kainate receptor activity, cyclothiazide was replaced with 0.15 mg/ml Concanavalin-A.

Cannabidiol protection against AMPA/kainate mediated neurotoxicity is illustrated in FIG. 1B, where LDH in the media was used as an index of cell injury. The neuroprotective effect of cannabidiol was similar to that observed in the NMDA mediated toxicity model (FIG. 1A). Cannabidiol prevented neurotoxicity (maximum protection 80. $\pm$ .17%) with an EC<sub>50</sub> of 2-4  $\mu\text{M}$  (specifically about 3.3  $\mu\text{M}$ ). Comparable results were obtained with either the AMPA receptor ligand, fluorowillardiine or the kainate receptor specific ligand, 4-methyl-glutamate (data not shown). Hence cannabidiol protects similarly against toxicity mediated by NMDA, AMPA or kainate receptors.

Unlike cannabidiol, THC is a ligand (and agonist) for the brain cannabinoid receptor. The action of THC at the cannabinoid receptor has been proposed to explain the ability of THC to protect neurons from NMDAR toxicity in vitro. However in AMPA/kainate receptor toxicity assays, THC and cannabidiol were similarly protective (FIG. 2A), indicating that cannabinoid neuroprotection is independent of cannabinoid receptor activation. This was confirmed by inclusion of cannabinoid receptor antagonist SR-141716A in the culture media (SR in FIG. 2B). See Mansbach et al., *Psychopharmacology* 124:315-22, 1996, for a description of SR-141716A. Neither THC nor cannabidiol neuroprotection was affected by cannabinoid receptor antagonist (FIG. 2B).

## EXAMPLE 5

### Cyclic Voltametry Studies or ReDox Potentials

To investigate whether cannabinoids protect neurons against glutamate damage by reacting with ROS, the antioxidant properties of cannabidiol and other cannabinoids were assessed. Cyclic voltametry, a procedure that measures the ability of a compound to accept or donate electrons under a variable voltage potential, was used to measure the oxidation potentials of several natural and synthetic cannabinoids. These studies were performed with an EG&G Princeton Applied Research potentiostat/galvanostat (Model 273/PAR 270 software, NJ). The working electrode was a glassy carbon disk with a platinum counter electrode and silver/silver chloride reference. Tetraethylammonium chloride in acetonitrile (0.1 M) was used as an electrolyte. Cyclic voltametry scans were done from +0 to 1.8 V at scan rate of 100 mV per second. The reducing ability of cannabidiol (CBD), THC, HU-211, and BHT were measured in this fashion. Anandamide, a cannabinoid receptor ligand without a cannabinoid like structure, was used as a non-responsive control. Each experiment was repeated twice with essentially the same results.

Cannabidiol, THC and the synthetic cannabinoid HU-211 all donated electrons at a similar potential as the antioxidant BHT. Anandamide (arachidonyl ethanolamide) did not undergo oxidation at these potentials (FIG. 3). Several other natural and synthetic cannabinoids, including cannabidiol, nabilone, and levantrodol were also tested, and they too exhibited oxidation profiles similar to cannabidiol and THC (data not shown).

## EXAMPLE 6

### Iron Catalyzed Dihydrorhodamine Oxidation (Fenton Reaction)

The ability of cannabinoids to be readily oxidized, as illustrated in Example 5, indicated they possess antioxidant properties comparable to BHT. The antioxidant activity of BHT was examined in a Fenton reaction, in which iron is catalyzed to produce ROS. Cannabidiol (CBD) and tetrahydrocannabinol (THC) were evaluated for their ability to prevent oxidation of dihydrorhodamine to the fluorescent compound rhodamine. Oxidant was generated by ferrous catalysis (dithionite reduced ferric citrate) of t-butyl hydroperoxide in a 50:50 water:acetonitrile (v/v) solution. Dihydrorhodamine (50  $\mu\text{M}$ ) was incubated with 300  $\mu\text{M}$  t-butyl hydroperoxide and 0.5  $\mu\text{M}$  iron for 5 minutes. After this time, oxidation was assessed by spectrofluorimetry (Excit=500 nm, Emiss=570 nm). Various concentrations of cannabinoids and BHT were included to examine their ability to prevent dihydrorhodamine oxidation.

Cannabidiol, THC and BHT all prevented dihydrorhodamine oxidation in a similar, concentration dependent manner (FIG. 4), indicating that cannabinoids have antioxidant potency comparable to BHT.

To confirm that cannabinoids act as antioxidants in the intact cell, neurons were also incubated with the oxidant t-butyl hydroperoxide and varying concentrations of cannabidiol (FIG. 5A). The t-butyl hydroperoxide oxidant was chosen for its solubility in both aqueous and organic solvents, which facilitates oxidation in both cytosolic and membrane cell compartments. Cell toxicity was assessed 18-20 hours after insult by measuring lactate dehydrogenase (LDH) release into the culture media. All experiments were conducted with triple or quadruple values at each point and all plates contained positive (glutamate alone) and baseline controls. The assay was validated by comparison with an XTT based metabolic activity assay. As shown in FIG. 5A, cannabidiol protected neurons against ROS toxicity in a dose related manner, with an EC<sub>50</sub> of about 6  $\mu\text{M}$ . The maximum protection observed was 88. $\pm$ .9%.

Cannabidiol was also compared with known antioxidants in an AMPA/kainate toxicity protocol. Neurons were exposed to 100  $\mu\text{M}$  glutamate and equimolar (5  $\mu\text{M}$ ) cannabidiol,  $\alpha$ -tocopherol, BHT or ascorbate (FIG. 5B). Although all of the antioxidants attenuated glutamate toxicity, cannabidiol was significantly more protective than either  $\alpha$ -tocopherol or ascorbate. The similar antioxidant abilities of cannabidiol and BHT in this chemical system (FIG. 4), and their comparable protection in neuronal cultures (FIG. 5B), implies that cannabidiol neuroprotection is due to an antioxidant effect.

## EXAMPLE 7

### In vivo Rat Studies

The middle cerebral artery of chloral hydrate anesthetized rats was occluded by insertion of suture thread into it. The animals were allowed to recover from the anesthetic and move freely for a period of two hours. After this time the suture was removed under mild anesthetic and the animals allowed to recover for 48 hours. Then the animals were tested for neurological deficits, sacrificed, and the infarct volume calculated. To examine the infarct volume, animals were anesthetized, ex-sanguinated, and a metabolically active dye (3-phenyl tetrazolium chloride) was pumped throughout the body. All living tissues were stained pink by the dye, while morbid regions of infarcted tissue remained white. Brains were then fixed for 24 hours in formaldehyde, sliced and the infarct volumes measured.

One hour prior to induction of ischemia 20 mg/kg of cannabidiol was administered by intra-peritoneal injection (ip) in a 90% saline:5% emulphor 620 (emulsifier):5% ethanol vehicle. A second ip 10 mg/kg dose of cannabidiol was administered 8 hours later using the same vehicle. Control animals received injections of

vehicle without drug. IV doses would be expected to be 3-5 times less because of reduction of first pass metabolism.

The infarct size and neurological assessment of the test animals is shown Table 1.

TABLE 1 Cannabidiol protects rat brains from ischemia damage Volume of Infarct Behavioral Deficit (mm<sup>3</sup>)  
Score Animal Drug Control Drug Control 1 108.2 110.5 3 2 2 83.85 119.6 4 4 3 8.41 118.9 3 4 4 75.5 177.7 1  
4 5 60.53 33.89 1 3 6 27.52 255.5 1 5 7 23.16 143 1 4 Mean 55.3 137.0 2.0 3.7 SEM 13.8 25.7 0.5 0.4 p =  
0.016 significant p = 0.015 significant \*Neurological scoring is performed on a subjective 1-5 scale of  
impairment. 0 = no impairment, 5 = severe (paralysis)

This data shows that infarct size was approximately halved in the animals treated with cannabidiol, which was also accompanied by a substantial improvement in the neurological status of the animal.

These studies with the nonpsychotropic marijuana constituent, cannabidiol, demonstrate that protection can be achieved against both glutamate neurotoxicity and free radical induced cell death. THC, the psychoactive principle of cannabis, also blocked glutamate neurotoxicity with a potency similar to cannabidiol. In both cases, neuroprotection is unaffected by the presence of a cannabinoid receptor antagonist. These results therefore surprisingly demonstrate that cannabinoids can have useful therapeutic effects that are not mediated by cannabinoid receptors, and therefore are not necessarily accompanied by psychoactive side effects. Cannabidiol also acts as an anti-epileptic and anxiolytic, which makes it particularly useful in the treatment of neurological diseases in which neuroanatomic defects can predispose to seizures (e.g. subarachnoid hemorrhage).

A particular advantage of the cannabinoid compounds of the present invention is that they are highly lipophilic, and have good penetration into the central nervous system. The volume of distribution of some of these compounds is at least 100 L in a 70 kg person (1.4 L/kg), more particularly at least 250 L, and most particularly 500 L or even 700 L in a 70 kg person (10 L/kg). The lipophilicity of particular compounds is also about as great as that of THC, cannabidiol or other compounds that have excellent penetration into the brain and other portions of the CNS.

Cannabinoids that lack psychoactivity or psychotoxicity are particularly useful embodiments of the present invention, because the absence of such side effects allows very high doses of the drug to be used without encountering unpleasant side effects (such as dysphoria) or dangerous complications (such as obtundation in a patient who may already have an altered mental status). For example, therapeutic antioxidant blood levels of cannabidiol can be 5-20 mg/kg, without significant toxicity, while blood levels of psychoactive cannabinoids at this level would produce obtundation, headache, conjunctival irritation, and other problems. Particular examples of the compounds of the present invention have low affinity to the cannabinoid receptor, for example a  $K_{sub.i}$  of greater than 250 nM, for example  $K_{sub.i}$  of 500-1000 nM. A compound with a  $K_{sub.i}$  of 1000 nM is particularly useful, which compound has essentially no psychoactivity mediated by the cannabinoid receptor.

Cannabidiol blocks glutamate toxicity with equal potency regardless of whether the insult is mediated by NMDA, AMPA or kainate receptors. Cannabidiol and THC have been shown to be comparable to the antioxidant BHT, both in their ability to prevent dihydrorhodamine oxidation and in their cyclic voltametric profiles. Several synthetic cannabinoids also exhibited profiles similar to the BHT, although anandamide, which is not structurally related to cannabinoids, did not. These findings indicate that cannabinoids act as antioxidants in a non-biological situation, which was confirmed in living cells by showing that cannabidiol attenuates hydroperoxide induced neurotoxicity. The potency of cannabidiol as an antioxidant was examined by comparing it on an equimolar basis with three other commonly used compounds.

In the AMPA/kainate receptor dependent neurotoxicity model, cannabidiol neuroprotection was comparable to the potent antioxidant, BHT, but significantly greater than that observed with either  $\alpha$ -tocopherol or ascorbate. This unexpected superior antioxidant activity (in the absence of BHT tumor promoting activity) shows for the first time that cannabidiol, and other cannabinoids, can be used as antioxidant drugs in the treatment (including prophylaxis) of oxidation associated diseases, and is particularly useful as a neuroprotectant. The therapeutic potential of nonpsychoactive cannabinoids is particularly promising, because of the absence of psychotoxicity, and the ability to administer higher doses than with psychotropic cannabinoids, such as THC. Previous studies have also indicated that cannabidiol is not toxic, even when chronically administered to humans or given in large acute doses (700 mg/day).

## EXAMPLE 8

### Effect of Cannabidiol on Lipoxygenase Enzymes

This example describes in vitro and in vivo assays to examine the effect of cannabidiol (CBD) on three lipoxygenase (LO) enzymes: 5-LO, 12-LO and 15-LO.

#### In vitro Enzyme Assay

The ability of CBD to inhibit lipoxygenase was examined by measuring the time dependent change in absorption at 234 nm following addition of 5 U of each lipoxygenase (rabbit 15-LO purchased from Biomol (PA), porcine 12-LO purchased from Cayman chemicals (MI)) to a solution containing 10  $\mu$ M (final concentration) linoleic acid.

Enzyme studies were performed using a u.v. spectrophotometer and a 3 ml quartz cuvette containing 2.5 ml of a stirred solution of 12.5  $\mu$ M sodium linoleic acid (sodium salt) in solution A (25 mM Tris (pH 8.1), 1 mM EDTA 0.1% methyl cellulose). The reaction was initiated by addition of 0.5 ml enzyme solution (10 U/ml enzyme in solution A) and recorded for 60 seconds. Lipoxygenase exhibits non-Michaelis-Menten kinetics, an initial "lag" (priming) phase followed by a linear phase which is terminated by product inhibition. These complications were reduced by assessing enzyme activity (change in absorption) over the "steepest" 20 second period in a 60 second run time. Recordings examined the absorption at 234 nm minus the value at a reference wavelength of 280 nm. Linoleic acid was used as the substrate rather than arachidonic acid, because the products are less inhibitory to the enzyme, thereby providing a longer "linear phase".

#### Cell Purification and Separation

Human platelets and leukocytes were purified from buffy coat preparations (NIH Blood Bank) using a standard Ficoll based centrifugation method used in blood banks. Prior to use, cells were washed three times to eliminate contaminating cell types. Cultured rat basophilic leukemia cells (RBL-2H3) were used as a source of 5-lipoxygenase.

#### In vivo Determination of Lipoxygenase Activity

Cells were incubated with arachidonic acid and stimulated with the calcium ionophore A23187. Lipids were extracted and separated by reverse phase HPLC. Product formation was assessed as the area of a peak that co-eluted with an authentic standard, had a greater absorbance at 236 nm than at either 210 or 280 nm, and the formation of which was inhibited by a lipoxygenase inhibitor.

Cell pellets were triturated in DMEM culture media, aliquoted and pre-incubated for 15 minutes with 20

. $\mu$ M arachidonic acid and varying concentrations of cannabidiol and/or 40 . $\mu$ M nordihydroguaiaretic acid (a lipoxygenase inhibitor). Platelets and leukocytes were also pre-incubated with 80 . $\mu$ M manolide (Biomol) to prevent phospholipase A2 activation. Product formation was initiated by addition of 5 . $\mu$ M A23187 and incubation for 10 minutes at 37.degree. C. At the end of the incubation, the reaction was stopped by addition of 15% 1M HCl and 10 ng/ml prostaglandin B2 (internal standard). Lipids were extracted with 1 volume of ethyl ether, which was dried under a stream of nitrogen. Samples were reconstituted in 50% acetonitrile:50% H.sub.2 O and separated by reverse phase HPLC using a gradient running from 63% acetonitrile: 37% H.sub.2 O:0.2% acetic acid to 90% acetonitrile (0.2% acetic acid) over 13 minutes.

### Measurement of NMDAr Toxicity

The ability of 12-HETE (12-(s)-hydroxy-eicosatetraenoic acid, the product of the action of 12-lipoxygenase on arachidonic (eicosatetraenoic) acid) to protect cortical neurons from NMDAr toxicity was measured as described in Example 3. The 12-HETE (0.5 . $\mu$ g/ml) was added either during ischemia (co-incubated with the glutamate), during post-ischemia (co-incubated with the DMEM after washing the cells), or during both ischemia and post-ischemia.

### Results

Using semi-purified enzyme preparations, the effect of CBD on rabbit 15-LO and porcine 12-LO was compared. As shown in FIGS. 6A and B, CBD is a potent competitive inhibitor of 15-LO with an EC.sub.50 of 598 nM. However, CBD had no effect on the 12-LO enzyme.

Using whole cell preparations, the effect of CBD on 5- and 12-LO enzymes was investigated. As shown in FIG. 7A, CBD inhibited 5-LO in cultured rat basophilic leukemia cells (RBL-2H3) with an EC.sub.50 of 1.92 . $\mu$ M. However, CBD had no effect on 12-LO, as monitored by the production of 12-HETE (the product of 12-LO), in either human leukocytes or platelets (FIGS. 7B and C). The leukocyte 12-LO is similar, while the platelet 12-LO is structurally and functionally different, from the porcine 12-LO used in the in vitro enzyme study.

The ability of 12-HETE to protect cortical neurons from NMDAr toxicity is shown in FIG. 8. To achieve best protection from NMDAr toxicity, 12-HETE was administered both during and post ischemia.

Therefore, CBD serves as a selective inhibitor of at least two lipoxygenase enzymes, 5-LO and 15-LO, but had no effect on 12-LO. Importantly, this is the first demonstration (FIG. 8) that the 12-LO product 12-HETE can play a significant role in protecting neurons from NMDAr mediated toxicity. Although the mechanism of this protection is unknown at the present time, 12-HETE is known to be an important neuromodulator, due to its ability to influence potassium channel activity.

### EXAMPLE 9

#### Methods of Treatment

The present invention includes a treatment that inhibits oxidation associated diseases in a subject such as an animal, for example a rat or human. The method includes administering the antioxidant drugs of the present invention, or a combination of the antioxidant drug and one or more other pharmaceutical agents, to the subject in a pharmaceutically compatible carrier and in an effective amount to inhibit the development or progression of oxidation associated diseases. Although the treatment can be used prophylactically in any patient in a demographic group at significant risk for such diseases, subjects can also be selected using more specific criteria, such as a definitive diagnosis of the condition. The administration of any exogenous

antioxidant cannabinoid would inhibit the progression of the oxidation associated disease as compared to a subject to whom the cannabinoid was not administered. The antioxidant effect, however, increases with the dose of the cannabinoid.

The vehicle in which the drug is delivered can include pharmaceutically acceptable compositions of the drugs of the present invention using methods well known to those with skill in the art. Any of the common carriers, such as sterile saline or glucose solution, can be utilized with the drugs provided by the invention. Routes of administration include but are not limited to oral, intracranial ventricular (icv), intrathecal (it), intravenous (iv), parenteral, rectal, topical ophthalmic, subconjunctival, nasal, aural, sub-lingual (under the tongue) and transdermal. The antioxidant drugs of the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Such medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, lipid carriers such as cyclodextrins, proteins such as serum albumin, hydrophilic agents such as methyl cellulose, detergents, buffers, preservatives and the like. Given the low solubility of many cannabinoids, they may be suspended in sesame oil.

Given the excellent absorption of the compounds of the present invention via an inhaled route, the compounds may also be administered as inhalants, for example in pharmaceutical aerosols utilizing solutions, suspensions, emulsions, powders and semisolid preparations of the type more fully described in Remington: The Science and Practice of Pharmacy (19<sup>sup</sup>.th Edition, 1995) in chapter 95. A particular inhalant form is a metered dose inhalant containing the active ingredient, in a suspension or a dispersing agent (such as sorbitan trioleate, oleyl alcohol, oleic acid, or lecithin, and a propellant such as 12/11 or 12/114).

Embodiments of the invention comprising pharmaceutical compositions can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions as would be known to those of skill in the art. The compositions are preferably in the form of a unit dose in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, injectable and infusible solutions, for example a unit dose vial, or a metered dose inhaler. Effective oral human dosage ranges for cannabidiol are contemplated to vary from about 1-40 mg/kg, for example 5-20 mg/kg, and in particular a dose of about 20 mg/kg of body weight.

If the antioxidant drugs are to be used in the prevention of cataracts, they may be administered in the form of eye drops formulated in a pharmaceutically inert, biologically acceptable carrier, such as isotonic saline or an ointment. Conventional preservatives, such as benzalkonium chloride, can also be added to the formulation. In ophthalmic ointments, the active ingredient is admixed with a suitable base, such as white petrolatum and mineral oil, along with antimicrobial preservatives. Specific methods of compounding these dosage forms, as well as appropriate pharmaceutical carriers, are known in the art. Remington: The Science and Practice of Pharmacy, 19<sup>th</sup> Ed., Mack Publishing Co. (1995), particularly Part 7.

The compounds of the present invention are ideally administered as soon as a diagnosis is made of an ischemic event, or other oxidative insult. For example, once a myocardial infarction has been confirmed by electrocardiograph, or an elevation in enzymes characteristic of cardiac injury (e.g. CKMB), a therapeutically effective amount of the cannabinoid drug is administered. A dose can also be given following symptoms characteristic of a stroke (motor or sensory abnormalities), or radiographic confirmation of a cerebral infarct in a distribution characteristic of a neurovascular thromboembolic event. The dose can be given by frequent bolus administration, or as a continuous IV dose. In the case of cannabidiol, for example, the drug could be given in a dose of 5 mg/kg active ingredient as a continuous intravenous infusion; or hourly intramuscular injections of that dose.

## EXAMPLE 10

The following table lists examples of some dibenzopyran cannabinoids that may be useful as antioxidants in the method of the present invention.

##STR13## ##STR14## Compound R.sub.19 R.sub.20 R.sub.21 R.sub.22 R.sub.23 R.sub.24 R.sub.25 R.sub.26 H 5 7-OH-.DELTA..sup.1 -THC CH.sub.2 OH H H H H H C.sub.5 H.sub.11 H 6 6.alpha.-OH-.DELTA..sup.1 -THC CH.sub.3 .alpha.-OH H 7 6.beta.-OH-.DELTA..sup.1 -THC CH.sub.3 .beta.-OH 8 1"-OH-.DELTA..sup.1 -THC CH.sub.3 OH H 9 2"-OH-.DELTA..sup.1 -THC CH.sub.3 OH 10 3"-OH-.DELTA..sup.1 -THC CH.sub.3 OH 11 4"-OH-.DELTA..sup.1 -THC CH.sub.3 OH H 12 6.alpha.,7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH .alpha.-OH H 13 6v,7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH .beta.-OH 14 1",7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH OH H 15 2",7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH OH H 16 3",7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH OH H 17 4",7-diOH-.DELTA..sup.1 -THC CH.sub.2 OH OH 18 1",6.beta.-diOH-.DELTA..sup.1 -THC CH.sub.3 .beta.-OH OH 19 1",3"-diOH-.DELTA..sup.1 -THC CH.sub.3 OH OH 20 1",6.alpha.,7-triOH-.DELTA..sup.1 -THC CH.sub.2 OH .alpha.-OH OH H 21 .DELTA..sup.1 -THC-6-one CH.sub.3 .dbd.O 22 Epoxyhexahydrocannabinol CH.sub.3 (EHC)\* 23 7-oxo-.DELTA..sup.1 -THC CHO H 24 .DELTA..sup.1 -THC-7"-oic acid COOH H 25 .DELTA..sup.1 -THC-3"-oic acid CH.sub.3 C.sub.2 H.sub.4 COOH H 26 1"-OH-.DELTA..sup.1 -THC-7"-oic acid COOH OH H 27 2"-OH-.DELTA..sup.1 -THC-7"-oic acid COOH OH H 28 3"-OH-.DELTA..sup.1 -THC-7"-oic acid COOH OH H 29 4"-OH-.DELTA..sup.1 -THC-7"-oic acid COOH OH H 30 3",4",5"-trisor-2"-OH-.DELTA..sup.1 - COOH C.sub.2 H.sub.4 OH THC-7-oic acid H 31 7-OH-.DELTA..sup.1 -THC-2"-oic acid CH.sub.2 OH CH.sub.2 COOH H 32 6.beta.-OH-.DELTA..sup.1 -THC-2"-oic acid CH.sub.3 .beta.-OH CH.sub.2 COOH H 33 7-OH-.DELTA..sup.1 -THC-3"-oic acid CH.sub.2 OH C.sub.2 H.sub.4 COOH H 34 6.beta.-OH-.DELTA..sup.1 -THC-3"-oic acid CH.sub.3 .beta.-OH C.sub.2 H.sub.4 COOH H 35 6.alpha.-OH-.DELTA..sup.1 -THC-4"-oic acid CH.sub.3 .alpha.-OH C.sub.3 H.sub.6 COOH H 36 2",3"-dehydro-6U-OH-.DELTA..sup.1 - CH.sub.3 .alpha.-OH C.sub.3 H.sub.4 COOH THC-4"-oic acid H 37 .DELTA..sup.1 -THC-1",7-dioic acid COOH COOH H 38 .DELTA..sup.1 -THC-2",7-dioic acid COOH CH.sub.2 COOH H 39 .DELTA..sup.1 -THC-3",7-dioic acid COOH C.sub.2 H.sub.4 COOH H 40 .DELTA..sup.1 -THC-4",7-dioic acid COOH C.sub.3 H.sub.6 COOH H 41 1",2"-dehydro-.DELTA..sup.1 -THC-3",7- COOH C.sub.2 H.sub.2 COOH dioic acid H 42 .DELTA..sup.1 -THC-glucuronic acid CH.sub.3 gluc.sup..dagger. H 43 .DELTA..sup.1 -THC-7-oic acid COO gluc.sup..dagger. glucuronide \*Epoxy group in C-1 and C-2 positions .sup..dagger. Glucuronide Note: R-group substituents are H if not indicated otherwise.

Chemical structures of some of the dibenzopyran cannabinoids are shown below. ##STR15## ##STR16## ##STR17##

## EXAMPLE 11

### Examples of Structural Analogs of Cannabidiol

The following table lists examples of some cannabinoids which are structural analogs of cannabidiol and that may be useful as antioxidants in the method of the present invention. A particularly useful example is compound CBD, cannabidiol.

Compound R.sub.19 R.sub.20 R.sub.21 R.sub.22 R.sub.23 R.sub.24 R.sub.25 R.sub.26 ##STR18## ##STR19## 44 CBD CH.sub.3 H H H H H H C.sub.5 H.sub.11 45 7-OH--CBD CH.sub.2 OH 46 6.alpha.-CH.sub.3 .alpha.-OH 47 6.beta.- CH.sub.3 .beta.-OH 48 1"- CH.sub.3 OH 49 2"- CH.sub.3 OH 50 3"- CH.sub.3 OH 51 4"- CH.sub.3 OH 52 5"- CH.sub.3 C.sub.4 H.sub.8 CH.sub.2 OH 53 6,7-diOH--CBD CH.sub.2 OH OH 54 3",7-diOH--CBD CH.sub.2 OH OH 55 4",7-diOH--CBD CH.sub.2 OH OH 56 CBD-7-oic acid COOH 57 CBD-3"-oic acid CH.sub.3 C.sub.2 H.sub.4 COOH ##STR20## ##STR21## 58 CBN CH.sub.3 H H H H H H C.sub.5 H.sub.11 59 7-OH--CBN CH.sub.2 OH 60 1"-OH--CBN CH.sub.3 OH 61

2"-OH--CBN CH.sub.3 OH 62 3"-OH--CBN CH.sub.3 OH 63 4"-OH--CBN CH.sub.3 OH 64 5"-OH--CBN CH.sub.3 C.sub.4 H.sub.8 CH.sub.2 OH 65 2"-7-diOH--CBN CH.sub.2 OH OH 66 CBN-7-oic acid COOH 67 CBN-1"-oic acid CH.sub.3 COOH 68 CBN-3"-oic acid CH.sub.3 C.sub.2 H.sub.4 COOH Note: R-group substituents are H if not indicated otherwise.

The invention being thus described, variation in the materials and methods for practicing the invention will be apparent to one of ordinary skill in the art. Such variations are to be considered within the scope of the invention, which is set forth in the claims below.

\* \* \* \* \*



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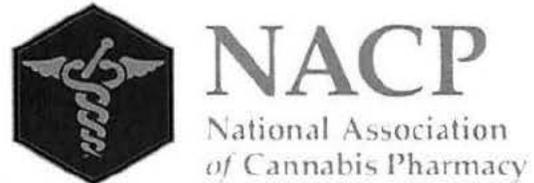
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REGISTRATION: JUNE 28<sup>TH</sup>, 2014 (16.00 – 18.00)

WELCOME RECEPTION: 18.30 – 20.00

DAY 1  
SUNDAY, JUNE 29<sup>TH</sup>

7.00	BREAKFAST		
8.15	WELCOME AND OPENING REMARKS		
<b>ORAL SESSION 1. CANNABINOIDS AND PATHOPHYSIOLOGY</b> <i>CHAIRS: STEVE ALEXANDER AND MAURO MACCARRONE</i>			
8.30	Fabio Arturo Iannotti, Enrico Mazzarella, Ester Pagano, Elisabetta Gazzero, Raffaele Capasso and Vincenzo Di Marzo	STUDY OF THE EXPRESSION PROFILE AND PHARMACOLOGICAL ROLE OF THE CB1 RECEPTOR IN DUCHENNE MUSCULAR DYSTROPHY (DMD) MUSCLES: A NEW OPPORTUNITY TO REINFORCE MUSCLE REPAIR AND LOCOMOTOR ACTIVITY	1
8.45	Giulia Donvito and Barbara Costa	PALMITOYLETHANOLAMIDE IN A RAT MODEL OF OSTEOARTHRITIS: ITS ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTIVENESS IN COMPARISON WITH NIMESULIDE AND ACETAMINOPHEN	2
9.00	Marie-Chantal Larose, Caroline Turcotte, Cyril Martin, Véronique Provost, Michel Laviolette and Nicolas Flamand	MECHANISMS OF HUMAN EOSINOPHIL MIGRATION INDUCED BY THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL	3

9.15	Irina Bronova, Katalin Erdelyi, Alex Makriyannis, Pal Pacher and Evgeny Berdyshev	PERIPHERAL TARGETING OF CBI CANNABINOID RECEPTORS PROTECTS FROM RADIATION- INDUCED PULMONARY FIBROSIS	4
9.30	Daniel J. Hermanson, Shu Xu, Naoko Brown, Jeffrey Reese, Sachin Patel and Lawrence J. Marnett	ENDOCANNABINOID AUGMENTATION BY SUBSTRATE- SELECTIVE COX-2 INHIBITORS: MECHANISM AND <i>IN VIVO</i> PROBE DEVELOPMENT	5
9.45	Laura Jimenez-Sanchez, Ruth Pazos, Hector Lafuente, Lorena Barata, Maria Ceprian, Martin Santos, Francisco Jose Alvarez and Jose Martinez- Orgado	ROLE OF 5HT1A RECEPTORS ON THE NEUROPROTECTIVE AND NEUROBEHAVIORAL EFFECTS OF CANNABIDIOL IN HYPOXIC- ISCHEMIC NEWBORN PIGS	6
10.00	<b>COFFEE BREAK</b>		
10.30	Shimon Ben-Shabat, Shiela Hauzner, Mor Cohen and Elie Beit-Yannai	PROTECTIVE ROLE OF THE CANNABINOID RECEPTOR SYSTEM IN AN <i>IN VITRO</i> MODEL OF AGE-RELATED MACULAR DEGENERATION (AMD)	7
10.45	Ariana Foinquinos, Katharina Schimmel, Jan Fiedler, Thomas Thum and Sandor Batkai	THE CANNABINOID-1 RECEPTOR IN CARDIAC FIBROSIS	8
11.00	Kevin Wilhelmsen, Samira Khakpour, Alphonso Tran and Judith Hellman	THE ENDOCANNABINOID N-ARACHIDONOYL DOPAMINE (NADA) AND WIN55,212-2 MODULATE THE INFLAMMATORY ACTIVATION OF HUMAN ENDOTHELIAL CELLS	9
11.15	Tamás Bíró, Attila Oláh, Dóra Bodnár, Lídia Ambrus, Attila G. Szöllösi, Nikolett Vasas, Judit Szabó-Papp, Ralf Paus, Michael Soeberdt and Christoph Abels	NOVEL INHIBITORS OF FATTY ACID AMIDE HYDROLASE EXERT REMARKABLE ANTI- INFLAMMATORY EFFECTS BOTH <i>IN VITRO</i> IN HUMAN KERATINOCYTES AND <i>IN VIVO</i> IN NC/TND MICE	10

11.30	Saja Baraghithy, Reem Smoum, Malka Attar-Namdar, Raphael Mechoulam and Itai Bab	STIMULATION OF BONE MASS BY A NOVEL METHYLATED OLEOYL SERINE DERIVATIVE	11
12.00	LUNCH		
13.00 - 15.00	POSTER SESSION 1		P1
15.00	<p><b>PRESIDENTIAL PLENARY SPEAKER</b></p> <p><i>CANNABINOIDS REVISITED?</i></p> <p><i>NEW TARGETS, CHEMISTRY AND PLANT SOURCES</i></p> <p><b>GIOVANNI APPENDINO, PH.D.</b></p> <p>Professor of Organic Chemistry, Università del Piemonte Orientale Department of Pharmaceutical Sciences, Novara, Italy</p>		
16.00	COFFEE BREAK		
<p><b>ORAL SESSION 2. PAIN AND TRPs</b></p> <p><i>CHAIRS: TIZIANA BISOGNO AND MARY LYNCH</i></p>			
16.30	Jenny Wilkerson, Sudeshna Ghosh, Ku-Lung Hsu, Benjamin F. Cravatt and Aron H. Lichtman	DIACYLGLYCEROL LIPASE BETA: NEW EVIDENCE FOR INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE	12

16.45	Richard A. Slivicki, Liting Deng, Pushkar M. Kulkarni, Maria Cascio, Roger G. Pertwee, Ganesh A. Thakur and Andrea G. Hohmann	POSITIVE ALLOSTERIC MODULATION OF CB1 WITH GAT211 SUPPRESSES PACLITAXEL-INDUCED NEUROPATHIC PAIN WHILE BYPASSING UNWANTED SIDE EFFECTS OF CB1 RECEPTOR ACTIVATION	13
17.00	Miren-Josune Canduela, Juan-Luis Mendizabal- Zubiaga, Amanda Sierra, Naiara Royo, Sara Peñasco, Leire Reguero, Nagore Puente and Pedro Grandes	ANATOMY AND FUNCTIONAL ROLE OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IN THE DENTATE GYRUS OF A KAINATE-INDUCED SEIZURES MOUSE MODEL	14
17.15	Heather B Bradshaw, Emma Lieshman, Jordyn M Stuart and Mark Connor	OVEREXPRESSION OF TRPV1 IN HEK CELLS DRIVES DRAMATIC CHANGES IN BASAL ENDOCANNABINOIDS AND RELATED LIPIDS WHICH ARE POTENTIATED WITH STIMULATION BY CAPSAICIN	15
<p><b>ORAL SESSION 3. DEVELOPMENTAL</b> <i>CHAIRS:</i> TIZIANA RUBINO AND SARA JANE WARD</p>			
17.30	Valerio Chiurchiù, Alessandro Leuti, Emanuela Talamonti and Mauro Maccarrone	ANANDAMIDE REGULATES MATURATION AND FUNCTION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS	16
17.45	Maria Morena, Andrea Peloso, Viviana Trezza, Matthew N. Hill and Patrizia Campolongo	LEARNING UNDER STRESS DIFFERENTIALLY AFFECTS CANNABINOID MODULATION OF SPATIAL MEMORY RETRIEVAL IN RATS	17
18.00	Piray Atsak, Daniela Hauer, Patrizia Campolongo, Gustav Schelling, Raquel V Fornari and Benno Roozendaal	THE ROLE OF ENDOCANNABINOID SIGNALING IN MEDIATING EFFECTS OF SEVERAL STRESS SYSTEMS IN MEMORY CONSOLIDATION	18

18.15	Andrea Martella, Rosa Maria Sepe, Cristoforo Silvestri, Oliana Carnevali, Paolo Sordino and Vincenzo Di Marzo	FUNCTIONAL CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM DURING ZEBRAFISH (DANIO RERIO) EMBRYONIC DEVELOPMENT	19
18.30	Emma Leishman, Ben Cornett, Ken Mackie and Heather B Bradshaw	EFFECTS OF DELETIONS IN FAAH ON THE ENDOCANNABINOID AND WIDE-RANGING-RELATED LIPIDOME IN EIGHT REGIONS OF THE MOUSE BRAIN	20
<p><b>ORAL SESSION 4. CANNABINOIDS AND CANCER</b>  <i>CHAIRS: TIZIANA RUBINO AND SARA JANE WARD</i></p>			
18.45	Paula Morales, Sandra Blasco-Benito, María Gómez-Cañas, Pilar Goya, Javier Fernández-Ruiz, Cristina Sánchez and Nadine Jagerovic	NOVEL CB2 SELECTIVE CANNABINOID-ORTHOQUINONES EFFECTIVE FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCER AND LACKING NON-TUMOR CELL TOXICITY	21
19.00	Christopher J. Fowler, Jenny Häggström, Mariateresa Cipriano and Peter Hammarsten	IDENTIFYING POTENTIAL UPSTREAM REGULATORS OF THE ENDOCANNABINOID SYSTEM IN PROSTATE CANCER	22
19.15	<b>DINNER</b>		

**DAY 2**  
**MONDAY, JUNE 30<sup>TH</sup>**

7.00	<b>BREAKFAST</b>		
<b>ORAL SESSION 5. CANNABINOID RECEPTORS AND SIGNALING</b> <i>CHAIRS: MICHELLE GLASS AND NEPHI STELLA</i>			
8.30	Chris Breivogel and Ankita Gupta	BETA-ARRESTIN2 APPEARS TO MEDIATE THE ACTIVITY OF CANNABINOIDS IN FEMALE MICE IN A MANNER THAT DIFFERS FROM MALES	23
8.45	Nilushi Karunaratne, Paul J White, Stewart Fabb, Meritxell Canals, Mark Connor and Daniel T Malone	SIGNALLING PROFILE OF CRIP1a: G-PROTEIN ACTIVATION AND SIGNAL TRANSDUCTION	24
9.00	Etienne Hebert- Chatelain, Tiffany Desprez, Edgar Soria, Luigi Bellocchio, Anna Delamarre, Arnau Busquets-Garcia, Laurie Robin, Nagore Puente, Jean-William Dupuy, Uzaskun Elezgarai, Rodrigue Rossignol, Federico Massa, Pedro Grandes, Giovanni Bénard and Giovanni Marsicano	MITOCHONDRIAL CANNABINOID RECEPTORS MEDIATE SPECIFIC EFFECTS OF CANNABINOIDS VIA SOLUBLE ADENYLYL CYCLASE (sAC)	25
9.15	Derek M. Shore, Diane L. Lynch, Michael C. Pitman and Patricia H. Reggio	MOLECULAR DYNAMICS STUDY OF A CB1 ENDOGENOUS ALLOSTERIC MODULATOR, LIPOXIN A4: DYNAMIC BEHAVIOR IN A LIPID BILAYER AND ENTRANCE INTO THE CB1 RECEPTOR	26

9.30	Alipi V. Naydenov, Marja Sepers, Katie Swinney, Lynn Raymond, Richard Palmiter and Nephi Stella	GENETIC RESCUE OF CBI RECEPTORS ON MEDIUM SPINY NEURONS PREVENTS LOSS OF EXCITATORY STRIATAL SYNAPSES BUT NOT MOTOR PHENOTYPE IN R6/2 MICE	27
9.45	Martin Kaczocha, Matthew W. Elmes, William T. Berger, KwanNok Leung, Iwao Ojima and Dale G. Deutsch	FATTY ACID BINDING PROTEINS (FABPS) ARE INTRACELLULAR CARRIERS FOR $\Delta^9$ -TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD)	28
10.00	Michelle Glass, Courtney M Breen, Erin Cawston, and Mark Connor	FUNCTIONAL SELECTIVITY IN CB1R PHARMACODYNAMICS	29
10.15	<b>COFFEE BREAK</b>		
<b>ORAL SESSION 6. CANNABIS USE AND ABUSE ISSUES</b> <i>CHAIRS: ZIVA COOPER AND RYAN VANDREY</i>			
10.45	Maju Mathew Koola, Hailey E. Turner, Deanna L. Kelly, Fang Liu, Jared A. Linthicum and David A. Gorelick	CANNABIS WITHDRAWAL IN ADULTS WITH MOOD DISORDERS	30
11.00	Zheng-Xiong Xi, Pretal Muldoon, Xiao-Fei Wang, Guo- Hua Bi, M. Imad Damaj, Aron H. Lichtman, Roger G. Pertwee and Eliot L. Gardner	EFFECTS OF $\Delta^8$ -TETRAHYDROCANNABIVARIN ( $\Delta^8$ -THCV) ON APPETITIVE EFFECTS OF COCAINE AND NICOTINE IN RODENTS	31
11.15	Ryan Vandrey, Edward J Cone, John M. Mitchell, Evan S. Herrmann, George E. Bigelow, Charles LoDico and Ron Flegel	EFFECT OF ROOM VENTILATION ON THE PHARMACODYNAMIC AND PHARMACOKINETIC EFFECTS OF SECONDHAND CANNABIS SMOKE EXPOSURE	32

11.30	Miriam Schneider, Chris M. Friemel, Laura Bindila and Beat Lutz	ADVERSE PEER-EXPERIENCES THROUGHOUT ADOLESCENCE IN MALE RATS PERSISTENTLY ALTER ETHANOL INTAKE AND ENDOCANNABINOID SIGNALING IN LATER LIFE	33
11.45	M. Haney, G. Bedi and Z.D. Cooper	NALTREXONE MAINTENANCE REDUCES CANNABIS SELF-ADMINISTRATION IN CANNABIS SMOKERS	34
12.00	<b>LUNCH</b>		
12.30	<b>NIDA CAREER INFOSESSION</b> "A LAYPERSON'S EXPECTATIONS OF CANNABIS AS MEDICINE: HOW SHOULD THE RESEARCH COMMUNITY RESPOND?"		
<b>ORAL SESSION 6 (CONT.) CANNABIS USE AND ABUSE ISSUES</b> <i>CHAIRS: ZIVA COOPER AND RYAN VANDREY</i>			
14.00	Antonia Manduca, Patrizia Campolongo, Louk J.M.J. Vanderschuren, Olivier J. Manzoni and Viviana Trezza	SOCIAL REWARD IS MEDIATED BY INTERACTING OPIOID AND CB1 CANNABINOID RECEPTORS IN THE NUCLEUS ACCUMBENS CORE IN ADOLESCENT RATS	35
14.15	Daniel Ziemianski, Rielle Capler, Rory Tekanoff, Anais Lacasse, Francesca Luconi and Mark A. Ware	CANNABIS IN MEDICINE: A NATIONAL EDUCATIONAL NEEDS ASSESSMENT AMONG CANADIAN PHYSICIANS	36
14.30	Jordyn Stuart, Samuel D. Banister, Courtney Breen, Michelle Glass, Michael Kassiou and Mark Connor	ACTIVITY OF SYNTHETIC CANNABINOID DRUGS OF ABUSE AT G PROTEIN COUPLED CB RECEPTORS AND CANNABINOID- SENSITIVE ION CHANNEL	37

14.45	Ziva D. Cooper, Richard W. Foltin and Margaret Haney	EFFECTS OF CANNABIS ON THE SUBJECTIVE-EFFECT RATINGS AND PHARMACOKINETICS OF SMOKED COCAINE	38
15.00	Philippe Lucas, Zachary Walsh, Kim Crosby, Robert Callaway, Lynne, Belle-Isle, Rielle Capler, Susan Holtzman, Bob Kay, Jamie Marshall, Trevor Stratton and Michael Woodsworth	SUBSTITUTION EFFECT IN 628 MEDICAL CANNABIS PATIENTS; RESULTS FROM THE CANNABIS ACCESS FOR MEDICAL PURPOSES SURVEY (CAMPS)	39
15.15	<b>COFFEE BREAK</b>		
<b>ORAL SESSION 7. METABOLISM</b> <i>CHAIRS: RANGAN MAITRA AND JENNY WILEY</i>			
15.45	Livio Luongo, Luigia Cristino, Roberta Imperatore, Serena Boccella, Stefania Petrosino, Francesca Guida, Piero Orlando, Vincenzo Di Marzo and Sabatino Maione	LEPTIN-CONTROLLED OREXIN/ENDOCANNABINOID INTERACTIONS IN THE MOUSE PERIAQUEDUCTAL GREY: ROLE IN THE REGULATION OF THE DESCENDING ANTINOCICEPTIVE PATHWAY	40
16.00	Rangan Maitra, Alan Fulp, Herbert Seltzman, Yanan Zhang and Timothy Fennell	<i>IN VIVO</i> EVALUATION OF THE PERIPHERALLY SELECTIVE CB1 RECEPTOR ANTAGONIST RTI-13329- 2 IN A MOUSE MODEL OF DIET- INDUCED WEIGHT GAIN	41
16.15	Natalia Murataeva, Alex Straiker and Ken Mackie	WHERE'S MY ENTOURAGE? THE CURIOUS CASE OF 2-OG AND 2-LG	42

16.30	Emma Leishman, Ben Cornett, Ken Mackie and Heather B Bradshaw	NAPE-PLD DELETION VIA AN ALTERNATIVE MECHANISM DRIVES SIGNIFICANT DECREASES IN AEA IN THE MOUSE BRAIN	43
<b>ORAL SESSION 8. CNS AND PSYCHIATRIC ISSUES</b> <i>CHAIRS: MATT HILL AND DANIELA PAROLARO</i>			
16.45	Rebecca J. Bluett, Brian Shonesy, Daniel Hermanson, Nolan Hartley, Teniel Ramikie, Lawrence Marnett, Danny Winder, Roger Colbran and Sachin Patel	BIDIRECTIONAL MANIPULATIONS OF 2-ARACHIDONOYLGLYCEROL CONTENT MODULATE ANXIETY- LIKE BEHAVIORS	44
17.00	Zach Walsh, Kim Crosby, Kelsey Lozenski and Susan Holtzman	CANNABIS, ANXIETY, AND PAIN: THE IMPORTANCE OF COPING STYLE	45
17.15	<b>BREAK</b>		
17.45	Luciana Leo, Suellen Almeida-Corrêa, Claudio Canetti, Olavo Amaral, Fernando Bozza and Fabricio Pamplona	ENDOGENOUS CBI ALLOSTERIC ENHANCER BALANCES AGE-RELATED COGNITIVE ALTERATIONS	46
18.00	Andras Bilkei-Gorzo, Onder Albayram, Anastasia Piyanova, Mona Dvir-Ginzberg, Astrid Draffehn, Itai Bab, Joachim Schultze, Ildiko Racz and Andreas Zimmer	$\Delta^9$ -THC RESTORES AGE-RELATED CHANGES IN THE BRAIN	47

18.15	<p>Arnau Busquets-Garcia,          Maria Gomis-González,          Laura Cutando, Raj K.          Srivastava, Antonio          Ortega-Álvaro, Luigi          Bellochio, Giovanni          Marsicano, Beat Lutz,          Rafael Maldonado          and Andrés Ozaita</p>	<p>A PERIPHERAL          ENDOCANNABINOID MECHANISM          FOR STRESS-INDUCED AMNESIA</p>	48
18.30	<p>Erica Zamberletti,          Marina Gabaglio,          Pamela Prini,          Tiziana Rubino          and Daniela Parolaro</p>	<p>PERSISTENT MICROGLIA          ACTIVATION WITHIN THE          PREFRONTAL CORTEX          CONTRIBUTES TO THE          DEVELOPMENT OF THE          DEPRESSIVE/PSYCHOTIC-          LIKE PHENOTYPE INDUCED          BY ADOLESCENT THC          EXPOSURE IN RATS</p>	49
18.45	<p>Michelle Sexton          and Dominic Corva</p>	<p>A MEDICAL ETHNOGRAPHIC          REPORT OF CANNABIS USE IN          PEDIATRIC INTRACTABLE          EPILEPSY (IE) PATIENTS</p>	50
19.00	<p>DINNER - ON YOUR OWN</p>		

**DAY 3**  
**TUESDAY, JULY 1<sup>ST</sup>**

7.00	<b>BREAKFAST</b>		
<p><b>NIDA SYMPOSIUM</b></p> <p><b>“TRP CHANNELS: THE ONLY TR(i)P YOU CAN HAVE ON CANNABINOIDS?”</b></p> <p><i>CHAIR: VINCENZO DI MARZO</i></p>			
8.15	<p><b>Pedro Grandes</b> Medicine and Dentistry Basque Country University Leioa, Spain</p>	<p>ANATOMY AND FUNCTIONAL ROLE OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IN THE DENTATE GYRUS OF A KAINATE- INDUCED SEIZURES MOUSE MODEL</p>	N1
8.45	<p><b>Thomas Voets</b> Laboratory of Ion Channel Research (LICR) Leuven, Belgium</p>	<p>MODULATION OF TEMPERATURE- SENSITIVE TRP CHANNELS</p>	N2
9.15	<p><b>Vincenzo Di Marzo</b> Endocannabinoid Research Group Institute of Biomolecular Chemistry Consiglio Nazionale delle Ricerche Pozzuoli, Italy</p>	<p>INTERACTIONS BETWEEN THE "ENDOCANNABINOIDOME" AND THE "TRPOME": A NEW "OME" FOR PHYTOCANNABINOIDS AND LIPID MEDIATORS?</p>	N3
9.45	<b>COFFEE BREAK</b>		

## ORAL SESSION 9. CANNABINOID GENETICS

CHAIRS: JAHAN MARCU AND ROGER PERTWEE

10.15	Kevin J. McKernan, Amir Zare, Lei Zhang, Jessica Spangler, Vasisht Tadigotla, Ted Foss, Christine Stanley, Melissa Pulliam, Robert W. Pulliam and Richard G. Boles	EXOME SEQUENCING OF FAMILIAL ATRIAL FIBRILLATION INFORMS POSITIVE TREATMENT WITH CANNABIDIOL	51
10.30	Mauro Maccarrone, Andrea Di Francesco, Bernardo Dell'Osso, Daniela Galimberti, A. Carlo Altamura and Claudio D'Addario	EPIGENETIC REGULATION OF THE ENDOCANNABINOID SYSTEM IN HUMAN PSYCHIATRIC DISORDERS	52
10.45	J.M. van Gool, I. Heitland, L. Groenink and J.M.P. Baas	GENETIC DIFFERENCES IN THE CB1/CNR1 GENE MODULATE FEAR EXTINCTION IN HUMANS	53
11.00	John M. McPartland and Geoffrey W. Guy	A QUESTION OF RANK: USING DNA BARCODES TO CLASSIFY CANNABIS SATIVA AND CANNABIS INDICA	54
11.15	<p style="text-align: center;"><b><u>SPECIAL ICRS SPEAKER</u></b></p> <p style="text-align: center;">OPTOGENETIC APPROACHES TO STUDYING REWARD AND SUBSTANCE ABUSE DISORDERS</p> <p style="text-align: center;"><b>ANTONELLO BONCI, PH.D.</b> National Institutes of Health</p>		

12.15	LUNCH	
13.15 - 15.00	POSTER SESSION 2	P2
15.00 -	OUTING	

Notes:

**DAY 4**  
**WEDNESDAY, JULY 2<sup>ND</sup>**

7.00	<b>BREAKFAST</b>		
8.15  Due to scheduling conflict	Luigia Cristino, Giovanna Morello, Roberta Imperatore, Fabiana Piscitelli, Letizia Palomba and Vincenzo Di Marzo	OREXIN/ENDOCANNABINOID/ LEPTIN INTERACTION AFFECTS HYPOTHALAMIC TAU PHOSPHORILATION BY GLYCOGEN SYNTHASE KINASE-3BETA ACTIVATION	55
<b>ORAL SESSION 10. FOCUS ON CB2</b> <i>CHAIRS: JOSÉE GUINDON AND ANDREAS ZIMMER</i>			
8.30	Anne-Caroline Schmöle, Ramona Göhrs, Önder Albayram, Daniele Bano, Pierluigi Nicotera, Judith Alferink and Andreas Zimmer	CANNABINOID RECEPTOR 2 DEFICIENCY ALTERS NEUROINFLAMMATION IN AN ALZHEIMER'S DISEASE MOUSE MODEL	56
8.45	Ming Gao, Zhengxiong Xi and Jie Wu	SELECTIVE ACTIVATION OF CB2Rs ELIMINATES VTA DOPAMINE NEURONAL BURSTING FIRING IN RODENTS	57
9.00	Liting Deng, Josée Guindon, Benjamin L. Cornett, Alexandros Makriyannis, Ken Mackie and Andrea G. Hohmann	CHRONIC CANNABINOID CB2 AGONIST REVERSES PACLITAXEL NEUROPATHY WITHOUT TOLERANCE, CB1-MEDIATED WITHDRAWAL OR SIDE EFFECTS	58
9.15	Josée Guindon, Liting Deng, Emma Leishman, Heather B. Bradshaw and Andrea G. Hohmann	THE COMBINATION OF AMITRIPTYLINE WITH FAAH OR MGL INHIBITORS IN CHEMOTHERAPY- INDUCED PERIPHERAL NEUROPATHY IS CB2 MEDIATED	59
9.30	Pritesh Kumar and Zhao-Hui Song	TAMOXIFEN IS AN ALLOSTERIC MODULATOR OF THE CB2 CANNABINOID RECEPTOR	60

9.45	Shaojuan Zhang, Pin Shao, Ningyang Jia, Qin Tong, Xiang-qun Xie, Christina Bagia, Jelena M. Janjic, Ying Ding and Mingfeng Bai	TARGETING CB2 RECEPTOR AS A NEW PHOTOTHERAPY APPROACH	61
10.00	COFFEE BREAK		
<p align="center"><b>ORAL SESSION 10 (CONT). FOCUS ON CB2</b>  <i>CHAIRS: JOSÉE GUINDON AND ANDREAS ZIMMER</i></p>			
10.30	Vanessa Petrucci, Andrea Chicca, Juan Manuel Viveros-Paredes and Jürg Gertsch	PEPTIDE ENDOCANNABINOIDS (PEPCANS) ARE PAMs OF CB2 RECEPTORS AND INVOLVED IN THE INNATE IMMUNE RESPONSE	62
10.45	Eef L. Theunissen, Pim Heckman, Elizabeth B. de Sousa Fernandes Perna, Kim P. C. Kuypers, Anke Sambeth, Arjan Blokland, Jos Prickaerts, Stefan W. Toennes and Johannes G. Ramaekers	REVERSING THC-INDUCED IMPAIRMENT OF VERBAL MEMORY IN HEALTHY HUMANS	63
11.00	Slava Rom, Holly Dykstra, Nancy Reichenbach, Viviana Zuluaga- Ramirez and Yuri Persidsky	SELECTIVE STIMULATION OF CANNABIOID TYPE 2 RECEPTORS (CB2) IN MONOCYTES PREVENTS THEIR ENGAGEMENT OF BRAIN ENDOTHELIUM PROTECTING BLOOD BRAIN BARRIER	64
11.15	Jenny L. Wiley, Brian F. Thomas, Hongfang Yang and Anu Mahadevan	CB2 AGONISTS: HOW SELECTIVE ARE THEY?	65

11.30	<p>Mario van der Stelt, Partha Mukhopadhyay, Marc Baggelaar, Zongxian Cao, Katalin Erdelyi, Filomena Fezza, Bogna Ignatowska- Jankowska, Marc Ruben, Resat Cinnar, George Kunos, Aron Lichtman, Mauro Maccarrone and Pal Pacher</p>	<p>PERIPHERALLY RESTRICTED, SELECTIVE CANNABINOID CB2 RECEPTOR AGONIST LEI-101 PREVENTS CISPLATIN-INDUCED NEPHROPATHY</p>	66
11.45	<p>Jean-Michel Adam, Christian M. Apfel, Stefanie Bendels, Caterina Bissantz, Jürgen Fingerle, Ivan Formentini, Jürgen Funk, Uwe Grether, Sabine Grüner, Atsushi Kimbara, Matthias Nettekoven, Giorgio Ottaviani, Camille Perret, Mark Rogers- Evans, Stephan Röver, Franz Schuler, Tanja Schulz-Gasch and Christoph Ullmer</p>	<p>TRIAZOLOPYRIMIDINES – A NOVEL CLASS OF HIGHLY POTENT, HIGHLY SELECTIVE AND <i>IN VIVO</i> ACTIVE CB2 AGONISTS</p>	67
12.00	<p>LUNCH</p>		
13.00 – 15.00	<p>POSTER SESSION 3</p>		P3

**ORAL SESSION 11.**  
**BEYOND THC: CANNABIDIOL AND OTHER COMPONENTS**

*CHAIRS:* LUCIANO DE PETROCELLIS AND MICHELLE SEXTON

15.00	Attila Oláh, Lidia Ambrus, Attila G. Szöllösi, Christos C. Zouboulis, Ralf Paus and Tamás Bíró	“WEED AGAINST ZIT?” – EXPLORATION OF THE MECHANISMS OF THE COMPLEX ANTI-ACNE ACTIONS OF CANNABIDIOL	68
15.15	Jeffrey C. Raber, Sytze Elzinga, Mark E. Raber, Bradley J. Douglass, Cameron Miller, Aaron Kendziorek, Justin Fishedick and Dale Gieringer	CANNABINOID AND TERPENOID PROFILING OF CANNABIS IN CALIFORNIA AND WASHINGTON	69
15.30	Richard C. Kevin, David J. Allsop, Wendy Swift and Iain S. McGregor	“SMELLS LIKE GOOD WEED”: THC LEVELS IN CANNABIS MAY BE PREDICTED BY MONOTERPENE HEADSPACE CONCENTRATIONS	70
15.45	Sándor Bátkai, Partha Mukhopadhyay, Katalin Erdélyi, Jürg Gertsch and Pál Pacher	PROTECTIVE EFFECT OF THE PHYTOCANNABINOID BETA- CARYOPHYLLENE IN LIVER ISCHEMIA REPERFUSION INJURY	71
16.00	Alex Naftaly, Ester Fride (Z”L), Jürg Gertsch and Sharon Anavi-Goffer	EFFECT OF BETA-CARYOPHYLLENE ON PHENCYCLIDINE-INDUCED BEHAVIOURAL CHANGES	72
16.15	Douglas E Brenneman, Dean Petkanas and William A. Kinney	CANNABIDIOL PROVIDES PROTECTION FROM ETHANOL AND AMMONIUM TOXICITY IN A HIPPOCAMPAL MODEL OF HEPATIC ENCEPHALOPATHY	73

16.30	Andrea Chicca, Diego Caprioglio, Alberto Minassi, Vanessa Petrucci, Salome Gachet, Giovanni Appendino, Orazio Tagliatalata-Scafati and Jurg Gertsch	NATURAL PRODUCT-DERIVED CANNABIMIMETICS AS SOURCE OF POLYPHARMACOLOGY IN THE ENDOCANNABINOID SYSTEM	74
16.45	Alline Campos, Aline Miranda, Fatima Brant, Fabiana Machado, Francisco Guimaraes and Antonio Teixeira	CANNABIDIOL REPEATED TREATMENT INCREASES SURVIVAL AND PROMOTES RESCUE OF COGNITIVE FUNCTION IN A MURINE MODEL OF CEREBRAL MALARIA	75
17.00	Maria Ceprian, Maria Ruth Pazos, Federica Penna, Laura Jimenez-Sanchez, Martin Santos and Jose Martinez	CANNABIDIOL PROMOTES OLIGODENDROCYTE SURVIVAL AFTER HYPOXIA-ISCHEMIA IN NEWBORN RATS	76
17.15	Nadia Solowij, Erika van Hell, Samantha Broyd, Lisa-marie Greenwood, Jelena Novakovic, Camilla Beale, Dave Martelozzo, Arno Hazekamp and Rodney Croft	EFFECTS OF CBD ALONE AND IN COMBINATION WITH THC ON COGNITIVE PERFORMANCE	77
17.30	James Brodie, Vincenzo Di Marzo and Geoffrey Guy	THE THERAPEUTIC HANDSHAKE: CRAFTING MEDICINES FOR COMPLEX MALADIES	78

17.45	Manoela V. Fogaça, Alline C. Campos and Francisco S. Guimarães	THE ANXIOLYTIC-LIKE EFFECT OF CANNABIDIOL ADMINISTRATION IN CHRONICALLY STRESSED MICE IS MEDIATED BY THE ENDOCANNABINOID SYSTEM: INVOLVEMENT OF NEUROGENESIS AND AUTOPHAGY	79
18.00	Jahan P. Marcu	RESULTS FROM AUDITING MEDICAL CANNABIS FACILITIES IN THE UNITED STATES IN 2014	80
18.15	<b>ICRS BUSINESS MEETING</b>		
20.00	<b>ICRS BANQUET</b>		

**POSTER SESSION 1: TOPICS A - E**  
**DAY 1, SUNDAY, JUNE 29<sup>TH</sup>: 13:00 - 15:00**

**TOPIC A. CANNABINOIDS AND PATHOPHYSIOLOGY**

<p>Michiel Balvers, Pim Koelink,  Aletta Kraneveld,  Mieke Poland,  Jocelijn Meijerink  and Renger Witkamp</p>	<p align="center">THE N-3 N-ACYLETHANOLAMIDE  DHEA IMPROVES MANIFESTATIONS  OF EXPERIMENTAL MURINE COLITIS</p>	<p align="center">P1-1</p>
<p>Valerio Chiurchiù, Emanuela  Talamonti, Alessandro Leuti  and Mauro Maccarrone</p>	<p align="center">THE ENDOCANNABINOID SYSTEM IS  A NOVEL MODULATOR OF HUMAN  MACROPHAGE PLASTICITY  AND POLARIZATION</p>	<p align="center">P1-2</p>
<p>Caroline Turcotte, Simona  Zarini, Stéphanie Jean, Cyril  Martin, Véronique Provost,  Adam Uzieblo, Robert C  Murphy and Nicolas Flamand</p>	<p align="center">PROSTAGLANDIN D<sub>2</sub>-GLYCEROL  AND E<sub>2</sub>-GLYCEROL INHIBIT HUMAN  NEUTROPHIL FUNCTIONS</p>	<p align="center">P1-3</p>
<p>Mark Jones, Marie Smith,  Susan Anderson  and Saoirse E. O'Sullivan</p>	<p align="center">EVIDENCE THAT THE NOVEL  ENDOCANNABINOID VIRODHAMINE  INCREASES OSTEOBLAST  PROLIFERATION: A ROLE  FOR CB2 AND GPR55</p>	<p align="center">P1-4</p>
<p>Valerie CM Shang, David A  Kendall and Richard E Roberts</p>	<p align="center">THE EFFECT OF CANNABINOIDS ON  HUMAN BRONCHIAL EPITHELIAL CELL  PERMEABILITY</p>	<p align="center">P1-5</p>

<p>Ku-Lung Hsu, Katsunori Tsuboi, Alexander Adibekian, Holly Pugh, Kim Masuda, Sudeshna Ghosh, Manuel Sanchez-Alavez, Aron Lichtman, Bruno Conti and Benjamin F. Cravatt</p>	<p>INACTIVATION OF DIACYLGLYCEROL LIPASE IN INFLAMMATORY DISEASE</p>	<p>P1-6</p>
<p>Jocelijn Meijerink, Zheng Wang, Mieke Poland, Jean-Paul ten Klooster and Renger Witkamp</p>	<p>DOCOSAHEXAENOYL-SEROTONIN (DHA-5-HT), AN INTESTINAL CONJUGATE OF SEROTONIN AND DHA EXHIBITS ANTI-INFLAMMATORY PROPERTIES</p>	<p>P1-7</p>
<p>Michael M. McDonald, Amos Matsiko, Adolfo López Noriega, Hugo D. Kieran, Aoife Gowran, Kevin J. Mulhall, Fergal J. O'Brien and Veronica A. Campbell</p>	<p>THE DEVELOPMENT OF A CANNABINOID-CONTAINING COLLAGEN-GAG SCAFFOLD FOR USE IN ORTHOPAEDIC TISSUE ENGINEERING STRATEGIES</p>	<p>P1-8</p>
<p>Valerio Chiurchiù, Emanuela Talamonti, Mirko Lanuti Tiziana Bisogno and Mauro Maccarrone</p>	<p>ENDOCANNABINOIDS DIFFERENTIALLY MODULATE NLRP3 INFLAMMASOME ACTIVATION IN PRIMARY HUMAN MACROPHAGES</p>	<p>P1-9</p>
<p>Michel Laviolette, Marie-Chantal Larose, Caroline Turcotte, Véronique Provost, Cyril Martin, Catherine Laprise and Nicolas Flamand</p>	<p>METABOLISM OF EXOGENOUS ARACHIDONOYL-ETHANOLAMIDE AND 2-ARACHIDONOYL-GLYCEROL BY HUMAN EOSINOPHILS</p>	<p>P1-10</p>
<p>Amina M. Bagher, Robert B. Laprairie, Melanie E.M. Kelly and Eileen M. Denovan-Wright</p>	<p>INFLUENCE OF THE DOPAMINE RECEPTOR TYPE 2 (D2) ANTAGONIST ON THE CANNABINOID RECEPTOR TYPE 1 (CB1) FUNCTION</p>	<p>P1-11</p>
<p>Attila Oláh, Nóra Czakó, Levente Molnár, Stefania Petrosino, Teresa Aveta, Vincenzo Di Marzo, Béla Fülesdi and Tamás Bíró</p>	<p>EFFECTS OF CONTROLLED, TRANSIENT HYPEROXIA ON THE SERUM LEVELS OF DIFFERENT ENDOCANNABINOIDS – A HUMAN PILOT STUDY</p>	<p>P1-12</p>

<p>Herbert H. Seltzman, Yatendra Mulpuri and Igor Spigelman</p>	<p>THE PERIPHERALLY-RESTRICTED CANNABINOID RECEPTOR AGONIST PRNMI ALLEVIATES CISPLATIN INDUCED PERIPHERAL NEUROPATHY</p>	<p>P1-13</p>
<p>Mustafa Karwad, Karen L. Wright, Michael Larvin, Jonathan Lund and Saoirse E. O'Sullivan</p>	<p>OLEOYLETHANOLAMIDE (OEA) AND PALMITOYLETHANOLAMIDE (PEA) MODULATE INTESTINAL PERMEABILITY IN AN <i>IN VITRO</i> ISCHAEMIA/REPERFUSION MODEL</p>	<p>P1-14</p>
<p>Ya Wang, Pierluigi Plastina, Michiel Balvers, Mieke Poland, Bartolo Gabriele, Jean-Paul Vincken, Renger Witkamp and Jocelijn Meijerink</p>	<p>N-ACYLDOPAMINES DERIVED FROM POLYUNSATURATED OMEGA-3 FATTY ACIDS EXERT ANTI-INFLAMMATORY EFFECTS IN MOUSE MACROPHAGES</p>	<p>P1-15</p>
<p>Natalia Malek, Katarzyna Popiolek-Barczyk, Joanna Mika, Barbara Przewlocka and Katarzyna Starowicz</p>	<p>RATIONALE FOR TARGETING THE ENDOCANNABINOID SYSTEM TO MANAGE NEUROINFLAMMATION IN LPS-ACTIVATED PRIMARY MICROGLIAL CULTURES</p>	<p>P1-16</p>
<p>Haley A. Vecchiarelli, Catherine M. Keenan, J. Megan Gray, Mohammad Bashashati, Keith A. Sharkey and Matthew N. Hill</p>	<p>COLITIS ALTERS CENTRAL ENDOCANNABINOID CONTENT</p>	<p>P1-17</p>
<p>Ulrike Taschler, Martina Schweiger, Martin A. Storr, Rudolf Schicho and Robert Zimmermann</p>	<p>MONOGLYCERIDE LIPASE DEFICIENCY CAUSES INTESTINAL CANNABINOID RECEPTOR DESENSITIZATION</p>	<p>P1-18</p>
<p>Elizabeth A. Cairns, Michele L. Archibald, Alex J. Straiker, Pushkar M. Kulkarni, Ganesh A.Thakur, William H. Badridge and Melanie E.M. Kelly</p>	<p>MODIFYING CB1 RECEPTOR SIGNALLING TO REDUCE IOP IN A MOUSE MODEL OF OCULAR HYPERTENSION</p>	<p>P1-19</p>

## TOPIC B. PAIN AND TRPS

<p>Bright N Okine, Manish K. Madasu, Fiona McGowan, Brendan Harhen, Michelle Roche and David P. Finn</p>	<p>DIRECT ADMINISTRATION OF N-PALMITOYLETHANOLAMIDE INTO THE RAT MEDIAL PREFRONTAL CORTEX REDUCES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR VIA A CB1 RECEPTOR-MEDIATED MECHANISM</p>	<p>P1-20</p>
<p>Mireille Alhouayek, Baptiste Buisseret, Owein Guillemot-Legrin and Giulio G. Muccioli</p>	<p>COMPARISON OF THE EFFECTS OF INHIBITION OF ABHD6 AND MAGL ON INFLAMMATION</p>	<p>P1-21</p>
<p>Samira Khakpour, Kevin Wilhelmsen, Alphonso Tran and Judith Hellman</p>	<p>INVESTIGATION OF THE ROLE OF THE TRPV1 CHANNEL IN ENDOTHELIAL INFLAMMATION</p>	<p>P1-22</p>
<p>Martin Kaczocha, Syed Azim, James Nicholson, Mario J. Rebecchi, William Galbavy, Tian Feng and Helene Benveniste</p>	<p>ENDOCANNABINOID LEVELS AND FUNCTIONAL DISABILITY STATUS IN PATIENTS WITH PAINFUL OSTEOARTHRITIS</p>	<p>P1-23</p>
<p>William Notcutt, Cheryl Phillips, Phillipe Lacoux, Adrian Shanks, Viji Vijayakulasingam and Laura Baldock</p>	<p>A RETROSPECTIVE DESCRIPTION OF THE USE OF NABILONE IN UK CLINICAL PRACTICE</p>	<p>P1-24</p>
<p>Pierluigi Plastina, Mieke Poland, Jocelijn Meijerink, Bartolo Gabriele and Renger Witkamp</p>	<p>HYDROXYTYROSYL OLEATE, AN ESTER ANALOGUE OF OLEOYL DOPAMINE (OLDA), SHOWS ANTI-INFLAMMATORY PROPERTIES <i>IN VITRO</i></p>	<p>P1-25</p>

<p>Molly S. Crowe, Emma Leishman, Ramesh Gujjar, Anu Mahadevan, Matthew Banks, Heather Bradshaw and Steven G. Kinsey</p>	<p>ATTENUATING NEUROPATHIC PAIN THROUGH DUAL INHIBITION OF CYCLOOXYGENASE AND MONOACYLGLYCEROL LIPASE</p>	<p>P1-26</p>
<p>Iryna Khasabova, Cutler Lewandowski, Xu Yao, Justin Paz, Natalya Burlakova, Donald Simone and Virginia Seybold</p>	<p>ANANDAMIDE MEDIATES THE ANTIHYPERALGESIC EFFECT OF 2AG IN A MURINE MODEL OF CHEMOTHERAPY-INDUCED NEUROPATHY</p>	<p>P1-27</p>
<p>Sara R. Nass and Steven G. Kinsey</p>	<p>THE MONOACYLGLYCEROL LIPASE INHIBITOR JZL184 ATTENUATES HYPERALGESIA INDUCED BY COLLAGEN-INDUCED ARTHRITIS</p>	<p>P1-28</p>
<p>Peggy Schneider, Laura Bindila, Beat Lutz, Rainer Spanagel and Miriam Schneider</p>	<p>LONG-TERM SOCIAL REJECTION DURING ADOLESCENCE ALTERS PAIN PERCEPTION AND AFFECTS THE ENDOCANNABINOID SYSTEM IN ADULT FEMALE RATS</p>	<p>P1-29</p>
<p>Luciano De Petrocellis, Stefania Petrosino, Aniello Schiano Moriello, Santiago Cerrato, Mariella Fusco, Anna Puigdemont and Vincenzo Di Marzo</p>	<p>“ENTOURAGE” EFFECTS OF PALMITOYLETHANOLAMIDE: ENHANCEMENT OF 2-AG ACTION AT TRPV1 CHANNELS AND OF 2-AG LEVELS <i>IN VITRO</i> AND <i>IN VIVO</i></p>	<p>P1-30</p>
<p>Torsten Lowin, Angelika Gräber and Rainer H. Straub</p>	<p>ANTI-INFLAMMATORY EFFECTS OF THE CB1/CB2 AGONIST WIN55212,2 ARE DEPENDENT ON TRPV1, TRPA1 AND AMPK IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS SYNOVIAL FIBROBLASTS</p>	<p>P1-31</p>
<p>Manish K. Madasu, Bright N. Okine, Weredeselam M. Olango, Michelle Roche and David P. Finn</p>	<p>DIFFERENTIAL EFFECTS OF PHARMACOLOGICAL MODULATION OF TRPV1 IN THE LATERAL PERIAQUEDUCTAL GREY ON FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR IN SPRAGUE-DAWLEY AND WISTAR-KYOTO RATS</p>	<p>P1-32</p>

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<p>Lindsay Silva, Rita Black and Diana Dow-Edwards</p>	<p>AGE OF EXPOSURE AFFECTS BEHAVIORAL RESPONSE TO DELTA-9-TETRAHYDROCANNABINOL DURING THE PERI-PUBERTAL PERIOD IN THE RAT</p>	<p>P1-34</p>
<p>Justine Renard, Michael Loureiro, Walter J. Rushlow and Steven R. Laviolette</p>	<p>LONG-TERM EFFECTS OF ADOLESCENT THC EXPOSURE ON ADULTHOOD PSYCHOPATHOLOGY</p>	<p>P1-35</p>
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<p>Anja Goepfrich and Miriam Schneider</p>	<p>MODULATORY INFLUENCE OF THE DEVELOPING ENDOCANNABINOID SYSTEM ON COGNITIVE ABILITIES DURING ADOLESCENT BRAIN DEVELOPMENT IN RATS</p>	<p>P1-37</p>
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<p>Seok-Woo Park and Myung-Whun Sung</p>	<p>CYCLOOXYGENASE-2-DEPENDENT GROWTH INHIBITION OF ARACHIDONOYL ETHANOLAMIDE ON LARYNGEAL CANCER CELLS</p>	<p>P1-41</p>
<p>Stan L. Banks, Miro Golinski, Jeff Howard, Dana Hammell and Audra L. Stinchcomb</p>	<p>DEVELOPMENT OF A <math>\Delta^9</math>-THC PRODRUG TRANSDERMAL DELIVERY SYSTEM FOR PREVENTION OF ACUTE AND DELAYED NAUSEA AND VOMITING ASSOCIATED WITH INITIAL AND REPEAT COURSES OF EMETOGENIC CANCER CHEMOTHERAPY</p>	<p>P1-42</p>
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<p>N. Rielle Capler and Lynda G. Balneaves</p>	<p>A REVIEW OF THE USE OF HERBAL CANNABIS AND CANNABIS EXTRACTS FOR THE TREATMENT OF CANCER AND CANCER-RELATED SYMPTOMS</p>	<p>P1-44</p>

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Jagjeet Mnpotra, Diane Lynch, Alan Grossfield, Nicholas Leioatts, Michael Pitman and Patricia Reggio	ROLE OF INTRACELLULAR LOOPS IN THE HYDRATION OF GDP: RESULTS FROM MOLECULAR DYNAMICS SIMULATIONS OF THE 2-AG ACTIVATED CANNABINOID RECEPTOR SUBTYPE 2 / Gi PROTEIN COMPLEX	P1-49
Khalil Eldeeb, Sandra Leone- Kabler, Lawrence C. Blume and Allyn C. Howlett	CB1 RECEPTOR INTRACELLULAR LOOP 4 MUTATION MODULATES G PROTEIN ACTIVATION AND CAMP PRODUCTION IN HUMAN NEUROBLASTOMA CELLS	P1-50

**POSTER SESSION 2: TOPICS E - H**  
**DAY 3, TUESDAY, JULY 1<sup>ST</sup>: 13:15 - 15:00**

**TOPIC E (CONT.) CANNABINOID RECEPTORS AND SIGNALING**

<p>Matthew W. Buczynski,          Melissa A. Herman, Ku-Lung          Hsu, Luis A. Natividad,          Cristina Irimia, Ilham Y. Polis,          Holly Pugh, Jae Won Chang,          Micah J. Niphakis,          Benjamin F. Cravatt,          Marisa Roberto          and Loren H. Parsons</p>	<p align="center">CHRONIC NICOTINE EXPOSURE          DIMINISHES INHIBITORY CONTROL          OF VTA DA NEURONS THROUGH          ENHANCED DIACYLGLYCEROL          LIPASE-MEDIATED          2-ARACHIDONOYLGLYCEROL          SIGNALING</p>	<p align="center">P2-1</p>
<p>Alex Straiker, Sherry Hu,          Karl Spork, Emma Leishmann          and Heather Bradshaw</p>	<p align="center">A GPR119-BASED SIGNALING SYSTEM          IN THE MURINE EYE REGULATES          INTRAOCULAR PRESSURE IN A          SEX-DEPENDENT MANNER</p>	<p align="center">P2-2</p>
<p>Richard S Priestley,          Sarah A. Nickolls,          Stephen P.H. Alexander          and David A Kendall</p>	<p align="center">CANNABINOID CB1 RECEPTOR-          MEDIATED ERK1/2 RESPONSES -          EVIDENCE OF G<sub>1/0</sub> PROTEIN-          INDEPENDENT SIGNALLING          AND AGONIST BIAS</p>	<p align="center">P2-3</p>
<p>Toru Uyama, Manami Inoue,          Yoko Okamoto, Naoki          Shinohara, Tatsuya Taii,          Masahiro Watanabe, Iffat Ara          Sonia Rahman, Kazuhito          Tsuboi, Tomohito Inoue, Akira          Tokumura and Natsuo Ueda</p>	<p align="center">PEROXISOMAL DYSFUNCTION BY          PLA/AT FAMILY PROTEINS IS NOT          RELATED TO THEIR NAPE-FORMING          N-ACYLTRANSFERASE ACTIVITY</p>	<p align="center">P2-4</p>
<p>Jayendra Z. Patel, Stephen          Ahenkorah, Yahaya Adams,          Susanna M. Saario, Teija          Parkkari, Juha R. Savinainen,          Jarmo T. Laitinen          and Tapio Nevalainen</p>	<p align="center">LORATADINE ANALOGUES          AS MAGL INHIBITORS</p>	<p align="center">P2-5</p>

Dow P. Hurst, Diane L. Lynch, Derek M. Shore, Michael C. Pitman and Patricia H. Reggio	MODELING THE ORG27569 INDUCED CB1/BETA-ARRESTIN 1 COMPLEX THAT ACTIVATES AN ARRESTIN BIASED PATHWAY	P2-6
William A. Devane and David P. Finn	PHARMACOLOGICAL CHARACTERISATION OF A BINDING SITE FOR [ <sup>3</sup> H]LEELAMINE	P2-7
Abhijit R. Kulkarni, Pushkar M. Kulkarni, Anisha Korde, Nicolai Zvonok, Maria Grazia Cascio, Alexandros Makriyannis, Roger G. Pertwee and Ganesh A. Thakur	DESIGN, SYNTHESIS AND BIOCHEMICAL EVALUATION OF NOVEL ELECTROPHILIC AND PHOTOAFFINITY COVALENT PROBES TO MAP THE CB1 RECEPTOR ALLOSTERIC SITE(S)	P2-8
Saoirse O'Sullivan, Sara Goodacre and Jonathan Yee	REVIEW OF EVIDENCE FOR CANNABINOID RECEPTORS AND ENDOCANNABINOID SIGNALLING IN INVERTEBRATES WITH A SPECIFIC FOCUS ON ARACHNIDS	P2-9
Carmen Rodríguez-Cueto, Mariluz Hernández-Gálvez, Cecilia J. Hillard, Patricia Maciel, Javier Fernández-Ruiz and María Gómez-Ruiz	ANALYSIS OF THE ENDOCANNABINOID SIGNALING SYSTEM IN BRAIN STRUCTURES OF SCA-3 TRANSGENIC MICE	P2-10
June Penman, Emanuel Ferreira Lopes and Andrew J. Irving	THE N-ACYL AMINO ACIDS, N-ARACHIDONOYL-L-SERINE AND N-ARACHIDONOYL GLYCINE, ACTIVATE GPR55	P2-11
Christoph Porazik, Anke Witting and Boris Fergert	SIMULTANEOUS DETERMINATION OF ENDOCANNABINOID AND PROSTAGLANDIN BIOMARKERS AND OF MONOACYLGLYCEROL LIPASE INHIBITOR EXPOSURE USING A NEW AND FAST LC-MS/MS-METHOD	P2-12
Marcus R. Goetz, Oskar Koch, Eduardo Munoz E and Bernd L. Fiebich	EFFECTS OF NOVEL SEMI-SYNTHETIC CANNABINOIDS ON CB1 AND CB2 RECEPTORS THROUGH BINDING AND SIGNALLING	P2-13

Jonathan Yee, Saoirse O’Sullivan and Sara Goodacre	REVIEW OF THE EVIDENCE FOR CANNABINOID RECEPTORS IN INVERTEBRATES WITH A SPECIFIC FOCUS ON ARACHNIDS	P2-14
<b>TOPIC F. CANNABIS USE AND ABUSE ISSUES</b>		
Luigi L. Romano and Arno Hazekamp	CANNABIS OIL – CHEMICAL EVALUATION OF AN UPCOMING CANNABIS-BASED MEDICINE	P2-15
Jennifer Johnston, Nicholas Lintzeris, David J. Allsop, Anastasia Suraev, Jessica Booth, Dean S. Carson, David Helliwell, Adam Winstock and Iain S. McGregor	LITHIUM CARBONATE IN THE MANAGEMENT OF CANNABIS WITHDRAWAL: A RANDOMIZED PLACEBO-CONTROLLED TRIAL IN AN INPATIENT SETTING	P2-16
Kim Crosby and Zach Walsh	CANNABIS USE AND PERPETRATION OF INTIMATE PARTNER VIOLENCE: THE MODERATING ROLE OF PROBLEMATIC ALCOHOL USE	P2-17
Brian F. Thomas, Richard C. Daw, Poonam G. Pande, Anderson O. Cox, Alex L. Kovach, Kenneth H. Davis, Jenny L. Wiley, Kenneth S. Rehder and Megan Grabenauer	WHAT GOES UP IN SMOKE: THE IDENTIFICATION AND PHARMACOLOGICAL CHARACTERIZATION OF SYNTHETIC CANNABINOID PYROLYSIS PRODUCTS FORMED DURING SMOKING	P2-18
Sonia Aroni, Claudia Sagheddu, Marco Pistis and Anna Lisa Muntoni	NEURONAL CIRCUITS UNDERLYING CANNABINOID WITHDRAWAL	P2-19

<p>Kiri L.Wills, Kiran Vemuri, Alana Kalmar, Cheryl L. Limebeer, Alexandros Makriyannis and Linda A. Parker</p>	<p>DELIVERY OF THE CBI ANTAGONIST, AM251, BILATERALLY TO THE CENTRAL NUCLEUS OF THE AMYGDALA AND ITS EFFECTS ON MORPHINE WITHDRAWAL</p>	<p>P2-20</p>
<p>Yong Liu, Ying Hu, Peng Zhong and Qing-song Liu</p>	<p>BDNF INTERACTS WITH ENDOCANNABINOIDS TO REGULATE COCAINE-INDUCED SYNAPTIC PLASTICITY IN MOUSE MIDBRAIN DOPAMINE NEURONS</p>	<p>P2-21</p>
<p>Regina Nelson</p>	<p>INTEGRAL FRAMES: INTERDISCIPLINARY TOOLS SUPPORTING CANNABIS RESEARCH</p>	<p>P2-22</p>
<p>Travis Grim, Jason Weibelhaus, Steve Negus and Aron Lichtman</p>	<p>C57BL6/J DEVELOP TOLERANCE TO CP55,940 IN INTRACRANIAL SELF- STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE</p>	<p>P2-23</p>
<p>Tasha Ahmad and Steven R. Laviolette</p>	<p>CANNABINOID CBI RECEPTOR TRANSMISSION IN THE BASOLATERAL AMYGDALA BI-DIRECTIONALLY CONTROLS THE MOTIVATIONAL PROPERTIES OF OPIATES VIA FUNCTIONAL EXCITATORY INPUTS TO THE NUCLEUS ACCUMBENS SHELL</p>	<p>P2-24</p>
<p>Kristin M. Gabella, Margaret S. Jones, Molly S. Crowe, Sara R. Nass and Steven G. Kinsey</p>	<p>RIMONABANT-PRECIPITATED <math>\Delta^9</math>-THC WITHDRAWAL ALTERS MARBLE BURYING AND STRUGGLING BEHAVIORS IN MICE</p>	<p>P2-25</p>
<p>Lena Bergström, Shima Momeni, Hanna Eriksson- Röhnisch and Erika Roman</p>	<p>FATTY ACID AMIDE HYDROLASE (FAAH) ACTIVITY IN DIFFERENT RAT BRAIN REGIONS AFTER TWO WEEKS TREATMENT WITH ETHANOL</p>	<p>P2-26</p>

## TOPIC G. METABOLISM

<p>Tania Muller, Stephanie Troy-Fioramonti, Laurent Demizieux, Joseph Gresti, Maria Bouam, Bruno Vergès and Pascal Degrace</p>	<p>ACTIVATION OF ADIPOSE TISSUE CANNABINOID RECEPTORS 1 (CB1R) ALTERS ANTILIPOLYTIC ACTION OF INSULIN AND INCREASES LIPOLYSIS IN MICE</p>	<p>P2-27</p>
<p>Stephanie Troy-Fioramonti, Laurent Demizieux, Joseph Gresti, Bruno Vergès and Pascal Degrace</p>	<p>PERIPHERAL ENDOCANNABINOID SYSTEM ACTIVATION INHIBITS INTESTINAL GLUCOSE ABSORPTION AND IMPROVES POSTPRANDIAL GLYCEMIA IN LEAN AND OBESE MICE</p>	<p>P2-28</p>
<p>Maria Scherma, Valentina Satta, Roberto Collu, Liana Fattore, Paola Fadda and Walter Fratta</p>	<p>PHARMACOLOGICAL MODULATION OF THE ENDOCANNABINOID SYSTEM IN A RODENT MODEL OF “ACTIVITY-BASED ANOREXIA”</p>	<p>P2-29</p>
<p>Oliver Linsell, Khaled Greish and John C. Ashton</p>	<p>SYNTHESIS AND CHARACTERIZATION OF A NANOMICELLE CANNABINOID FORMULATION</p>	<p>P2-30</p>
<p>Jessica Karlsson and Christopher J. Fowler</p>	<p>INHIBITION OF ENDOCANNABINOID METABOLISM BY THE METABOLITES OF IBUPROFEN AND FLURBIPROFEN</p>	<p>P2-31</p>
<p>Sandra Gouveia-Figueira, Jana Späth, Angela Zivkovic and Malin L. Nording</p>	<p>DIETARY EFFECTS ON CIRCULATING OXYLIPINS AND ENDOCANNABINOIDS</p>	<p>P2-32</p>
<p>Giovanna Morello, Roberta Imperatore, Fabiana Piscitelli, Daniela Sarnataro, Letizia Palomba, Luciano De Petrocellis, Luigia Cristino and Vincenzo Di Marzo</p>	<p>OREXIN-A ENHANCES 2-AG BIOSYNTHESIS VIA CB1/OX-1R HETEROMERS IN THE NEURONS OF THE MOUSE HYPOTHALAMIC ARCUATE NUCLEUS</p>	<p>P2-33</p>

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## ICRS2014 - PRESIDENTIAL PLENARY SPEAKER

### CANNABINOIDS REVISITED? NEW TARGETS, CHEMISTRY AND PLANT SOURCES

GIOVANNI APPENDINO, PH.D.

*Dipartimento di Scienze del Farmaco, Largo Donegani 2, 28100 Novara, Italy*

The pharmacological potential of Cannabis has long been reductively identified with the one of THC, its psychotropic constituent. While undoubtedly a major player for the medicinal exploitation of Cannabis, THC is not the only constituent of this plant having pharmacological potential, nor cannabinoids are the only class of bioactive compounds present in this plant. These issues are now well established, but less known is the fact that cannabinoids as well as compounds with cannabinoid activity are not unique to Cannabis.

Cannabinoids are the result of the biogenetic merging of a polyketide-derived alkylresorcinol and an isopropenyl residue. This leitmotiv is not uncommon in natural products, being documented in both lower (liverworts) and higher plants. In hemp cannabinoids, the isoprenyl residue is, with a single exception, of the monoterpene type, while the resorcinyl alkyl residue has five, or more rarely, a lower number of carbons. However, a large and diverse group of "cannabinoids" also occurs in South-African members of the genus *Helichrysum*. While cannabigerol (CBG) and its acidic precursor (pre-CBG) have both been isolated from *H. umbraculigerum* Less.,<sup>1</sup> most *Helichrysum* cannabinoids are derived from the phenethyl version of these compounds, where a phenyl ring replace the three terminal carbons of the classic pentyl chain of hemp cannabinoids. Surprisingly, the biological profile of these compounds is totally unknown, despite their close similarity to hemp cannabinoids and the use of some *Helichrysum* species to induce a trance state not unlike the one associated to the recreational use of hemp. The potential of *Helichrysum* cannabinoids to affect the metabotropic (CBs), ionotropic (TRPs) and trascription factors (PPARs) end-points of hemp cannabinoids will be discussed.

From a pharmacological standpoint, cannabinoids are compounds capable to interact with the metabotropic cannabinoid receptors CB<sub>1</sub> and CB<sub>2</sub>. While CB<sub>1</sub> is rather selective in terms of natural products ligands, CB<sub>2</sub> is more promiscuous, and can be modulated by different structural classes of natural products. The most surprisingly ligand of CB<sub>2</sub> is, undoubtedly, the sesquiterpene b-caryophyllene (**3**), the only hydrocarbon capable to bind in a specific and potent way a macromolecular biological end-point, and structure-activity relationships for this interaction will be discussed, highlighting the potential of the sesquiterpene framework to modify various end-points of endogenous eicosanoids. While b-caryophyllene is a major constituent of the essential oil from hemp, this plant also contains a host of bioactive and unique phenolics capable to interact with inflammatory targets and especially the enzymatic formation of eicosanoids (PGs, LTs). This inhibitory activity on the production of inflammatory eicosanoids complements the mimicry of regulatory eicosanoids (AEA, 2AG) typical of cannabinoids, qualifying Cannabis as a source of compounds capable to selectively modulate endolipid signaling and beneficially affect its pathological imbalance in several pathological settings.

1. Bohlmann, F.; Hoffmann, E. Cannabigerol-aenlich Verbindungen aus *Helichrysum umbraculigerum*. *Phytochemistry* **1979**, *18*, 1371-1374.

2014 PRESIDENTIAL PLENARY SPEAKER - SUNDAY, JUNE 29<sup>TH</sup> @ 15.00

# NIDA SYMPOSIUM

*TUESDAY, JULY 1<sup>ST</sup>*

## TRP CHANNELS: THE ONLY TR(I)P YOU CAN HAVE ON CANNABINOIDS?

CHAIR: VINCENZO DI MARZO

### **Pedro Grandes**

Medicine and Dentistry  
Basque Country University  
Leioa, Spain

### **Thomas Voets**

Laboratory of Ion Channel Research (LICR)  
Leuven, Belgium

### **Vincenzo Di Marzo**

Endocannabinoid Research Group  
Institute of Biomolecular Chemistry  
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Pozzuoli, Italy

# **ANATOMY AND FUNCTIONAL ROLE OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IN THE DENTATE GYRUS OF A KAINATE-INDUCED SEIZURES MOUSE MODEL**

Miren-Josune Canduela, Juan-Luis Mendizabal-Zubiaga, Amanda Sierra, Naiara Royo, Sara Peñasco, Leire Reguero, Nagore Puente and **Pedro Grandes**

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The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions where the channel has physiological roles. Nevertheless, the anatomical localization of TRPV1 is well established in the periphery, but it is a matter of debate in the brain. We have recently demonstrated a high TRPV1 localization at the dentate molecular perforant path synapses (Puente et al., 2014). However, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is not yet characterized anatomically.

To this goal, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were in dentate granule cell dendrites receiving symmetric synapses. The labelling was mostly confined to postsynaptic membranes and was distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 distribution pattern at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice.

We have also investigated the effect of an intrahippocampal injection of kainic acid (50 nl of 20 mM) on the trigger of kainate-induced seizures in WT and TRPV1-KO mice, a mouse model of medial temporal lobe epilepsy (MTLE). The behavioral score revealed that seizures were milder in TRPV1-KO than in WT during kainate-induced status epilepticus. Prior to kainate injection, a significant increase of cannabinoid 1 (CB1) receptor immunoreactivity in the inner 1/3 of the molecular layer and an increase of DAGL- $\alpha$  and NAPE-PLD optical densities were observed in dentate gyrus of TRPV1-KO versus WT. These findings may suggest that the absence of TRPV1 in the dentate gyrus triggers some adaptative changes that may be beneficial in the control of epileptic seizures.

Acknowledgements: Funding for P. Grandes' laboratory is provided by Ministerio de Economía y Competitividad (BFU2012-33334); The Basque Country Government grant IT764-13, and by Red de Trastornos Adictivos, RETICS, Instituto de Salud Carlos III, grant number: RD07/0001/2001. Funding for A. Sierra's laboratory is provided by Ministerio de Economía y Competitividad (BFU2012-32089).

# **MODULATION OF TEMPERATURE-SENSITIVE TRP CHANNELS**

**Thomas Voets**

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Thermosensing is initiated at sensory nerve endings, where alterations in temperature lead to swift changes in the gating of specific temperature-sensitive ion channels. This finally results in electrical signals that are conveyed to the CNS, where they are translated into a sense of temperature, ranging from ice cold to burning hot. Dysregulation of the thermal sensitivity of sensory neurons can lead to thermal allodynia, hyperalgesia and chronic pain. Temperature-sensitive ions therefore represent attractive targets for novel analgesic drugs.

In the last decades, temperature-sensitive Transient Receptor Potential (TRP) channels have been put forward as the main thermosensitive elements in sensory neurons. In this lecture, current insights into the molecular nature of TRP channels involved in cold and heat sensation will be discussed, and examples will be provided of the mechanistic basis and potential therapeutic implications of TRP channel modulation by chemical ligands.

# INTERACTIONS BETWEEN THE "ENDOCANNABINOIDOME" AND THE "TRPOME": A NEW "OME" FOR PHYTOCANNABINOIDS AND LIPID MEDIATORS?

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In 1999, it was proposed that anandamide, apart from CB1 and CB2 receptors, also activates transient receptor potential of vanilloid type-1 (TRPV1) channels. Over the last decade, this interaction has been shown to occur both in peripheral tissues and brain, during both physiological and pathological conditions. TRPV1, as well as other vanilloid-type TRP channels (namely TRPV2, TRPV3 and TRPV4), transient receptor potential of melastatin type-8 (TRPM8) channels and transient receptor potential of ankyrin type-1 (TRPA1) channels, are activated also by non-physiological high or low temperatures, and are thus grouped in that subfamily of the "TRPome" (including more than 60 members) known as "thermo-TRPs".

TRPV1 channels, can be activated also by another less abundant endocannabinoid, *N*-arachidonoyl-dopamine (NADA), and other unsaturated *N*-acyl-dopamines, and have been proposed by some authors to act as ionotropic endocannabinoid receptors. Furthermore, both anandamide and NADA antagonize TRPM8 channels. Recently, also 2-arachidonoylglycerol was found to exert activity at TRPV1, and we have confirmed this action, which, however, is exerted at concentrations higher than those required to anandamide to activate this channel. Unsaturated *N*-acyl-serotonins, which were previously known to act as inhibitors of fatty acid amide hydrolase, and hence as "indirect" CB1 and CB2 agonists, are instead *antagonists* of TRPV1 and were recently found to occur in animal tissues. Finally, several other members of the "endocannabinoidome", such as the anandamide congeners, oleoylethanolamide and palmitoylethanolamide, also activate TRPV1, whereas some *N*-acyl-taurines activate both TRPV1 and TRPV4. It is likely that also other members of the large family of endogenously occurring amides between fatty acids and amino acids or amine transmitters influence the activity of TRP channels.

Interestingly, although exhibiting in most cases very low affinity for, and functional activity at, CB1 and CB2 receptors, non-THC phytocannabinoids, such as cannabidiol, cannabigerol and cannabichromene, also interact at low micromolar or submicromolar concentrations with "thermo-TRPs", which mediate in part the pharmacological activity of these compounds. Thus, both the "endocannabinoidome" and the "phytocannabinoidome" overlap in part with the "TRPome".

I will discuss the latest discoveries on this subject from my and other laboratories and touch upon the complications of pharmacologically manipulating the endocannabinoid system in view of it being part of a wide "endocannabinoidome" with multiple signaling characteristics.

SPECIAL ICRS SPEAKER

OPTOGENETIC APPROACHES TO STUDYING  
REWARD AND SUBSTANCE ABUSE DISORDERS

ANTONELLO BONCI, PH.D.

*National Institutes of Health*

The ventral tegmental area (VTA), nucleus accumbens (NAC) and prefrontal cortex (PFC) are all part of the limbic system and play a fundamental role in motivation, reward- and drug-dependent behaviors. A few years ago, my laboratory has shown that drugs of abuse such as cocaine can produce long-term synaptic plasticity and that the duration of such plasticity is dependent upon the modality of drug or reward administration. By applying a multidisciplinary approach that includes electrophysiology, optogenetics and behavioral procedures, my laboratory has produced a series of studies aimed at defining the pathways that control and modulate reward and drug-dependent behaviors. During my presentation, I will present the latest data on the cellular mechanisms and pathways that underlie reward substance use disorders.

# STUDY OF THE EXPRESSION PROFILE AND PHARMACOLOGICAL ROLE OF THE CB1 RECEPTOR IN DUCHENNE MUSCULAR DYSTROPHY (DMD) MUSCLES: A NEW OPPORTUNITY TO REINFORCE MUSCLE REPAIR AND LOCOMOTOR ACTIVITY

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Duchenne muscular dystrophy (DMD) is a hereditary myopathy that causes the progressive degeneration of skeletal muscle tissue. It represents one of the most common forms of muscular dystrophy, which mainly affects young males, since it is determined by alterations present in the gene encoding for the structural protein dystrophin, which is on the X chromosome. The loss of dystrophin function causes irreversible muscle damage (1). Prognosis is still poor, given that death of affected patients occurs in most cases within 10-15 years of age due to respiratory failure.

We recently showed that in both murine and human skeletal muscle cells the stimulation of CB1 receptor impairs myotube formation and inversely increases the rate of myoblast proliferation (2). The purpose of this study was to investigate the potential implication of CB1 during DMD progression. Towards this aim, we examined CB1 expression and function in skeletal muscles of *Dmd<sup>mdx</sup>* mice, which represents the most utilized animal model of DMD (3). We found that in all the affected skeletal muscles investigated, among all the genes belonging to the endocannabinoid system, *Cnr1* and *Cnr2* showed the highest degree of up-regulation at weeks 5-6, the point of disease onset. Similar changes were observed for the *Pax7*, *Myod*, *Myf-5* genes. These genes are largely expressed in muscle satellite cells (SC) and serve to initiate and drive SC differentiation to replace injured muscle fibers (4). These changes were also detected in the muscles of children affected by DMD both at 3 and 7 years of age. Intriguingly, we found that CB1 stimulation by ACEA, anandamide and 2-AG (1-3  $\mu$ M) increased the proliferation rate of primary human SC. By bio-informatic analysis we found putative consensus sites for PAX7 in both mouse and human *Cnr1* gene. By means of a luciferase reporter assay we provide preliminary evidence that functional PAX7 binding sites are located proximal to, and hence may regulate the expression of, *Cnr1*. Importantly, treatment of *Dmd<sup>mdx</sup>* mice with the CB1 inverse agonist rimonabant (0.5mg/kg IP, 3 times a week for 3 weeks), resulted in a marked increase in locomotor activity as assessed by the rotarod assay. In conclusion, all these findings indicate a novel role for CB1 in the development of degenerative muscle disease, perhaps by affecting muscle differentiation and repair processes, thus making of this receptor a potential therapeutic target for the treatment of such disorders.

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## **PALMITOYLETHANOLAMIDE IN A RAT MODEL OF OSTEOARTHRITIS: ITS ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTIVENESS IN COMPARISON WITH NIMESULIDE AND ACETAMINOPHEN**

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Osteoarthritis (OA) is the most prevalent joint disease that reduced quality of life. Acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs) are employed for pain relief associated with OA. However, their prolonged use induces serious side effects. So the identification of alternative drugs is crucial for the OA pathology. Starting from previous preliminary data presented at 21<sup>st</sup> Annual Symposium on the Cannabinoids (Costa B., Russo D., Ronzulli D., and Comelli F.; Experimental osteoarthritis in rats is attenuated by oral administration of palmitoylethanolamide (2011) 21<sup>st</sup> Annual Symposium on the Cannabinoids International Cannabinoid Research Society, Research Triangle Park, NC, USA, Page 7), the aim of this present study is to confirm the anti-inflammatory, and antinociceptive efficacy of palmitoylethanolamide (PEA), an endogenous lipid analogous of the endocannabinoid anandamide, in the well known OA model induced by intrapatellar injection of MIA, and especially to compare its effect with that evoked by nimesulide and acetaminophen, in order to propose the OA treatment with PEA in clinic. PEA 50 mg/kg, nimesulide 10 mg/kg, and acetaminophen 300 mg/kg were orally administered for 21 consecutive days starting from the day after the pathology induction. As expected, rats developed a significant knee swelling, as index of inflammation. We observed that PEA was able to abolish knee swelling, as nimesulide, and acetaminophen treatment. Additionally, as further index of inflammation, the day after MIA-injection, rats developed a significant decrease in thermal withdrawal latency. Treatment of MIA rats with PEA resulted in a significant relief of thermal hyperalgesia, as observed after nimesulide, and acetaminophen administration. After MIA-injection, rats also developed mechanical allodynia, as an index of chronic pain. Treatment of MIA rats with PEA resulted in a significant, even if partial, relief of mechanical allodynia, as nimesulide, and acetaminophen treatment. However, PEA anti-allodynic effectiveness was major than that elicited by nimesulide, and acetaminophen. We also evaluated the motor functionality by a walking track analysis. In particular, according with the footprints, the SFI (Sciatic Functional Index) value was calculated: a value approximately around zero indicates a normal locomotor function, while a value close to -100 indicates a significant impairment of locomotor function. As expected, intra-articular injection of MIA resulted in a significant increase of joint discomfort. PEA treatment completely restored locomotor functionality and this effect remained stable after one week of treatment, as nimesulide and acetaminophen treatment. However, only PEA treatment preserved such an effect at the end of the treatment. In addition, we observed a mild/moderate cartilage damage after MIA injection (large chondral erosions, and exposure of subchondral bone). Repeated PEA treatment preserved cartilage from damage, conversely to repeated nimesulide, and acetaminophen treatment. OA patients show elevated levels of pro-algogen mediators such as the nerve growth factor (NGF) in their synovial fluid. Starting from these observations, we also determined the NGF levels in the synovial fluid of MIA rats. Only PEA treatment completely restored the physiological NGF level. In conclusion, we demonstrated that the repeated administration of PEA reduced knee swelling, mechanical allodynia, thermal hyperalgesia, motor impairment, and slowed the degradation of cartilage interposition in MIA-induced osteoarthritis model. PEA efficacy was superimposable, and in some cases greater than that evoked by nimesulide and acetaminophen, two of the most drugs used for OA treatment, so suggesting a therapeutic use of PEA in clinic, without causing side effect.

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## MECHANISMS OF HUMAN EOSINOPHIL MIGRATION INDUCED BY THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL

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**BACKGROUND.** Eosinophils are leukocytes involved in numerous inflammatory diseases such as asthma and possibly obesity. How they migrate to the tissues is not completely defined but is recognized to involve chemokines and/or bioactive lipids, noteworthy prostaglandin (PG) D<sub>2</sub> and 5-oxo-eicosatetraenoate (5-KETE). In mice, eosinophils might play an important role in adipose tissue maintenance. Although the chemoattractants recruiting eosinophils to the latter are unknown, it has been suggested that IL-5 might play a role. The endocannabinoid 2-arachidonoyl-glycerol (2-AG), found in the adipose tissue, activates eosinophils, is recognized to activate the cannabinoid receptors CB<sub>1</sub> and CB<sub>2</sub> and can regulate leukocyte functions through its metabolites. Herein, we investigated the mechanisms involved in the 2-AG-induced migration of human eosinophils.

**RESULTS.** IL-5 increased eosinophil migration induced by 2-AG from ~5% to ~40%. This was mimicked by IL-3 and GM-CSF but not by IL-13 or IFN- $\gamma$ . The effect of IL-5 was rapid and blocked by the Lyn inhibitor PP2. In contrast, IL-5 did not increase the migration of eosinophils induced by prostaglandin D<sub>2</sub> and 5-oxo-eicosatetraenoate (5-KETE). The effect of 2-AG on migration was prevented by the CB<sub>2</sub> antagonists AM-630 and SR144528, but was not mimicked by anandamide or other CB<sub>2</sub> agonists. 2-AG, but not anandamide, also induced the biosynthesis of leukotrienes and eoxins, which was totally blocked by the 2-AG hydrolysis inhibitors MAFP and JZL-184. Using inhibitors, we determined that the 2-AG-induced migration of eosinophils involved 2-AG hydrolysis and 15-lipoxygenase, in contrast to that mediated by 5-KETE. However, lipoxin A<sub>4</sub>, EXC<sub>4</sub>, 15-HETE, or 15-HETE-glycerol did not recapitulate the effect of 2-AG.

**CONCLUSIONS.** The 2-AG-induced migration of human eosinophils requires IL-5. The effects of 2-AG likely involve MAG lipase, unidentified 15-lipoxygenase metabolites, and the CB<sub>2</sub> receptor.

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## **PERIPHERAL TARGETING OF CB1 CANNABINOID RECEPTORS PROTECTS FROM RADIATION-INDUCED PULMONARY FIBROSIS**

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Radiation-induced pulmonary fibrosis (RIF) is a severe complication of thoracic radiotherapy which limits its dose, intensity, and duration. CB1 cannabinoid receptor-mediated signaling emerges as an active promoter of liver and skin fibrogenesis; however, there is no information if CB1 cannabinoid receptors are involved in pulmonary fibrotic diseases. Here we tested a hypothesis that CB1 cannabinoid receptors take active part in the onset and progression of pulmonary fibrogenesis using a mouse model of RIF.

Our mouse model of RIF consists of 20 Gy irradiation applied to thoracic area of C57Bl/6 mice with head, abdomen, and other parts of the body shielded from irradiation. C57Bl/6 mice are sensitive to irradiation and develop RIF and die between 15 and 19 weeks post-irradiation when such a dose of irradiation is applied to thoracic area. The treatment of C57 BL/6 mice with peripherally restricted CB1 antagonist AM6545 (1 mg/kg i.p., 3x/week) from the day animals received thoracic irradiation increased animal survival and significantly delayed the onset of RIF as confirmed by decreased upregulation of markers of fibrosis and inflammation and by decreased lung tissue collagen deposition and inflammation in comparison to solvent-treated controls. The use of global CB1 knockout mice generated on the same C57BL/6 background offered similar protection from RIF. Our study provides the first evidence that radiation-induced pulmonary fibrosis can be controlled through selective targeting of CB1-mediated signaling.

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## ENDOCANNABINOID AUGMENTATION BY SUBSTRATE-SELECTIVE COX-2 INHIBITORS: MECHANISM AND *IN VIVO* PROBE DEVELOPMENT

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The development of endocannabinoid (eCB) degradation inhibitors has significantly advanced the therapeutic potential of eCB signaling for a variety of pathological conditions. We and others have previously demonstrated that COX-2 regulates brain eCB levels through oxygenation of AEA and 2-AG. While COX-2 inhibitors increase eCBs levels, they also modulate arachidonic acid (AA) and prostaglandin (PG) levels. We have developed novel probes termed “substrate-selective” COX-2 inhibitors that selectively augment eCBs without inhibiting PG production.

Substrate-selective COX-2 inhibition arises from the fact that although COX-2 is a structural homodimer, it is a functional heterodimer. Kinetic analyses have revealed that substrate-selective COX-2 inhibitors bind in the allosteric COX-2 subunit and act as non-competitive inhibitors of eCB oxygenation in the catalytic subunit. We have found that mutation of Leu-531 to Ala results in the abrogation of substrate-selective COX-2 inhibition by (*R*)-flurbiprofen, lumiracoxib, and mefenamic acid. A crystal structure of lumiracoxib bound to murine L531A reveals that this lack of inhibition is not a result of altered inhibitor binding. Taken together, these findings indicate that binding of a substrate-selective inhibitor to the allosteric COX-2 subunit causes a rotation of Leu-531 in the catalytic COX-2 subunit such that a steric clash with the glycerol head group of 2-AG occurs, leading to non-competitive inhibition of 2-AG.

Previous studies have identified LM-4131 as an *in vivo* substrate-selective COX-2 inhibitor; however, it does not have optimal *in vivo* pharmacological properties. We screened a small library of promising *in vitro* substrate-selective COX-2 inhibitors and identified lumiracoxib as a potent *in vivo* substrate-selective COX-2 inhibitor. Lumiracoxib increases brain AEA and 2-AG in acute and chronic dosing regimens up to 8 hours after treatment, but also increases the levels of AA and 2-OG. Co-treatment of lumiracoxib with the MAGL inhibitor JZL-184 abolishes the increase in AA and 2-OG, suggesting that inhibition of COX-2 by lumiracoxib leads to a transient increase in 2-AG and subsequent hydrolysis by MAGL to AA. In addition, lumiracoxib is soluble in aqueous buffers and is orally bioavailable. The duration of action of lumiracoxib and the lack of PG inhibition in chronic studies identifies lumiracoxib as an improved *in vivo* substrate-selective COX-2 inhibitor for studying eCB augmentation by COX-2.

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## ROLE OF 5HT1A RECEPTORS ON THE NEUROPROTECTIVE AND NEUROBEHAVIORAL EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS

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**Background:** Blockade of 5HT1A receptors (5HT1AR) antagonizes the neuroprotective effects of cannabidiol (CBD) in the first 6 hours after a hypoxic-ischemic (HI) insult in newborn pigs (Pazos et al, Neuropharmacology 2013). The aim of the present work was to study whether or not 5HT1A blockade interferes with CBD neuroprotective and neurobehavioral effects in a longer period-72 h. **Methods:** 1 day-old piglets were studied for 72 h after a HI insult (carotid clamp and FiO<sub>2</sub> 10% for 20 min). Thirty min after HI piglets received vehicle (HV, n=8) or CBD 1 mg/kg single dose or in 3 repeated doses u.i.d (respectively, HC1, n=5; and HC3, n=6), alone or with the 5HT1AR antagonist WAY100630 1 mg/kg/12 h, 15 min before the corresponding CBD dose (respectively, HC1W, n=4; HC3W, n=4). Non HI piglets served as controls (SHM, n=4). Every 24 h brain activity was assessed by amplitude-integrated EEG (aEEG) and a neurobehavioral score was carried out including eating behaviour assessment. Object-related and social playfulness activity was assessed by video recording, and anxiety was quantified by the restless time during holding for aEEG recording. At the end of the experiment the brain was obtained. Proton magnetic resonance spectroscopy (H<sup>+</sup>-MRS) was made on frozen brain samples to assess neuroprotection by neuronal and astroglial damage quantification by lactate/n-acetyl aspartate (Lac/NAA) or myoinositol/creatine (ml/Cr) ratios, respectively. **Results:** CBD treatment restored brain activity (aEEG) and neurobehavioral performance 72 h postHI (Table). CBD blunted HI-increase of Lac/NAA and decrease of ml/Cr. In addition, CBD induced an anxiolytic effect and restored playfulness. There were no differences between HI piglets receiving single dose or three doses of CBD. CBD neuroprotective effects as shown by brain activity and motor performance and H<sup>+</sup>-MRS biomarkers were blunted by 5HT1AR antagonism in animals receiving CBD single dose but not in those receiving CBD for three days. CBD effects on anxiety and playfulness responses, however, were reversed by 5HT1AR antagonism in all CBD-treated animals no matter the dosage schedule. The 5HT1AR antagonist did not worsen HI effects on vehicle-treated animals and had no effects on sham animals.

Item	SHM	HV	HC1	HC3	HC1W	HCW3
aEEG (μV)	18(2)	14(1)	20(3)	19(2)	<b>12(3)</b>	18(2)
Lac/NAA	0.87(0.1)	2.17(0.4)	0.96(0.1)	1.1(0.1)	<b>2.3 0.5)</b>	1.1(0.1)
ml/Cr	1.6 (0.1)	1.3(0.1)	1.42(0.1)	1.42(0.1)	<b>1.2(0.1)</b>	1.4 (0.1)
NBS (pts)	35.5(0.5)	29(2)	35(1)	34.6(1)	<b>32.6(1)</b>	34.5(1)
Eating bhv (pts)	4.6(0.3)	3.5(0.4)	4.8(0.1)	4.8(0.2)	<b>3.1(0.3)</b>	4.4(0.1)
Restless (min)	3.5(1)	6.7(1)	2.2(1)	2.4(1)	<b>5.2(1)</b>	<b>6.3(1)</b>
Object play (%)	14(3)	17(8)	22(6)	24(8)	<b>10(2)</b>	<b>11(3)</b>
Social play (%)	52(6)	23(8)	40(6)	37(8)	<b>16(5)</b>	<b>15(6)</b>

Items measured 72 h postHI. *Italic:* p<0.05 vs. SHM. **Bold:** p<0.05 vs HC

**Conclusions:** 5HT1AR activation is involved in CBD neuroprotection in the first 24 hours postHI. Later on CBD is able to induce neuroprotection by 5HT1AR-independent mechanisms. 5HT1AR activation mediates anxiolytic and some behavioural effects of CBD.

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## **PROTECTIVE ROLE OF THE CANNABINOID RECEPTOR SYSTEM IN AN *IN-VITRO* MODEL OF AGE-RELATED MACULAR DEGENERATION (AMD)**

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Accumulation in the retinal pigment epithelium (RPE) cells of A2E, a pyridinium bis-retinoid, has the potential to cause RPE cell death and may contribute to the RPE cell atrophy that is observed in AMD. The cannabinoid receptor system is presented in human RPE cells and is intimately involved in the oxidative damage process, including that associated with AMD. It has been shown that levels of endocannabinoids are significantly increased in retinal tissue from AMD donors.

This study aimed to investigate the effects on pro-inflammatory agents and specific oxidative intracellular signalling related proteins- MAPKs, by *in vitro* AMD-A2E model, in the presence and absence of cannabinoids (HU-210 and HU-308), endocannabinoid (anandamide), non-psychoactive cannabinoid (CBD) and cannabinoid antagonists (SR-141716A [CB1] and SR-144528 [CB2]). By using this unique *in vitro* model, which includes A2E-loaded RPE cells exposed to blue light irradiation, we can mimic the oxidative stress taking place in AMD and leading to inactivation of the proteasome.

Experiments were conducted to investigate the ability of cannabinoids and endocannabinoids to attenuate the effect of A2E on the pro-inflammation agent's secretion and MAPKs pathway activation. When the RPE cells were exposed to A2E and blue light, anandamide and CBD, successfully and significantly, reduced the expression and secretion of IL-8 by deactivation of the p38 MAPK. Irradiated A2E-loaded RPE cells suppressed the expression and secretion of MCP-1, while anandamide, HU-210, HU-308 and CBD lead to up-regulation of MCP-1. A significant decrease in several MAPK signals was also determined in the AMD-A2E model, in the presence of the cannabinoids. The above signals were measured in presence and absence of the selective CB1 and CB2 antagonists for pathways clarification.

The results support our hypothesis that the newly discovered cannabinoid receptor system may attenuate AMD generated by the accumulation of A2E and may contribute to delaying or arresting A2E-related toxicity to the RPE. We assume that inactivation of the proteasome by A2E is a mechanistic link between oxidative stress and altered inflammatory responses. The down-regulation of MCP-1, under extensive oxidative stress by A2E, may have physiological consequences since it was reported that MCP-1 knockout mice developed AMD-like phenotypes. Therefore up-regulation of MCP-1 by the cannabinoids is suggested for neuroprotection in AMD.

## THE CANNABINOID-1 RECEPTOR IN CARDIAC FIBROSIS

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The endocannabinoid system is an emerging target in chronic cardiac diseases, owing to its involvement in fibrogenesis, inflammation and cell death. The deregulation of this system has been implicated in myocardial infarction and subsequent heart failure. We have shown previously that chronic CB<sub>1</sub> blockade is cardioprotective in cardiac injury models such as diabetes and doxorubicin toxicity. A recent study suggested that CB<sub>1</sub> antagonists improve cardiac function and reduces adverse remodeling after myocardial infarction, but the exact mechanism of these beneficial effects is still unknown. The aim of our study was to identify signaling pathways and investigate their involvement in the chronic effect of CB<sub>1</sub> inhibition in cardiac fibrosis and left ventricular remodeling.

In a mouse model of cardiac fibrosis, angiotensin II (AII, 3 mg/kg/day) was administered by osmotic minipumps for 14 days. CB<sub>1</sub> receptor antagonist (rimonabant, 5 mg/kg), or vehicle was given every second day during the AII administration period. At the end of 2 weeks, hemodynamic parameters were measured by non-invasive echocardiography. Cardiac pressure volume catheter was used to evaluate global functional parameters. Cardiac tissue was collected for histologic and biochemical evaluation. Two weeks of AII infusion significantly increased systolic pressure in all groups, irrespective of treatment. Although no difference in systolic parameters was found between the groups, cardiac dysfunction was shown by altered myocardial performance index, which was significantly prevented by CB<sub>1</sub> antagonist treatment ( $p < 0.01$ ). There was also a significant reduction ( $p < 0.05$ ) of the left ventricular mass in the group of mice treated with the CB<sub>1</sub> antagonist. Fibrosis, assessed by collagen deposition, was significantly reduced in the CB<sub>1</sub> antagonist group by one fold. Reduced fibrosis was confirmed by downregulation of pro-fibrotic genes e.g. CTGF and Colla1 in the same group. In vitro studies, using activated 3T3 cells, suggest that CTGF is downregulated after rimonabant treatment. Fibrogenic activation of primary fibroblast isolated from mice hearts induced extracellular matrix genes expression e.g. Colla1, CTGF and fibrillin, which was sensitive to CB<sub>1</sub> antagonist treatment.

In conclusions, we found that chronic CB<sub>1</sub> antagonist treatment in AII-induced mice preserved LV function and cardiac fibrosis was reduced with concomitant downregulation of fibrogenic genes. The study helps to better understand the anti-fibrotic action of chronic CB<sub>1</sub> treatment. Novel generation of CB<sub>1</sub> inhibitors, devoid of neuropsychiatric side-effects, may be therapeutically explored in chronic heart failure.

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## **THE ENDOCANNABINOID N-ARACHIDONOYL DOPAMINE (NADA) AND WIN55,212-2 MODULATE THE INFLAMMATORY ACTIVATION OF HUMAN ENDOTHELIAL CELLS**

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Recent reports have shown that cannabinoids possess immunomodulatory activity and suggest that they may be able to regulate the activation of the endothelium in response to inflammatory mediators. However, it remains unclear which receptors and what mechanisms are responsible for this modulation. Understanding the specific role of cannabinoids in endothelial cell (EC) activation is critical, since ECs are centrally involved in the pathogenesis of organ injury in acute inflammatory disorders. ECs express cytokines and chemokines, which facilitate the trafficking of leukocytes to organs, and can decrease vascular barrier function. We hypothesized that ECs express cannabinoid receptors and enzymes required for cannabinoid metabolism, and that cannabinoid signaling pathways modulate endothelial inflammatory responses. We find that primary human ECs from multiple organs express the cannabinoid receptors CB<sub>1</sub>R, GPR18, and GPR55, as well as the ion channel TRPV1. In contrast to leukocytes, CB<sub>2</sub>R is only minimally expressed in some EC populations. Furthermore, ECs express all of the known endocannabinoid (eCB) metabolic enzymes. Examining a diverse panel of cannabinoids, we also demonstrate that the synthetic cannabinoid WIN55,212-2 and the eCB *N*-arachidonoyl dopamine (NADA), but neither anandamide nor 2-arachidonoylglycerol, reduce EC inflammatory responses induced by bacterial lipopeptide, LPS, and TNF $\alpha$ . We find that endothelial CB<sub>1</sub> and CB<sub>2</sub> receptors are necessary for the effects of NADA, but not those of WIN55,212-2. Furthermore, TRPV1 appears to counter the anti-inflammatory properties of WIN55,212-2 and NADA, but conversely, in the absence of these cannabinoids, its inhibition exacerbates the inflammatory response in ECs activated with LPS. These data indicate that the eCB system can modulate the inflammatory activation of the endothelium and may have important implications for a variety of acute inflammatory disorders that are characterized by EC activation.

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**NOVEL INHIBITORS OF FATTY ACID AMIDE HYDROLASE EXERT  
REMARKABLE ANTI-INFLAMMATORY EFFECTS BOTH *IN VITRO*  
IN HUMAN KERATINOCYTES AND *IN VIVO* IN NC/TND MICE**

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High prevalence of skin diseases (e.g. atopic dermatitis [AD]), characterized by pathological cutaneous inflammatory processes, highlights the necessity of invention of novel, highly targeted therapeutic approaches, preferable possessing favorable side-effect profiles. It has recently been shown that the cutaneous endocannabinoid (eCB) tone is one of the key “gate-keepers” of the cutaneous inflammatory processes (Karsak et al, Science, 2007). Since fatty acid amide hydrolase (FAAH), a key enzyme involved in the degradation of the prototypic eCB anandamide, is an important regulator of this tone, in the current study, we intended to explore the potential anti-inflammatory activity of certain novel FAAH-inhibitors (compound -440 and -479) both *in vitro* in human epidermal keratinocytes (KC) and *in vivo* in NC/Tnd mice.

First, we confirmed that FAAH is indeed expressed in KCs. Moreover, quite intriguingly, we have also found that its expression was highly increased upon Toll-like receptor (TLR)-2, -3 or -4 activation, suggesting that its up-regulation and the subsequent decrease in the eCB tone might contribute to the pro-inflammatory action of the TLR-signaling. Next, by monitoring the expression of various pro-inflammatory target genes (interleukin [IL]-1 $\alpha$ , IL1 $\beta$ , IL6, IL8), whose expressional alterations specifically indicated the development of the cellular inflammatory response of KCs upon lipoteichoic acid (LTA; a TLR2-activator) treatments (24 hrs), we investigated the putative anti-inflammatory effects of the inhibition of FAAH. We found that co-administration of the novel FAAH-inhibitors was able to prevent pro-inflammatory action of LTA both at the mRNA and at the released protein levels (IL6 and -8). Since these effects were effectively abrogated by the co-antagonism of CB1 and CB2 cannabinoid receptors, it can be proposed that they were indeed mediated by the elevation of the cutaneous eCB tone. Finally, since both inhibitors showed negligible cytotoxic effects on KCs following long-term (72 hrs) administration, we tested their efficiency *in vivo* by using the well-known animal model of AD, the NC/Tnd mice. We found that both inhibitors improved clinical score and reduced ear thickness of these mice statistically significantly; moreover, compound -440 reduced scratching behavior as well.

Taken together, our *in vitro* and *in vivo* data strongly argue for that the investigated novel FAAH-inhibitors are potent anti-inflammatory agents by targeting the “very first-line” players (i.e. the epidermal keratinocytes) of the cutaneous inflammatory responses. Therefore, our findings may encourage one to systematically explore, next in appropriate clinical trials, their putative therapeutic efficiency in cutaneous inflammatory diseases such as e.g. AD.

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## STIMULATION OF BONE MASS BY A NOVEL METHYLATED OLEOYL SERINE DERIVATIVE

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Bone mass is determined by a continuous remodeling process, whereby the mineralized matrix is being removed by osteoclasts and subsequently replaced with newly formed bone tissue produced by osteoblasts. Several endogenous fatty acyl amides are present in bone and affect bone cells. Of these compounds, oleoyl serine (OS) was found to be the most potent anti-osteoporotic agent in both *in vitro* and *in vivo* models. OS can be rapidly hydrolyzed by amidases that limit its activity. Here we tested whether the OS activity can be enhanced by adding a methyl group adjacent to the amide group, thus hindering the enzyme action. Two such derivatives were tested, oleoyl  $\alpha$ -methyl serine (HU-671) and 2-methyl-oleoyl serine (HU-681), in which the added methyl group is on the respective serine and fatty acid part of OS. The results show that HU-671 stimulates the number of osteoblasts in culture with a peak effect at a concentration 10-fold lower than that of OS, whereas HU-681 had its peak activity at a concentration an order of magnitude higher than that of OS. OS and HU-671, inhibit *ex vivo* osteoclast formation peaking at  $10^{-11}$  and  $10^{-13}$  M, respectively, in a culture of bone marrow derived monocytes incubated with M-CSF and RANKL. When osteoclasts are grown on dentin slices, both compounds inhibit *ex vivo* bone resorption expressed as a reduction in the number of resorption pits. HU-681 affects neither osteoclastogenesis nor pit formation. As in the case of OS, the effects of HU-671 and HU-681 on osteoblasts are blocked by pertussis toxin and the MEK/Erk1,2 inhibitor PD98059, suggesting that all three compounds target a signaling pathway consisting of a Gi-protein coupled receptor and Erk1,2. In a mouse model for osteoporosis daily treatment for 6 weeks with OS or HU-671 completely rescues bone loss. The increase in bone density consists primarily of enhanced trabecular thickness. The most effective dose of HU-671 is 0.5 mg/kg/day, an order of magnitude lower compared to OS. The reversal of bone loss resulted from both increased bone formation and decreased bone resorption. These results, based on quantitative morphology, were confirmed by determination of the serum bone remodeling markers osteocalcin (bone formation) and type 1 collagen C-terminal crosslinks (bone resorption). Taken together, these data suggest that methylation interferes with amidase activity, thus enhancing the OS *in vitro* and *in vivo* effects by extending its availability to target cells. In addition, the present data provides a preclinical proof for further development HU-671-based anti-osteoporotic therapy. The potential advantages of such therapy are the concomitant bone anabolic and anti-resorptive activity. HU-671 has logP of 5.17, closely matching the gold standard for oral bioavailability.

## **DIACYLGLYCEROL LIPASE BETA: NEW EVIDENCE FOR INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE**

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Diacylglycerol lipase (DAGL), the enzyme responsible for generation of 2-arachidonylglycerol (2-AG), represents a particularly interesting component of the endogenous cannabinoid (endocannabinoid) system as a potential therapeutic target to treat pain. This enzyme also regulates formation of arachidonic acid in mouse peritoneal macrophages in response to inflammatory insults. As DAGL- $\beta$  inhibition leads to decreases in lipopolysaccharide (LPS)-induced prostaglandins (PGs) and proinflammatory cytokines, we hypothesized that inhibiting this enzyme will reverse nociceptive behavior in laboratory animal models of inflammatory and neuropathic pain. Both the inflammatory model of intraplantar LPS (2.5  $\mu$ g) and chronic constriction injury of the sciatic nerve (CCI) model of neuropathic pain produce robust increases in sensitivity to light mechanical touch, or allodynia, as assayed with the von Frey test, and increased thermal sensitivity, or thermal hyperalgesia, as assayed in the hotplate test. Both allodynia and thermal hyperalgesia are sensory-discriminative pain evoked behaviors, but do not encompass the affective-motivational aspect of pain, which is often seen clinically. Pain-depressed behavioral models may therefore be additionally beneficial in determining the therapeutic potential of experimental compounds for the treatment of pain. Here a form of pain-depressed behavior in mice, digging, was examined via the marble burying assay.

Both systemic and local intraplantar injection of the selective DAGL- $\beta$  inhibitor KT109 reversed LPS-induced allodynia in a time- and dose-dependent manner. As KT109 also has off target effects at ABHD6, we tested KT195, a compound structurally related to KT109 that selectively inhibits ABHD6 but is inactive against DAGL- $\beta$ . Importantly, systemic or intraplantar injection of KT195 had no effect on LPS-induced allodynia. Moreover, DAGL- $\beta$  knockout mice displayed significant reductions in LPS-induced allodynia. The anti-allodynic effects of intraplantar KT109 were verified as being local, and not systemic, as its intraplantar administration into the contralateral paw did not reduce LPS-induced hyperalgesia or allodynia. Interestingly, neither intrathecal nor intracerebroventricular injection of KT109 reversed LPS-induced allodynia, consistent with a peripheral site of action. Systemic KT109 (40 mg/kg) also reversed CCI-induced allodynia and thermal hyperalgesia. Experiments using transgenic mice indicated that these antinociceptive effects did not require CB1 or CB2 receptors. Systemically administered KT109 also restored CCI-depressed digging behavior, suggesting that inhibiting DAGL- $\beta$  also reverses the negative emotional component often associated with pain. Taken together, these findings suggest that DAGL- $\beta$  represents a provocative target to treat both inflammatory and neuropathic pain.

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## POSITIVE ALLOSTERIC MODULATION OF CB<sub>1</sub> WITH GAT211 SUPPRESSES PACLITAXEL-INDUCED NEUROPATHIC PAIN WHILE BYPASSING UNWANTED SIDE EFFECTS OF CB<sub>1</sub> RECEPTOR ACTIVATION

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Activation of cannabinoid CB<sub>1</sub> receptors suppresses pathological pain but also produces unwanted central side effects (e.g. psychoactivity) that constrain therapeutic dosing. We hypothesized that positive allosteric modulation of the CB<sub>1</sub> receptor would suppress neuropathic pain produced by chemotherapy treatment without producing cardinal signs of CB<sub>1</sub> receptor activation (i.e. hypothermia, catalepsy, hypoactivity and tail-flick antinociception). We evaluated a positive allosteric modulator of the CB<sub>1</sub> receptor, GAT211, for anti-allodynic efficacy in a model of chemotherapy-induced peripheral neuropathy produced by paclitaxel treatment. Responsiveness to mechanical and cold stimulation was assessed before, during and after establishment of paclitaxel-induced neuropathic pain. Anti-allodynic efficacy of GAT211 was assessed following acute and chronic dosing initiated during the maintenance phase of paclitaxel-induced allodynia. Effects of GAT211 on cardinal signs of CB<sub>1</sub> receptor activation were also evaluated in a modified tetrad. Acute treatment with GAT211 (20 mg/kg i.p.) failed to produce antinociception in the tail-flick test, catalepsy in the ring test, motor ataxia or hypothermia. Moreover, GAT211 did not alter basal nociceptive thresholds to mechanical or cold stimuli in the absence of paclitaxel. However, GAT211 suppressed paclitaxel-induced mechanical and cold allodynia following both acute and chronic administration. Therapeutic efficacy was preserved over a chronic dosing period of 8 days with no appreciable signs of tolerance to antinociceptive efficacy. By contrast, repeated dosing with the prototypic classical cannabinoid  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) produced tolerance to the antinociceptive effects of the cannabinoid over the same dosing interval. Finally, the CB<sub>1</sub> antagonist rimonabant (10 mg/kg i.p.) did not produce CB<sub>1</sub>-dependent withdrawal signs in mice treated chronically with GAT211. The results of these experiments suggest that positive allosteric modulation of the CB<sub>1</sub> receptor with GAT211 does not produce unwanted CNS side-effects commonly associated with CB<sub>1</sub> receptor activation. Our results also suggest that GAT211 remains efficacious in suppressing paclitaxel-induced allodynia following both acute and chronic dosing. Thus, positive allosteric modulation of CB<sub>1</sub> represents an alternate route for harnessing the therapeutic potential of the endocannabinoid signaling system to suppress neuropathic pain without unwanted CNS side effects. Allosteric modulation of CB<sub>1</sub> thus represents an analgesic strategy that may be exploited to bypass antinociceptive tolerance and detrimental CNS side-effects observed with prototypical cannabinoid agonists such as  $\Delta^9$ -THC.

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## **ANATOMY AND FUNCTIONAL ROLE OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IN THE DENTATE GYRUS OF A KAINATE-INDUCED SEIZURES MOUSE MODEL**

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The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions where the channel has physiological roles. Nevertheless, the anatomical localization of TRPV1 is well established in the periphery, but it is a matter of debate in the brain. We have recently demonstrated a high TRPV1 localization at the dentate molecular perforant path synapses (Puente et al., 2014). However, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is not yet characterized anatomically.

To this goal, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were in dentate granule cell dendrites receiving symmetric synapses. The labelling was mostly confined to postsynaptic membranes and was distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 distribution pattern at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice.

We have also investigated the effect of an intrahippocampal injection of kainic acid (50 nl of 20 mM) on the trigger of kainate-induced seizures in WT and TRPV1-KO mice, a mouse model of medial temporal lobe epilepsy (MTLE). The behavioral score revealed that seizures were milder in TRPV1-KO than in WT during kainate-induced status epilepticus. Prior to kainate injection, a significant increase of cannabinoid 1 (CB1) receptor immunoreactivity in the inner 1/3 of the molecular layer and an increase of DAGL- $\alpha$  and NAPE-PLD optical densities were observed in dentate gyrus of TRPV1-KO versus WT. These findings may suggest that the absence of TRPV1 in the dentate gyrus triggers some adaptative changes that may be beneficial in the control of epileptic seizures.

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## **OVEREXPRESSION OF TRPV1 IN HEK CELLS DRIVES DRAMATIC CHANGES IN BASAL ENDOCANNABINOIDS AND RELATED LIPIDS WHICH ARE POTENTIATED WITH STIMULATION BY CAPSAICIN**

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Use of expression systems such as TRPV1-transfected HEK cells are an important molecular tool to study a range of pharmacological and biochemical pathways. Our groups routinely use TPR-HEK expression systems to determine novel ligand activity as well as push our understanding of intracellular cascades with known TRP activators. One aspect of cellular signaling that has been somewhat left out of this equation is the potential bioactive lipid modulation with activation by TRPs. Here, to test the hypothesis that activation of TRPV1 will drive changes in the cellular lipidome, we treated TRPV1-transfected HEK cells (hereafter called TRPV1-HEKs) with 100nM capsaicin for 5 minutes and then quenched the reaction with 5 volumes of methanol, cells were scraped from the flask and the cell/methanol solution was spiked with 100pmols of D8NAGly and then put on ice for 2 hours. At two hours, the solution was vortexed and then centrifuged at 19,000 x g for 15 min at 24C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS. The same protocol was used to compare non-transfected HEK cells with TRPV1-HEKs.

Activation by capsaicin in TRPV1-transfected HEK cells produced a dramatic increase in 2-AG as well as Anandamide and a variety of additional structurally related lipids, however, no significant changes were measured in arachidonic acid. Release/production of 2-AG after TRPV1 activation suggests that this may be a mechanism of action for the synergistic interactions of TRPV1 and the Endocannabinoid system. In terms of overall lipidomics findings, one of the most striking differences were observed between the basal lipid levels of the WT HEK cells and the basal levels. Screening over 80 lipids between the two cell lines reveals that 34 distinct lipids in the screen were significantly different between the WT and the TRPV1-HEKs. Most notably, the levels of AEA and related N-acyl ethanolamines were significantly increased, whereas, the level of 2-AG, arachidonic acid and PGs were significantly decreased in the TPRV1-HEKs compared to the WTs. These data highlight new findings in the signaling pathways associated with TRPV1 activation as well as demonstrating that the overexpressing of TRPV1 alone is enough to dramatically change the lipidome of the cell.

## ANANDAMIDE REGULATES MATURATION AND FUNCTION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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Dendritic cells (DC) are the most important professional antigen-presenting cells of the immune system and regulate the balance between immunity and tolerance. They play a major role in innate immune responses to infection as well as in linking innate and adaptive immunity. They exist in two different states that reflect their functional activity: immature (immDC) and mature (matDC) dendritic cells. ImmDC have high phagocytic activity and express low levels of antigen-presenting molecules (HLA-DR) and co-stimulatory activation markers (CD80 and CD86); however matDC have a central role in T cell activation, due to low phagocytic activity and high expression levels of HLA-DR activation markers. Although DC possess a fully functional endocannabinoid system, yet no evidence for a modulatory role of anandamide (AEA) on DC maturation and activation has been reported.

ImmDC and matDC were obtained from peripheral blood monocytes and were first differentiated into immDC for 6 days with granulocyte-macrophage colony stimulator factor and interleukin-4, and then matured with LPS for 2 additional days in presence or absence of AEA. We found that AEA-treated immDCs showed a remarkable decrease in surface activation markers (HLA-DR, CD80 and CD86) and an increase in their phagocytic activity. Conversely, AEA-treated matDCs expressed significantly higher levels of such surface markers, with no significant effects on their phagocytic activity. Finally, matDC were also tested for their ability to activate T cells upon DC/T cells co-cultures and we found that T cells released reduced levels of tumor necrosis factor- $\alpha$  following co-culture with AEA-treated matDC. These findings demonstrate for the first time that AEA, on one hand is able to directly affect DC capacity of processing the antigens and of co-stimulating nearby T cells, on the other hand it also affects the inflammatory immune responses of those co-stimulated T cells, thus suggesting that this endocannabinoid might play a key role in the regulation of the “innate-adaptive immune axis”.

## LEARNING UNDER STRESS DIFFERENTIALLY AFFECTS CANNABINOID MODULATION OF SPATIAL MEMORY RETRIEVAL IN RATS

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Literature evidence shows that variation in environmental aversiveness differentially influences spatial memory processes in rats (Akirav et al., *Learn Mem.* 11 (2004) 188-195; Salehi et al., *Learn Mem.* 17 (2010) 522-530). We have previously demonstrated that cannabinoid effects on memory are dependent on the aversiveness of environmental context and on the level of stress at the time of drug administration and/or training (Campolongo et al., *Front Behav Neurosci.* 20 (2012) 11; Campolongo et al., *Neuropsychopharmacology.* 38 (2013) 1276-1286). Moreover, we also showed that glucocorticoids interact with the hippocampal endocannabinoid system in impairing contextual aversive memory retrieval (Atsak et al., *Proc Natl Acad Sci U S A.* 109 (2012) 3504-3509).

Here we investigated the role of the hippocampal endocannabinoid system on spatial memory retrieval in rats trained under two experimental conditions that differed with respect to their training associated stress levels. To this aim adult Sprague Dawley rats were trained in a Morris Water Maze task at two different water temperatures (19° C and 25° C) in order to elicit different levels of emotional arousal (Salehi et al., *Learn Mem.* 17 (2010) 522-530). To test cannabinoid effects on spatial memory retrieval, the synthetic cannabinoid agonist WIN55,212-2, the AEA hydrolysis inhibitor URB597 or the 2-AG hydrolysis inhibitor JZL184 were bilaterally infused into the hippocampus 1 hr before the retrieval (probe) trial in separate groups of animals. We found that WIN55,212-2 impaired memory retrieval only in rats trained under more stressful conditions (19° C). Such effect was blocked by administration of the CB1 antagonist AM251. Interestingly, URB597 did not alter spatial memory retrieval performances in any of the two experimental conditions. However, highly comparable with WIN55,212-2 effects, JZL184 impaired spatial memory retrieval only in rats trained under higher stressful conditions *via* an interaction with CB1 receptors. Consistently, rats trained under higher stress displayed an increase in hippocampal 2-AG levels, both after the training and probe trials, and alterations in CB1 affinity and in the activity of the main 2-AG degradative enzyme MAGL after the probe trial than rats trained under less stressful conditions. The present findings indicate that the hippocampal endocannabinoid system plays a key role in mediating emotional arousal effects on spatial memory retrieval, shedding light on the neurobiological mechanism involved in the differential impact of stress on memory processes.

## THE ROLE OF ENDOCANNABINOID SIGNALING IN MEDIATING EFFECTS OF SEVERAL STRESS SYSTEMS IN MEMORY CONSOLIDATION

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Glucocorticoid hormones enhance the consolidation of long-term memory of emotionally arousing experiences. Prior studies indicated that emotional arousal induces noradrenergic activation within the basolateral complex of the amygdala (BLA) and glucocorticoids affect memory by rapidly influencing this noradrenergic activity. However, the time frame of this influence is too rapid to be compatible with the genomic mechanism of glucocorticoids and thus possibly mediated by a nongenomic mechanism. Recent evidence showed that glucocorticoids interact with endocannabinoids, a retrograde messenger system in the brain, in regulating the stress response. Here, we examined whether the endocannabinoid system is involved in regulating glucocorticoid effects on the BLA noradrenergic arousal system in enhancing memory consolidation. We found that posttraining blockade of the cannabinoid receptor type 1 (CB1) by AM251 in the BLA prevents the inhibitory avoidance memory enhancement as induced by co-infused glucocorticoid receptor agonist RU 28362 or by membrane-impermeable glucocorticoid CORT-BSA or by corticotropin-releasing factor receptor activation (CRF). These findings demonstrate that CB1 receptor activation in the BLA is essential in mediating memory enhancement induced by several neurohormonal stress systems such as glucocorticoids and CRF. Accordingly, activation of CB1 receptors by WIN55,212-2 in the BLA results in an enhancement of inhibitory avoidance memory; however, this effects is blocked by the beta-adrenoceptor antagonist propranolol co-infused into the BLA, suggesting that endocannabinoids regulate the memory-modulatory effects of glucocorticoids via fast influences on the noradrenergic system within the BLA. These findings have important implications for understanding local network functions within the BLA in regulating the effects of several neurohormonal stress responsive systems on neural plasticity and memory consolidation.

## FUNCTIONAL CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM DURING ZEBRAFISH (*DANIO RERIO*) EMBRYONIC DEVELOPMENT

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Over the last two decades the endocannabinoid system (ECS) has been intensively studied, and considerable advancements have been made in the understanding of its physiological roles in different vertebrate phyla. Zebrafish (*Danio rerio*) represents a low-cost and powerful vertebrate organism for disease modeling and drug screening applications (1). Very little is known about the developmental role of the endocannabinoid system in this teleost species, even though there is evidence indicating that this lipid signaling pathway is evolutionarily conserved within zebrafish (2,3).

In order to get initial insights into the role of the ECS during zebrafish development we utilized qPCR analysis to track the expression of 14 genes including the cannabinoid receptors as well as anandamide and 2-AG metabolic enzymes at different embryonic stages. Interestingly, the gene expression analysis revealed different trends for the genes regulating these two endocannabinoids. While anandamide catabolic and metabolic enzymes showed a similar time-dependent increase, indicating no net change in anandamide levels, two 2-AG hydrolyzing enzymes (*mgll*, *ptgs2b*) displayed a time specific down-regulation during the hatching period. Mirroring the gene expression trends, LC-MS quantifications confirmed that while anandamide levels remained constant over the time periods examined, 2-AG levels had a significant time-dependent increase with a peak corresponding to the hatching period. To further understand the function of 2-AG during zebrafish development, we studied the *mgll* spatial expression profile in zebrafish larvae by whole mount *in situ* hybridization. From early somitogenesis (14 hour post fertilization, hpf) and on, *mgll* was seen in several regions of the forebrain and hindbrain including the optic tectum and retinal ganglion cells of the eye. Given the abundance of 2-AG during zebrafish development, we also performed morpholino knock-down experiments on *dagla* and *mgll* genes in order to gain insight into their respective roles in the developing brain. Analysis of the morphant phenotypes, revealed that *mgll* and *dagla* morpholinos resulted in an expansion and loss of the midline barrier, respectively, at the level of the midbrain hindbrain boundary associated with aberrant patterns of neuronal fasciculation and axon trajectories. Intriguingly, only the *mgll*-deprived embryos displayed a retinal loss of neuronal innervation/fasciculation, but both gene morphants showed a thinner optic chiasm with respect to the control.

Our study shows for the first time that a complete and redundant ECS is able to actively produce endocannabinoids during zebrafish development. Furthermore, we provide evidence of *mgll* gene distribution inside distinct areas of the embryo CNS underling a putative role of 2-AG during neurogenesis and brain patterning. Indeed, morpholino experiments suggest a possible role of this endocannabinoid acting as paracrine mediator for axonal pathfinding and fasciculation, and possible implications in optic development.

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## EFFECTS OF DELETIONS IN FAAH ON THE ENDOCANNABINOID AND WIDE-RANGING-RELATED LIPIDOME IN EIGHT REGIONS OF THE MOUSE BRAIN

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Endogenous cannabinoids 2-AG, AEA, and the AEA metabolite *N*-arachidonoyl glycine (NAGly) are all derivatives of arachidonic acid (AA), a polyunsaturated fatty acid (PUFA) that is also a substrate for the production of prostaglandins (PGs). The directionality of the relationship among these classes of AA-derived lipids is an important biochemical factor in how enzymes that regulate each of the classes of lipids affect the available pools of the others. Fatty acid amide hydrolase (FAAH) is hypothesized to be responsible for the majority of AEA hydrolysis in the brain. Even though FAAH is best known for its role in AEA hydrolysis, it can also hydrolyze other *N*-acyl amides, such as the *N*-acyl ethanolamines. Therefore, deleting FAAH may have effects on levels of other bioactive lipids that are both AA-derived as well as other classes of lipids derived from other fatty acids. This study aims to elucidate the effects of genetic deletion of FAAH on the *N*-acyl amide, 2-acyl glycerol, and PG lipidome in the mouse striatum, hippocampus, cerebellum, thalamus, cortex, hypothalamus, midbrain and brainstem. Groups of 6 FAAH knockout (KO) mice were each compared to 6 age and sex matched wild-type (WT) mice from the same C57 genetic background. Animals were sacrificed, brains were removed and targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS.

Results here highlight those of AEA, 2-AG, NAGly, and PGs; however, many differences were observed in other lipids in these FAAH KO mice. Replicating previous studies, levels of AEA were higher in FAAH KO mice across all eight brain regions. *N*-arachidonoyl serine was likewise increased, but only in the striatum, thalamus, and brainstem. Interestingly, 2-AG levels were *significantly reduced* with regional specificity in the cerebellum, thalamus, and cortex of FAAH KO mice. FAAH KO mice displayed lower levels of NAGly and *N*-arachidonoyl GABA in all brain regions, and levels of 6 additional *N*-arachidonoyl acyl amides were significantly lowered by FAAH deletion as well. However, in stark contrast to the data from MAGL deletion presented here last year, deleting FAAH had no impact on levels of PGE<sub>2</sub> or free AA. These data replicate and greatly extend the finding that deletion of FAAH drives changes in a range of AA metabolites and extends this to a wider range of *N*-acyl amides. This pattern of shifts in substrates and products appears largely unique to AA derivatives, as the majority of effects on the *N*-acyl amide lipidome of other fatty acid derivatives showed an increase in levels throughout. These data demonstrate that FAAH has differential effects on lipid biosynthetic and metabolic pathways that are dependent on the specific fatty acid derivatives that are interacting with the enzyme and that AA derivatives have a unique interplay with FAAH that drives a variety of different biochemical outcomes.

**NOVEL CB<sub>2</sub> SELECTIVE CANNABINOID-ORTHOQUINONES EFFECTIVE FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCER AND LACKING NON-TUMOR CELL TOXICITY**

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Triple-negative breast cancer is characterized by tumors that do not express estrogen receptors (ER), progesterone receptors (PR), or HER-2 receptors. This type of cancer does not respond to endocrine therapy or other available targeted agents so it represents an important clinical challenge. Consequently, there is an evident need to develop new therapeutic strategies for the management of this disease. Chemotherapy with its well-known side effects is currently used as systemic treatment for this cancer. It is why selective toxicity for cancer, but not normal cells, is crucial in the discovery of potent antitumor drugs.

In that context, new chromenopyrazolediones have been designed and synthesized as anticancer agents using the multibiological target concept that involves quinone cytotoxicity and cannabinoid antiproliferative properties. In radioligand competition binding experiments, these compounds showed to be fully selective CB<sub>2</sub> cannabinoid receptor ligands with affinity in the nanomolar range, with affinity for the CB<sub>1</sub> receptor higher than 40 μM which eliminates any side psychotropic effect derived from their activity at these receptors. Concerning their antitumor activity, they decreased cell proliferation in human a triple negative breast cancer cell line (MDA-MB-231) using MTT assays. An additional important fact is their lack of significant cytotoxicity against normal human mammary epithelial cells (HMEC). Further mechanistic studies allowed us to determine that these antitumor effects were mediated through activation of CB<sub>2</sub> receptors and through induction of oxidative stress. As confirmed by western blot analysis using active caspase-3 as biomarker, these compounds induced apoptosis in the aforementioned breast cancer cell line.

In summary, we have designed and synthesized a series of chromenopyrazolediones, with selectivity for the CB<sub>2</sub> receptor, which may serve for the development of new antitumoral therapies for the treatment of those forms of aggressive and hormone-independent breast cancer.

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## IDENTIFYING POTENTIAL UPSTREAM REGULATORS OF THE ENDOCANNABINOID SYSTEM IN PROSTATE CANCER

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*Introduction:* A high CB<sub>1</sub> receptor expression in prostate cancer (Pca) tumour tissue is associated with a poor disease outcome (Chung et al., Eur J Cancer 45 [2009] 174-182). There are a number of downstream mediators of CB<sub>1</sub> receptor signalling in Pca (review, see Guindon & Hohmann, Br J Pharmacol 163 [2011] 1447-63), but little is known about upstream regulators of this receptor. In the present study, we have used a Bayesian network analysis of a well-characterised Pca database to identify potential candidates.

*Method:* The database for the study contained immunoreactive scores for tumour and non-malignant tissue for the following parameters: CB<sub>1</sub> receptor (CB<sub>1</sub>R), FAAH epidermal growth factor receptor (EGFR) and its phosphorylated form (pEGFR), the related growth factor receptor ErbB2, the EGFR modulator LRIG1, platelet-derived growth factor receptor β (PDGFRβ), androgen receptor (AR), von Willebrand factor (vWf), endoglin, hyaluronan and mast cell densities. Bivariate correlation analysis indicated that the CB<sub>1</sub>R was significantly correlated with FAAH, pEGFR, ErbB2 and LRIG1, and so these were used for the Bayesian analyses, which were conducted using the max-min hill-climbing method to create Directed Acyclic Graphs. Bootstrap analyses were conducted to assess the degree of robustness of the findings.

*Results:* For the non-malignant samples, two Directed Acyclic Graphs were constructed, one with three variables (CB<sub>1</sub>R, pEGFR and LRIG1, n=263) and one with four variables (as above + FAAH, n=221). In both cases, directionality pEGFR → CB<sub>1</sub>R was seen. For the tumour samples (n=267-274 depending upon the number of variables used), the same directionality was seen, but only when FAAH was included in the analysis. An additional pathway ErbB2 → FAAH was also found in the tumour samples.

*Conclusion:* The data suggest that EGFR may be an upstream regulator of CB<sub>1</sub> receptors. Together with data from the literature using cultured cells (e.g. Mimeault et al. Prostate 56 [2003] 1-12) the present analyses can be used to propose a model whereby EGFR, in addition to its other cellular actions (EGFR is well established as a pathogenic factor in Pca), increases CB<sub>1</sub> receptor densities which then respond to local endocannabinoids to feedback inhibit EGFR signalling as well as producing their own signalling responses. In tumour tissue, however, the increased expression of endocannabinoid metabolic enzymes (such as FAAH and cyclooxygenase-2) reduces the levels of the endocannabinoids, thus weakening this feedback regulatory pathway and hence allowing deleterious EGFR-mediated overactivity to proceed unchecked.

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## BETA-ARRESTIN2 APPEARS TO MEDIATE THE ACTIVITY OF CANNABINOIDS IN FEMALE MICE IN A MANNER THAT DIFFERS FROM MALES

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Previous studies of sex differences in the responses to cannabinoids have found that generally cannabinoids produce the same effects in both sexes, but mostly minor differences in the degree of some effects exist. Many of the differences have been attributed to differences in rates of metabolism or in percent muscle mass or fat distribution between the sexes. We have found evidence that cannabinoid receptor signal transduction with respect to beta-arrestin2 differs between male and female mice and may underlie some of the observed sex differences.

We have previously reported that male beta-arrestin2 knock-out mice exhibited greater temperature depression and antinociceptive effects than wild-type mice treated with  $\Delta^9$ -tetrahydrocannabinol (THC)<sup>1</sup>. *In vitro* studies showed that THC stimulated more [<sup>35</sup>S]GTP $\gamma$ S binding to brain membranes from beta-arrestin2 knock-out mice than from wild-type<sup>2</sup>. Other agonists tested in males, including CP55940, methanandamide and O-1812, did not show any differences between those genotypes for any of these assays performed in male mice.

More recent studies in female mice have shown very different effects from what was seen in males. The antinociceptive (but not rectal temperature) effects of THC obtained in wild-type females were nearly absent in beta-arrestin2 knock-outs. Similar to male mice, the effects of CP55940 did not differ between the genotypes in female mice. Currently, *in vitro* assays to assess [3H]SR141716A and [<sup>35</sup>S]GTP $\gamma$ S binding to brain membranes from female mice are underway.

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## SIGNALLING PROFILE OF CRIP1a: G-PROTEIN ACTIVATION AND SIGNAL TRANSDUCTION

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**Background:** Cannabinoid receptors are a family of G-protein coupled receptors that are involved in a wide range of physiological functions and diseases. Key regulators unique to cannabinoid receptors are cannabinoid receptor interacting proteins (CRIPs). Among them, CRIP1a was found to decrease the constitutive activity of the cannabinoid type-1 receptor (CB1R). The aim of this study was to gain an understanding of how CRIP1a modulates agonist-induced CB1 receptor function.

**Methods:** CB1R agonists, WIN55,212-2 (WIN), CP55,940 (CP), anandamide (AEA) and 2-arachydonylglycerol (2-AG) were used to investigate changes in signalling in the presence or absence of CRIP1a. Changes in K<sup>+</sup> channel signalling were determined using a Membrane Potential Assay in AtT20-hCB1 cells inherently expressing CRIP1a. Changes in ERK1/2 phosphorylation and cAMP accumulation were determined using AlphaScreen assays in HEK293-hCB1±CRIP1a cells. Changes in G-protein activation were determined using BRET technology also in HEK293-hCB1±CRIP1a cells, transiently transfected with Rluc8- and Venus-tagged constructs.

**Results:** *K<sup>+</sup> channel activation:* CRIP1a protein knockdown was observed in cells treated with CRIP1a-siRNA (20nM), 48 to 72 hours post-transfection (p<0.001). This siRNA-induced CRIP1a knockdown significantly increased both AEA and 2-AG-induced K<sup>+</sup> channel activation (p < 0.05) whilst no change was observed in response to WIN and CP. *ERK1/2 phosphorylation and cAMP accumulation:* Downstream signalling studies found no significant difference in adenylyl cyclase or MAP kinase activity in response to WIN, CP, AEA and 2-AG in CRIP1a expressing HEK293-hCB1 cells compared with HEK293-hCB1 cells not expressing CRIP1a. *G-protein activation:* ERK1/2 phosphorylation assays, routinely used to evaluate ligand-induced Gi/o-mediated signalling, demonstrated that addition of Rluc8 to CB1 did not alter the potency or efficacy of G protein-coupling following activation by WIN and CP. The pEC50 values were 6.80±0.04 and 6.76±0.25 for WIN treatment and 6.97±0.12 and 6.83±0.09 for CP, for untagged and Rluc8-tagged CB1R respectively. BRET kinetic studies were used to show ability of the CB1R to signal via specific Gai proteins, including Gai1, 2 and 3, in response to WIN, CP, AEA and 2-AG. Ongoing BRET studies are currently looking at changes in agonist mediated Gi/o protein activation in the presence of overexpressed CRIP1a.

**Conclusions:** These results suggest that CRIP1a modulates CB1 receptor signalling in the ligand- and pathway-specific manner. Overall, this study provides a greater understanding of the biological link between CRIP1a and CB1 and improves our knowledge of how CB1 receptor activity can be selectively altered.

## MITOCHONDRIAL CANNABINOID RECEPTORS MEDIATE SPECIFIC EFFECTS OF CANNABINOIDS VIA SOLUBLE ADENYLYL CYCLASE (sAC)

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**INTRODUCTION:** Recent evidence indicates that the cannabinoid type-1 CB1 receptor is present at brain mitochondrial membranes (mtCB1), where it can control cellular respiration, energy production and synaptic plasticity (Benard et al. *Nature Neuroscience* 2012, 15(4):558-64 and Hebert-Chatelain et al., *Molecular Metabolism* - *in press*). Cannabinoids exert potential therapeutic effects (e.g. analgesia), accompanied by important side effects (e.g. catalepsy and amnesia), but the specific molecular mechanisms and the brain region(s) involved are poorly understood. In this study, we asked whether mtCB1 receptors are involved in specific effects of cannabinoids.

**METHODS:** Biochemical, pharmacological and genetic approaches, together with stereotactic drug and virus applications and anatomical studies were used to identify the brain region(s) and the molecular mechanisms involved in specific behavioral effects of cannabinoids

**RESULTS:** The lipid cell-penetrant antagonist AM251 injected into the substantia nigra reticulata (SNr) blocked cannabinoid-induced analgesic and cataleptic effects. However, a peptide non-cell penetrant antagonist (hemopressin) injected in the same brain region blocked only analgesic effects, suggesting the participation of intracellular CB1 receptors in sedative effects. By biochemical studies in the brain, we found that mtCB1 receptors regulate brain bioenergetic processes via inhibition of the mitochondrial soluble adenylyl cyclase (sAC). Interestingly, intra-SNr injections of the sAC inhibitor KH7 blocked the cataleptic, but not the analgesic effect of cannabinoids. Additionally, we generated a mutant CB1 protein (DN22-CB1) lacking mitochondrial localization and function *in vitro*. Electron microscopic expression analysis of viral hippocampal re-expression of wild-type CB1 or DN22-CB1 in CB1-KO mice confirmed the lack of mitochondrial localization of DN22-CB1 *in vivo*. Importantly, viral re-expression of wild-type CB1 rescued cannabinoid-induced memory impairment in an object recognition task in CB1-KO mice, whereas re-expression of DN22-CB1 did not.

**CONCLUSIONS:** Cannabinoids exert analgesia independently of mtCB1 signaling. However, cannabinoid-induced catalepsy and memory impairment require mtCB1 receptor signaling. Thus, the study of smtCB1 receptors signaling represent a novel way to dissect beneficial (analgesia) from undesired side-effects (catalepsy and memory impairment) of cannabinoids.

**MOLECULAR DYNAMICS STUDY OF A CB<sub>1</sub> ENDOGENOUS ALLOSTERIC MODULATOR, LIPOXIN A<sub>4</sub>: DYNAMIC BEHAVIOR IN A LIPID BILAYER AND ENTRANCE INTO THE CB<sub>1</sub> RECEPTOR**

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The CB<sub>1</sub> endogenous, positive allosteric modulator, lipoxin A<sub>4</sub>, increases the equilibrium binding and efficacy of CP55,940 and anandamide (orthosteric agonists). Interestingly, lipoxin A<sub>4</sub> contains a carboxyl group; our calculations strongly suggest that this carboxyl group is negatively charged at physiological pH. This negative charge makes lipoxin A<sub>4</sub> unique among the cannabinoids, as the majority of other CB<sub>1</sub> orthosteric and allosteric ligands (endogenous and synthetic) are electrostatically neutral. The purpose of this study was to use molecular dynamics (MD) simulations to investigate how this negative charge may affect lipoxin A<sub>4</sub>'s dynamic behavior in a lipid bilayer, as well as its mechanism of binding.

We have previously reported that orthosteric cannabinoids may enter the receptor from the lipid bilayer, between TMH6/7 (Hurst et al. JBC 285:17954 (2010)). However, this method of entry may depend on a ligand's ability to partition into the lipid bilayer and reside at the correct height in the bilayer. To investigate if individual cannabinoids partition differently into the lipid bilayer, we have performed MD simulations on lipoxin A<sub>4</sub>, 2-arachidonoylglycerol (2-AG), CP55,940, and ORG27569 in a fully hydrated POPC bilayer. Simulations were run using the GPU-accelerated PME (Particle Mesh Ewald) AMBER12 package. The NPT ensemble was used to maintain temperature and pressure (T=300K, P=1.0bar). The CHARMM22 protein force field with CMAP corrections and the CHARMM 36 lipid force field were used in this study. 2-AG, CP55,940, and ORG27569 all partitioned completely into the lipid bilayer, with their headgroups/polar moieties (on average) residing in the glycerol region of the bilayer. In contrast, the charged headgroup of lipoxin A<sub>4</sub> resided (on average) higher up in the bilayer in the phosphocholine region. Of all the ligands studied, only lipoxin A<sub>4</sub> showed a tendency to partially leave the membrane by extending its headgroup into water. These results suggest that lipoxin A<sub>4</sub> may be able to enter CB<sub>1</sub> from a different portal than TMH6/7.

To investigate whether this unique behavior in the lipid bilayer impacts receptor binding, we also conducted MD simulations of lipoxin A<sub>4</sub> in the presence of CB<sub>1</sub> (embedded in a POPC lipid bilayer). In these simulations, we observed lipoxin A<sub>4</sub> partially leave the bilayer to reach over TMH1 and interact with the EC-3 loop, as well as the N terminus. These results suggest that lipoxin A<sub>4</sub> may enter the CB<sub>1</sub> receptor from lipid at the level of the extracellular loops, a very different approach route than seen for 2-AG, for example (Hurst et al. JBC 285:17954 (2010)). Altogether, these results may inform ligand design in the pursuit of specific binding/signaling outcomes.

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**GENETIC RESCUE OF CB<sub>1</sub> RECEPTORS ON MEDIUM SPINY NEURONS  
PREVENTS LOSS OF EXCITATORY STRIATAL SYNAPSES BUT  
NOT MOTOR PHENOTYPE IN R6/2 MICE**

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Huntington's disease (HD) is caused by an expanded polyglutamine repeat in the huntintin (htt) protein that disrupts gene expression, protein function and neurotransmission in specific neuronal populations resulting in characteristic motor, cognitive and affective symptoms. Histopathological hallmarks observed in both HD patients and genetic mouse models include the down-regulation of striatal synaptic proteins and reduction of dendritic spines on medium spiny neurons (MSNs), both of which are thought to mediate the predominant behavioral deficiencies associated with this neurodegenerative disease.

The down-regulation of cannabinoid CB<sub>1</sub> receptors on MSN terminals of HD patients and mouse models represents an early event that is hypothesized to participate in disease pathogenesis, and CB<sub>1</sub> receptor signaling is known to play a crucial role in neuronal survival and connectivity. We developed a new genetic mouse line that allows for the cell-specific genetic rescue of CB<sub>1</sub> receptor expression in MSNs of R6/2 mice. Detailed histological and electrophysiological studies show that rescuing CB<sub>1</sub> receptor function selectively in MSNs fully rescues the loss of excitatory synaptic markers (synaptophysin, vGlut1, vGlut2, and PSD95), MSN dendritic spine density and sEPSCs in MSNs. Remarkably, despite restoration of excitatory striatal input, motor impairment persisted in these mice. This results emphasizes the concept that behaviour is dependent on many different circuits and their interactions, and that it is not possible to predict rescue (or not) of behaviour based on function of a subset of synapses onto MSNs.

We conclude that CB<sub>1</sub> receptor functionality on MSNs controls the loss of striatal excitatory synapses in the R6/2 mouse model of HD, and that this loss can be uncoupled from the motor phenotype. Our results provide a deeper molecular understanding of the therapeutic potential of and limitations of cannabinoid-based therapeutics for treating patients with HD.

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## FATTY ACID BINDING PROTEINS (FABPs) ARE INTRACELLULAR CARRIERS FOR $\Delta^9$ -TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD)

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THC and CBD are hydrophobic compounds that are insoluble in aqueous media. In the blood, lipoproteins and albumin serve as carriers. To date intracellular transporters have not been described for THC and CBD. In theory they would require a carrier to transverse the aqueous milieu, for example, to enter the nucleus for signaling and the endoplasmic reticulum for catabolism. Fatty acid binding proteins (FABPs) have been identified as intracellular transporters for the endocannabinoid anandamide (AEA) and other N-acylethanolamines. We describe here how FABPs can also serve as transporters for the cannabinoids.

Computational methods were initially employed to determine the likelihood of FABP5 being a carrier for cannabinoids. MD simulation showed that both THC and CBD displayed scores consistent with appreciable binding. This analysis showed tighter binding of CBD to FABP5 than THC with  $\Delta G_{\text{binding}} = -41.67 \pm 0.81$  and  $\Delta G_{\text{binding}} = -36.63.67 \pm 1.85$ , respectively. Furthermore, modeling shows that THC and CBD were accommodated in the FABP5 pocket, in a manner similar to the fatty acid palmitate.

Binding studies of THC and CBD using a fluorescent NBD-stearate displacement assay indicated that these two cannabinoids bind to FABP3, FABP5 and FABP7 with low micromolar affinities. Interestingly, THC and CBD bind the FABPs as well as a variety of other compounds including various known transport inhibitors.

We next determined if FABP5 is an intracellular transporter for THC and CBD. HeLa cells are a suitable cell-type to examine interactions of exogenous cannabinoids with FABPs and their effects upon endocannabinoid transport. Furthermore, HeLa cells, which lack FAAH were transfected with human FAAH and AEA uptake was subsequently examined. This assay measures AEA uptake that is coupled to AEA breakdown by FAAH and to intracellular transport by FABP5, the primary FABP in HeLa cells. Both THC and CBD inhibited AEA uptake, with CBD being slightly more efficacious. The greater potency of CBD as an inhibitor of AEA uptake mirrored its higher affinity (lower  $K_i$ ) for FABP5. The FABP5 knockdown (shRNA), as expected, inhibited AEA uptake and neither of these two cannabinoids had any further effect upon uptake in the knockdown cells. Neither CBD nor THC inhibited human FAAH activity in cell homogenates. In conclusion, our work indicates that FABPs are intracellular carriers for  $\Delta^9$ -tetrahydrocannabinol and cannabidiol. This may help explain the observations of others that CBD and THC raise endocannabinoid levels *in vivo*.

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## FUNCTIONAL SELECTIVITY IN CB1R PHARMACODYNAMICS

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**Introduction.** Functional selectivity is a relatively recent concept in the G-protein coupled receptor field which describes the ability of a range of agonists to activate different pathways with different efficacy or potency (Kenakin et al, 2011). This has produced a major paradigm shift away from the suggestion that the receptor has a single “active” state and towards the idea that subtly different conformations of the receptor produce different downstream signalling events.

**Aim.** To investigate a large range of pharmacodynamic parameters of the CB1R following activation by a range of structurally diverse ligands.

**Methods.** The CB1R endogenous ligands: anandamide, 2-arachidonoyl glycerol; classical partial agonists: THC, BAY59-3074; and classical full agonists: CP55940, WIN55212-2 were used to investigate functional selectivity in a range of signalling assays in both HEK and AtT20 cells transfected with hCB1 receptors. Assays studied included competition binding assays, G protein activation, inhibition of cAMP accumulation, GIRK channel activation, CB1R desensitization, arrestin recruitment and internalisation.

**Results and Discussion.** Differences in rank order of agonist potency and efficacy suggest ligand dependent functional selectivity for some pharmacodynamic parameters. Analysis of this data using an operational model shows a strong signalling bias of all ligands towards the inhibition of cAMP compared to other Gi-linked pathways, probably reflecting amplification within this signalling pathway. Ligand specific bias was observed for GIRK activation by 2AG, with this pathway being maximally activated at very low receptor occupancy, suggesting strong intrinsic efficacy. CP55,940, often considered a “classical” cannabinoid was particularly poor at desensitizing the receptor, yet highly efficacious in internalising the receptor, suggesting these pathways are not always strongly linked together.

Kenakin T (2011). Functional selectivity and biased receptor signaling. *J Pharmacol Exp Ther* **336**(2): 296-302.

## CANNABIS WITHDRAWAL IN ADULTS WITH MOOD DISORDERS

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Cannabis use is prevalent among adults with mood disorders, but little is known about the experience of cannabis withdrawal in this population. We collected data about cannabis use patterns and the experience of cannabis withdrawal from 51 adults (two-thirds men) with mood disorders (26 bipolar, 24 major depression, 1 unspecified), using the Marijuana Quit Questionnaire, a 176-item, semi-structured questionnaire. The index quit attempt and subsequent withdrawal period was defined as their “most difficult” (self-defined) cannabis quit attempt without formal treatment.

42.4% of participants experienced a cannabis withdrawal syndrome (DSM-5 criteria) at some point in their life, of whom 70.4% used cannabis in response to withdrawal symptoms. At the start of their index quit attempt, participants were a mean age of 28 years (range 10-55 years) and using cannabis at least weekly. The quit attempt lasted 14.5 [41.8] months (median 2 months, range 1 day to 35 years). During the quit attempt, 95.5% of subjects reported  $\geq 1$  withdrawal symptom (mean [SD] 9.5 [6.1], median 9.0): the most frequently reported being irritability (76.5%), increased cannabis craving (74.5%), feeling depressed (68.6%), and feeling anxious (68.6%). The number of withdrawal symptoms was positively correlated with greater frequency and amount of cannabis use. Withdrawal symptoms often prompted actions to relieve them, including increased use of alcohol (41.5%), tobacco (48.2%), and cannabis (33.3%). These findings suggest that cannabis withdrawal syndrome is common among adults with mood disorders and is experienced similarly to other individuals with mental illness (e.g., schizophrenia) and people without serious psychiatric comorbidity. As is seen in other populations, cannabis withdrawal often serves as negative reinforcement for relapse during a quit attempt and may prompt increased use of other psychoactive substances.

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## EFFECTS OF $\Delta^8$ -TETRAHYDROCANNABIVARIN ( $\Delta^8$ -THCV) ON APPETITIVE EFFECTS OF COCAINE AND NICOTINE IN RODENTS

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Growing evidence suggests that blockade of brain cannabinoid CB<sub>1</sub> receptors or activation of brain cannabinoid CB<sub>2</sub> receptors attenuates the rewarding effects of cocaine or other addictive drugs such as nicotine.  $\Delta^8$ -Tetrahydrocannabivarin ( $\Delta^8$ -THCV) is a synthetic analogue of the plant cannabinoid  $\Delta^9$ -tetrahydrocannabivarin ( $\Delta^9$ -THCV), which exhibits CB<sub>1</sub> receptor antagonist and CB<sub>2</sub> receptor agonist profiles. Thus, dual CB<sub>1</sub> receptor blockade and CB<sub>2</sub> receptor activation might produce an additive or synergistic therapeutic anti-reward effect.

To test this hypothesis, we observed the effects of  $\Delta^8$ -THCV on cocaine and on nicotine self-administration and other addiction-related behavior. We found that systemic administration of  $\Delta^8$ -THCV (3, 10, 20 mg/kg, i.p.) failed to alter intravenous cocaine self-administration in wild-type or CB<sub>2</sub> receptor-knockout mice, but dose-dependently inhibited intravenous nicotine self-administration in alcohol-preferring rats and wild-type mice. Co-administration of  $\Delta^8$ -THCV and AM630, a selective CB<sub>2</sub> receptor antagonist, blocked  $\Delta^8$ -THCV's action in alcohol-preferring rats, and genetic deletion of CB<sub>2</sub> receptors (in CB<sub>2</sub> receptor-knockout mice) partially attenuated  $\Delta^8$ -THCV's action on nicotine self-administration. Also, systemic administration of  $\Delta^8$ -THCV (0.3-3 mg/kg, i.p.) inhibited nicotine-induced conditioned place preference and nicotine-seeking behavior in wild-type mice during extinction in the absence of nicotine and nicotine-associated cues. Further, CB<sub>2</sub> receptor-knockout mice show significantly lower levels of nicotine self-administration with longer inter-infusion intervals than their wild-type littermates, an effect similar to drug-taking behavior maintained by a higher dose of nicotine. Taken together, these findings suggest that: 1)  $\Delta^8$ -THCV may have anti-nicotine, but not anti-cocaine, therapeutic effects – partially mediated by activation of CB<sub>2</sub> receptors; and 2) deletion of CB<sub>2</sub> receptors appears to enhance nicotine's rewarding effects. Further studies are required to confirm these findings.

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## EFFECT OF ROOM VENTILATION ON THE PHARMACODYNAMIC AND PHARMACOKINETIC EFFECTS OF SECONDHAND CANNABIS SMOKE EXPOSURE

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There has been little controlled research conducted on the effects of exposure to secondhand cannabis smoke. Most research has focused on detection of cannabinoids in biological matrices; assessment of the subjective and physiologic effects of secondhand cannabis exposure is rare. Since seminal work conducted in the 1980s, technology for toxicology testing has evolved considerably and the average potency of “street” cannabis has increased more than 3-fold.

The present study was conducted to evaluate the pharmacodynamic and pharmacokinetic effects of secondhand cannabis exposure. Across 3 experimental sessions, cannabis potency (approximately 5% THC and 10-12% THC) and room ventilation (standard HVAC ventilation of 11 air changes per hour vs. unventilated) were studied. Six adult cannabis users and 6 drug-free adults who agreed to secondhand smoke exposure completed each session. Cannabis exposure occurred in a 910 cu.ft. plexiglass chamber. Seating position alternated between cannabis smokers and non-smokers, and smokers were allowed to smoke cannabis *ad libitum* for 60-minutes. Biological specimens and measures of subjective, cardiovascular, and cognitive performance effects [psychomotor ability (DSST), working memory (PASAT), divided attention] were obtained outside the exposure chamber at baseline and for up to 34 hours post-exposure. Here we present outcomes from non-smokers in the ventilated and unventilated test sessions during which cannabis containing 10-12% THC was smoked.

Active smokers consumed a considerable amount of cannabis in both ventilated (16.5 grams total) and unventilated (14.4 grams total) sessions. During the ventilated test session, secondhand smoke exposure did not affect subjective ratings of “drug effect”, heart rate, blood pressure, or divided attention. Psychomotor ability (DSST number correct) and working memory (PASAT total correct) improved in the first hour post-exposure relative to baseline (likely indicating a learning effect). THC was detected in whole blood by highly sensitive methods (LC/MS/MS) immediately following exposure in 4 of 6 non-smokers (min/max = 0.7/0.9 ng/mL), but was not detectable 30 minutes post-exposure. The metabolite, THCCOOH, was detected immediately in 2 of 6 individuals (min/max = 0.6/0.7 ng/mL), and one individual had detectable levels in blood 30 minutes post-exposure. During the unventilated exposure session, secondhand cannabis smoke did not affect heart rate, blood pressure, or cognitive performance. Subjective ratings of “drug effect” increased immediately post-exposure [mean ( $\pm$ SD) score of 23 (13) on a 100-pt VAS item], and remained above zero for an average of 1.8 hours. THC was detected in whole blood immediately following exposure (min/max = 1.2/5.6 ng/mL) and for an additional hour in all participants, and remained detectable 3 hours post-exposure for one individual. THC-COOH was detected in 5 of 6 individuals, peaked 30 minutes after exposure (min/max = 2.1/5.1 ng/mL), and remained detectable for up to 22 hours post exposure in 3 individuals. Detection of cannabinoids in urine and oral fluid similarly varied as a function of room ventilation and assay sensitivity.

Study results indicate that absorption of cannabinoids may occur during extreme secondhand exposure to cannabis smoke. Room ventilation had a significant impact on the degree of exposure and resultant pharmacodynamic effects. Though participants in the unventilated condition reported low to moderate levels of subjective intoxication, impairment was not observed on the cognitive domains assessed. Whether or not cannabinoids would be detected in biological specimens obtained for drug testing from individuals passively exposed to cannabis smoke will depend on the characteristics of the matrix (blood, urine, oral fluid, hair) and the sensitivity and specificity of the methodology (screening and confirmation cutoffs used and whether target of analysis is THC or THC-COOH).

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**ADVERSE PEER-EXPERIENCES THROUGHOUT ADOLESCENCE IN MALE RATS  
PERSISTENTLY ALTER ETHANOL INTAKE AND ENDOCANNABINOID SIGNALING IN  
LATER LIFE**

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Peer-interactions become particularly important during adolescence and hence, human teenagers display enhanced sensitivity towards social rejection, which might contribute to the development of neuropsychiatric disorders. Neurobiological consequences of social rejection during adolescence are have not yet been well investigated, and no appropriate animal model is available. Here, we studied the potential adverse consequences of inadequate social encounters in adolescent rats, which we propose as an operational model for adolescent peer-rejection. Male adolescent Wistar rats were either reared with other Wistar animals (control), or with age-matched rats from the less playful Fischer344 strain (inadequate social rearing; play-deprived). From day 65 on, all Wistar rats were again group-housed with same strain partners. Voluntary ethanol intake was assessed in Wistar animals throughout adolescence and in adulthood. In addition, levels of the endocannabinoids anadamide (AEA) and 2-arachidonylglycerol (2-AG), as well as protein levels of the CB1 receptor were measured in adult Wistar animals in various brain regions. Animals reared with inadequate social partners showed increased ethanol intake during adolescence as compared to controls, a feature which persisted into adulthood. The two groups exhibited differences in the expression levels of the CB1 receptor and concentration of endocannabinoids, which were most pronounced in the amygdala and the nucleus accumbens. In conclusion, adverse social experiences during adolescence result in distinct acute and persistent behavioral and neurobiological alterations which promote ethanol intake.

## NALTREXONE MAINTENANCE REDUCES CANNABIS SELF-ADMINISTRATION IN CANNABIS SMOKERS

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**Background:** Currently, approximately 24% of patients entering treatment for substance use disorders have a diagnosis of cannabis use disorder (CUD), yet their treatment outcome is poor, with a small minority of patients achieving continued abstinence following clinical treatment. There is a clear need to improve treatment outcome for CUD and pharmacological options are one important strategy to investigate. In light of the unavailability of cannabinoid receptor antagonists for clinical study, opioid receptor antagonists offer an indirect approach to reducing cannabis' positive subjective and reinforcing effects. Preclinical studies show that opioid antagonists reduce both the discriminative stimulus and reinforcing effects of CB1 receptor agonists, suggesting that opioid receptor antagonists could reduce cannabis abuse liability. However, in daily cannabis smokers, acute pretreatment with the opioid antagonist, naltrexone (12, 25, 50, 100 mg), *increased* rather than attenuate the positive subjective effects of cannabis (Cooper and Haney, 2010). Given that chronic antagonist administration can produce different effects on drug intoxication than acute antagonist pretreatment, this placebo-controlled, human laboratory study assessed the effects of active and inactive cannabis before, during and after chronic NTX administration.

**Method:** Non-treatment-seeking, cannabis smokers were randomized to receive NTX (50 mg) or placebo (0 mg) for 16 consecutive days. Each participant completed 10 laboratory sessions over 3-4 weeks: before NTX administration, after a single NTX administration, after 1 and 2 weeks of daily NTX administration, and 1 week after termination of NTX administration. At each timepoint, the reinforcing, subjective, psychomotor, and cardiovascular effects of active (5.5% THC) and inactive (0.0%) cannabis were assessed. Medication compliance was ensured by: observed capsule administration  $\geq 4$  times/week, plasma naltrexone measurement, and urine riboflavin levels. Cannabis self-administration was measured by having participants choose to pay \$1.00 for individual puffs of cannabis (0-3 puffs/session).

**Results:** Forty-nine participants, receiving either placebo (n=26M; 2F) or NTX (n=18M; 5F), completed the study. Demographic variables were comparable across the two groups, with participants reporting to smoke an average of 6 cannabis cigarettes/day, 6 days/week. The number of participants who failed to complete the study was also comparable across the 2 groups (placebo: n=8; NTX: n=9). In terms of outcome, maintenance on NTX significantly reduced cannabis self-administration compared to placebo. Those maintained on placebo had 7.6 (95% CI: 1.1, 51.8) times the odds of buying at least 1 puff of active cannabis compared to those receiving NTX. Rates of inactive cannabis self-administration were low and unaffected by NTX maintenance condition. NTX also reduced the positive subjective effects (e.g., ratings of 'Good Effect' and 'Friendly') of active cannabis relative to placebo. NTX did not significantly alter cannabis's cardiovascular effects.

**Discussion:** These results show that chronic naltrexone administration decreased active cannabis's reinforcing and subjective effects. These data suggest that clinical studies among patients motivated to reduce their cannabis use are warranted to determine if NTX may have use for the treatment of cannabis dependence.

This research was supported by US National Institute on Drug Abuse Grant DA19239 and DA09236

## SOCIAL REWARD IS MEDIATED BY INTERACTING OPIOID AND CB1 CANNABINOID RECEPTORS IN THE NUCLEUS ACCUMBENS CORE IN ADOLESCENT RATS

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Social play is an essential form of social interaction crucial for proper development of physical, cognitive and social capacities of young mammals, it is highly rewarding and it is modulated by interactions between the endocannabinoid anandamide and endogenous opioids (Trezza et al., *Trends Pharmacol. Sci.* 31 (2010) 463-469). The principal endocannabinoid 2-arachidonoylglycerol (2-AG) regulates emotional behaviors in rodents (Mulvihill and Nomura, *Life Sci.* 92 (2013) 492-497). However, its contribution in social play and the neural substrates underlying opioid-cannabinoid interactions in the modulation of this behavior remain unknown. To address this aim, we tested the effects of systemic administration of the 2-AG hydrolysis inhibitor JZL184, which prolongs the effects of locally released 2-AG (Long et al., *Nat. Chem. Biol.* 5 (2009) 37-44) in social play behavior.

Systemic administration of the 2-AG hydrolysis inhibitor JZL184 (1 mg/kg; i.p.) increased social play behavior in adolescent rats. These effects were blocked by systemic pre-treatment with either CB1 cannabinoid receptor antagonist SR141617A (0.1 mg/kg; i.p.) or the opioid receptor antagonist naloxone (1 mg/kg; s.c.). Thus, increasing 2-AG levels facilitates social play through CB1 cannabinoid and opioid receptor signaling.

Our previous studies demonstrated that anandamide and endogenous opioids act within the nucleus accumbens core (NAcc) to modulate social play behavior (Trezza et al., *J. Neurosci.* 31 (2011) 6362-6370; Trezza et al., *J. Neurosci.* 32 (2012) 14899-14908). Thus, we hypothesized that this brain region also underlies the 2-AG modulation of social play in adolescent rats. In line with this hypothesis, intra-NAcc infusion of SR141716A (3 µg/0.3 µl) antagonized the enhancement of social play induced by systemic administration of JZL184 (1 mg/kg; i.p.). Interestingly, intra-NAcc infusion of naloxone (0.5 µg/0.3 µl) also counteracted the increase in social play induced by systemic treatment with JZL184 (1 mg/kg; i.p.). Thus, stimulation of CB1 cannabinoid and opioid receptors within the NAcc is necessary and sufficient for 2-AG to modulate social play. Likewise, social play enhancement by systemic treatment with the opioid agonist morphine (1 mg/kg; s.c.) was prevented by intra-NAcc infusion of SR141716 (3 µg/0.3 µl).

Altogether, these data show that opioid and CB1 cannabinoid receptors crosstalk in the NAcc underlies cannabinoid and opioid stimulation of social play behavior in adolescent rats, extending previous findings indicating complex functional interactions between these two receptors.

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## **CANNABIS IN MEDICINE: A NATIONAL EDUCATIONAL NEEDS ASSESSMENT AMONG CANADIAN PHYSICIANS**

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The study was conducted to determine the educational needs of Canadian physicians regarding cannabis for therapeutic purposes (CTP). A national needs assessment survey was developed based on previous survey tools. The survey was approved by the Research Ethics Board of the McGill University Health Centre Research Institute and was provided online using LimeSurvey. Several national physician organizations and medical education organizations informed their members of the survey. The target audience was Canadian physicians. We sought to identify and rank using 5-point Likert scales the most common factors involved in decision making about using CTP in the following categories: knowledge, experience, attitudes, and barriers. Preferred educational approaches and physician demographics were collected. Gap analysis was conducted to determine the magnitude and importance of differences between perceived and desired knowledge on all decision factors.

Four hundred and twenty six responses were received, and physician responses were distributed across Canada consistent with national physician distribution. The most commonly identified factors influencing CTP concerned potential risks of using cannabis for medical purposes (4.23/5) and safety, warning signs and precautions for patients using medical cannabis (4.21/5). The largest gap between perceived current and desired knowledge levels was identified for dosing and the development of treatment plans (average gap=1.78).

We have identified several key educational needs among Canadian physicians regarding CTP. These data can be used to develop resources and educational programs to support clinicians in this area, as well as to guide further research to inform these gaps.

## ACTIVITY OF SYNTHETIC CANNABINOID DRUGS OF ABUSE AT G PROTEIN COUPLED CB RECEPTORS AND CANNABINOID-SENSITIVE ION CHANNEL

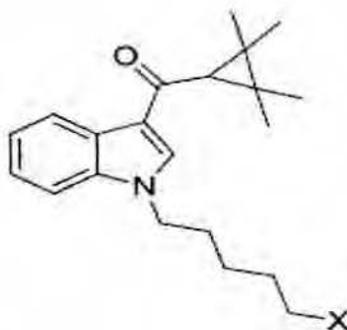
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The recreational use of synthetic cannabinoids (SCs) continues to present major health and regulatory concerns. Legal restrictions on the original SCs has led to the rapid chemical evolution, with the identity of available compounds constantly changing and their pharmacological properties essentially unknown. With the instances of hospitalization and death due to the misuse of these compounds on the rise, it is important to gain knowledge on the reactivity and toxicology of these compounds. We are establishing the pharmacological profile of SCs by using high throughput membrane potential assays for hCB1 and hCB2 activation of K channels in AtT-20 cells, as well as calcium elevation assays for TRPV1 and TRPA1 activity in HEK-293 cells. Here we focus on the SCs UR-144, fluorinated XLR-11, and 5-hydroxypentyl metabolite of XLR (SB5035), and compare their activity to  $\Delta^9$ -THC. All three drugs were agonists at hCB1 with pEC<sub>50</sub> values of  $6.3 \pm 0.05$ ,  $6.9 \pm 0.05$ , and  $5.5 \pm 0.3$  respectively. They were more potent agonists at CB2 with pEC<sub>50</sub> values of  $7.1 \pm 0.05$ ,  $8 \pm 0.15$ , and  $\sim 8.7$  respectively. When compared to  $\Delta^9$ -THC effects (pEC<sub>50</sub> value at hCB1  $7.3 \pm 0.05$  and CB2  $> 10\mu\text{M}$ ),  $\Delta^9$ -THC is more potent and efficacious at CB1 whereas the synthetics are CB2 preferring. We tested the SCs on the related TRP channels, TRPA1 and TRPV1, both of which are targets for cannabinoids. For the TRPA1 experiments, all data was compared to the effects of maximally effective concentration of cinnamaldehyde (300 $\mu\text{M}$ ). UR-144 (30  $\mu\text{M}$ ) activated TRPA1 to  $38 \pm 4\%$  of the cinnamaldehyde response. XLR-11 (pEC<sub>50</sub>  $5.1 \pm 0.05$ ) and SB5035 (pEC<sub>50</sub>  $4.95 \pm 0.02$ ) had similar efficacies to cinnamaldehyde. Both XLR-11 and SB5035 were more efficacious than  $\Delta^9$ -THC (pEC<sub>50</sub>  $5.0 \pm 0.3$ , max  $75 \pm 20\%$  of cinnamaldehyde). None of the SCs were TRPV1 agonists. Surprisingly, these new SCs are significantly less potent than  $\Delta^9$ -THC in at least one assay of hCB1 function, they are CB2 preferring, and they are relatively potent and efficacious agonists at the noxious chemical sensing ion channel TRPA1. TRPA1 activity could conceivably mediate some of the toxicity of SCs.

UR-144; X= H

XLR-11; X= F SB5035; X = OH



## EFFECTS OF CANNABIS ON THE SUBJECTIVE-EFFECT RATINGS AND PHARMACOKINETICS OF SMOKED COCAINE

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Despite the prevalence of concurrent cannabis and cocaine use, there is a paucity of controlled data addressing the interaction between these two drugs. The current study sought to establish the behavioral effects and pharmacokinetic interactions of cocaine and cannabis when smoked conjunctively. Nontreatment-seeking cocaine and cannabis smokers were recruited to participate in this within-subject, double-blind, inpatient study. Across six laboratory sessions, participants smoked inactive or active cannabis (0.0 or 5.6% THC) followed by four doses of 0, 12, or 25 mg of smoked cocaine separated by 14 minutes intervals. Throughout each session, subjective drug-effect ratings were measured and blood was drawn for pharmacokinetic analysis of plasma levels of parent drug compounds (cocaine and  $\Delta^9$ -THC) and respective metabolites (benzoylecgonine [BZ] and 11-nor-9-carboxy $\Delta^9$ -THC [THC-C]).

Eight male participants who smoked cocaine ( $5 \pm 2$  days/wk) and cannabis ( $3 \pm 1$  days/wk) have completed the study. Cocaine increased subjective ratings of positive drug effects ('Liking,' Good Effect'), drug quality ('Potency,' 'High Quality'), 'High,' and engendered greater ratings of the dose's monetary value relative to placebo ( $p \leq 0.05$ ); cocaine craving also increased under these conditions ( $p \leq 0.05$ ). Active cannabis decreased the subjective ratings of positive drug effects, drug quality, and subjective rating of the monetary value for the high cocaine dose (25 mg;  $p \leq 0.05$ ). However, active cannabis increased these ratings for the low cocaine dose (12 mg;  $p \leq 0.05$ ). Cannabis decreased cocaine craving for both cocaine doses ( $p \leq 0.05$ ). Cocaine increased plasma levels of cocaine and BZ ( $p \leq 0.0001$ ); cannabis attenuated cocaine-induced increases of BZ but did not affect cocaine plasma levels ( $p \leq 0.05$ ). Cannabis increased plasma THC and THCC levels ( $p \leq 0.001$ ); cocaine did not affect these levels.

These preliminary findings suggest that cannabis may decrease the positive subjective effects of larger doses of smoked cocaine and cocaine craving, and may also alter cocaine's metabolism. By assessing the interactions between cannabis and varying doses of cocaine, this study will provide clinically relevant information regarding the rationale for why these drugs are co-abused and the health risks associated with the combination.

Acknowledgements: This research was supported by US National Institute on Drug Abuse Grant DA19239, DA09236, and DA02775.

## **SUBSTITUTION EFFECT IN 628 MEDICAL CANNABIS PATIENTS; RESULTS FROM THE CANNABIS ACCESS FOR MEDICAL PURPOSES SURVEY (CAMPS)**

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### **Background**

With over 628 responses so far, the Cannabis Access for Medical Purposes Survey (CAMPS) is the largest polling of Canadian medical cannabis patients to date. This paper examines self-reported cannabis substitution for alcohol, pharmaceuticals and illicit substances from the CAMPS project.

### **Methods**

The CAMPS questionnaire is a 414 question cross-sectional survey that was made available to Canadian medical cannabis patients online and by hard copy in 2011 & 2012 in order to gather information on patient demographics, medical conditions and symptoms, patterns of medical cannabis use, cannabis substitution, and obstacles to safe access to medical cannabis. Responses were then analyzed in SPSS to identify statistically significant correlations with other patient characteristics.

### **Outcomes**

Overall, 86.6% of patients reported substituting cannabis for at least one other substance: 80.3% (n=504) of patients stated that they used cannabis as a substitute for prescriptions drugs, 51.7% (n=325) used cannabis as a substitute for alcohol, and 32.6% (n=205) used it as a substitute for illicit substances. The main reasons cited included “better symptom management” and “less adverse side-effects”. Patients who listed a greater number of symptoms were more likely to report cannabis substitution, and younger patients (below 30yrs old) were far more likely to substitute cannabis for prescription drugs, alcohol and illicit substances than older patients (50 and over).

### **Discussion**

This study adds to a growing amount of research suggesting that a large percentage of patients who use cannabis are substituting it for pharmaceutical drugs, alcohol and illicit substances. Consequently, cannabis may be serving a harm reduction function in some patients using high rates of pharmaceuticals and/or affected by problematic substance use issues. Further research should seek to differentiate between biomedical substitution for prescription pharmaceuticals and psychoactive drug substitution for addiction, and to better elucidate the mechanisms behind both. Additionally, research into cannabis as a treatment for problematic substance use in non-patient populations should be explored.

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# LEPTIN-CONTROLLED OREXIN/ENDOCANNABINOID INTERACTIONS IN THE MOUSE PERIAQUEDUCTAL GREY: ROLE IN THE REGULATION OF THE DESCENDING ANTINOCICEPTIVE PATHWAY

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In the ventrolateral periaqueductal gray (vlPAG), activation of excitatory output neurons projecting monosynaptically to OFF cells in the rostral ventromedial medulla (RVM) causes antinociceptive responses via OFF cells stimulation and ON cell inhibition<sup>1</sup>. We demonstrated that this descending nociceptive pathway is under the control of cannabinoid receptor type-1 (CB1)<sup>2</sup>. Moreover, 2-AG deeply affects nociception via CB1 stimulation, and its concentration is higher in the PAG and RVM of wt mice during neuropathic pain<sup>3</sup>. Orexins are hypothalamic peptides known to modulate arousal, feeding, reward and antinociception via orexin receptors (OX-R). In obese *ob/ob* leptin knock-out mice, OX-A expression increases in the fibers projecting to vlPAG and 2-AG levels increases in the vlPAG. Recently, Ho and collaborators demonstrated that orexin-A (OX-A), by activating OX-AR (OX-A receptor) in the vlPAG of rats, stimulates the synthesis of 2-AG and retrograde inhibition of the tonically active GABAergic circuit (disinhibition) thus inducing activation of descending nociceptive pathway<sup>4</sup>. On this basis we hypothesized the existence of a leptin-controlled orexin/endocannabinoid interaction in the modulation of the pain network leading to nociception. In this study we have validated this hypothesis using a combination of electrophysiological (*in vivo* recording), immunohistochemical (OX-A, OX-AR and CB1 single and multiple localization), ultrastructural (CB1/OX-A immunogold labeling on symmetric or asymmetric synapses) and behavioral (nociception in the "plantar test" and in spontaneous and tail-flick-related activities of RVM neurons) approaches in wt and *ob/ob* mice.

We observed that OFF (anti-nociceptive) and ON (pro-nociceptive) cells are more and less active, respectively, in *ob/ob* compared to wt. We found a significant increase of number and intensity of OX-A fibers in the PAG of *ob/ob* mice and this was accompanied by a two-fold increase of pre-prorexin mRNA expression in the LH compared to wt. OX-AR/DAGLalpha expression colocalized in a limited subset of PAG neurons through a electron microscopy approach. Moreover, CB1 receptors were expressed at symmetric synapses to OX-AR-expressing neurons thus suggesting an heterosynaptic pathway. The pharmacological blockade of the OX-R1 into the PAG produced pro-nociceptive effect in WT mice detected by both paw withdrawal and ON OFF cell activity. Interestingly, in the *ob/ob* mice the dose of the OX-AR antagonist able to generate the pronociceptive effect were double as compared to WT mice suggesting a change of this system in the absence of leptin. On the other hand, AM251, a selective CB1 antagonist also induced pro-nociceptive effect in wt mice and needed of lower dose in the *ob/ob* mice suggestin a tight cross-talk between leptin-orexin and cannabinoid systems. The endocannabinoid level measurements further confirmed the data.

**CONCLUSIONS:** Here we provide evidence supporting that the heterosynaptic endocannabinoid spread in the vlPAG after OX-AR activation is modulated by leptin. The leptin-related increase of OX-A signalling in PAG is accompanied by increased activation of OX-AR which are GqPCRs and could initiate the GqPCR-PLC-DAGL-2AG retrograde inhibition onto tonic GABAergic transmission in the vlPAG, leading to the potentiation of antinociception. Finally, we show that, beside the feeding and arousal, the orexin system could be highly involved in the pain modulation and its activity is possibly regulated by the leptin-cannabinoid system interaction.

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## IN VIVO EVALUATION OF THE PERIPHERALLY SELECTIVE CB1 RECEPTOR ANTAGONIST RTI-13329-2 IN A MOUSE MODEL OF DIET-INDUCED WEIGHT GAIN

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Cannabinoid receptor 1 (CB1R) antagonists have the potential to treat several important diseases such as obesity, drug addiction, diabetes, and liver disease. Regrettably, central nervous system (CNS) related adverse effects including depression and suicidal ideation were reported with non-tissue selective first generation CB1R antagonists. However, recent studies indicate that primarily targeting peripherally expressed CB1R with antagonists that have limited brain penetration is an attractive strategy for medications development. These compounds are expected to have limited adverse effects in patients while providing the metabolic benefits of CB1R antagonism. Over the last few years, our group has developed several novel CB1R antagonists with limited brain penetration based on the diphenyl purine scaffold of otenabant. One of these compounds, N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}methanesulfonamide (RTI-13329-2), was selected for further in vivo studies. RTI-13329-2 is extremely potent with apparent antagonist dissociation constant ( $K_e$ ) ~ 2.9 nM, CB1R affinity ( $K_i$ ) ~ 6.2 nM, and >150-fold selectivity for CB1R over CB2R (Fulp et al, *J Med Chem*, 55, 10022-32, 2012). Further, this compound has excellent metabolic stability, no noticeable interaction with hERG, and minimal effect on CYP3A4 induction. Pharmacokinetic studies indicated that this peripherally selective compound had ~10% brain penetration in both rats and mice. Consequently, RTI-13329-2 was tested in a mouse model of diet-induced weight gain. Male C57BL6 mice at ten weeks of age were fed a high-fat diet (HFD, 60% fat calories) for 4 weeks. Control mice were maintained on a normal diet (10% fat calories). Some animals on HFD were given otenabant (10 mg/kg) or RTI-13329-2 (1 or 10 mg/kg) orally once daily. Body weight and food consumption were closely monitored throughout the study. At the end of four weeks, animals were subjected to oral glucose testing prior to sacrifice. Animals on HFD gained significantly more weight than control animals. Treatment with otenabant and RTI-13329-2 abrogated weight gain in animals and improved glucose tolerance compared to animals on HFD. Livers of animals treated with otenabant and 13329-2 had significantly reduced levels of fat accumulation as well. In conclusion, RTI-13329-2 is a peripherally selective CB1R antagonist that produced beneficial effects in a rodent model of obesity and metabolic syndrome. Refinement of this class of compounds is currently underway.

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## WHERE'S MY ENTOURAGE? THE CURIOUS CASE OF 2-OG AND 2-LG

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2-Arachidonoylglycerol (2-AG) is the most abundant endogenous cannabinoid in the brain and is a high efficacy agonist at both cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>). Over the past years the synthesis, degradation and signaling of 2-AG have been investigated in some detail. However, several other endogenous monoacylglycerols have been isolated from various tissues, but their pharmacology has not been fully explored. Two of these are 2-linoleoylglycerol (2-LG) and 2-oleoylglycerol (2-OG), also a GPR119 agonist. The current data suggest that these compounds do not bind to the cannabinoid receptors. Nor do they affect intracellular free Ca<sup>2+</sup> levels or adenylyl cyclase activity in a CB<sub>1</sub>-dependent manner. However, the presence of these compounds has been reported to potentiate the activity of 2-AG and slow its breakdown, possibly through competitive inhibition of 2-AG degradation. This phenomenon has been dubbed the 'entourage effect' and may be a means to regulate synaptic activity.

To clarify the activity of these lipids at the CB<sub>1</sub> receptor we employed patch-clamp and cell-based assays. For the former we used cultured autaptic hippocampal neurons, i.e. self-synapsing neurons that have the necessary cellular machinery for several forms of endocannabinoid-mediated synaptic plasticity. This includes the 2-AG-, CB<sub>1</sub>-, and MAGL-dependent retrograde form of neuronal signaling known as depolarization-induced suppression of excitation (DSE), making it a useful model to test for a potential entourage effect. As expected, our electrophysiological data show that 2-OG and 2-LG do not inhibit neurotransmission via CB<sub>1</sub> when applied to autaptic neurons. However, these compounds fail to potentiate the 2-AG-dependent DSE. Instead 2-OG and 2-LG behave as antagonists at the CB<sub>1</sub> receptor, *attenuating* DSE. This result is inconsistent with an 'entourage effect'. Interestingly 2-OG and 2-LG do internalize CB<sub>1</sub> receptors in CB<sub>1</sub>-HEK cells, as shown by an on-cell western assay, indicating that these compounds do activate CB<sub>1</sub> receptors under some conditions. Our results suggest 1) that these compounds may serve as functional antagonists under certain conditions and, interestingly, 2) that these compounds may exhibit functional selectivity in their signaling. Our results suggest that the relationship between 2-AG and its congeners may be more nuanced than previously appreciated.

## NAPE-PLD DELETION VIA AN ALTERNATIVE MECHANISM DRIVES SIGNIFICANT DECREASES IN AEA IN THE MOUSE BRAIN

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A leading hypothesis of the biosynthesis of the endogenous cannabinoid AEA is that it is dependent on the availability of *N*-acyl-phosphatidylethanolamine (NAPE), wherein the production of AEA is the result of NAPE hydrolysis by a NAPE-specific phospholipase D (NAPE-PLD). This hypothesis was challenged with data showing AEA levels were unaffected in NAPE-PLD KO mice<sup>1</sup>. Here, we examine this hypothesis with an alternative strain of NAPE-PLD KO mice developed by Palmiter and Luquet that demonstrate behavioral characteristics and a lower rate of conversion of exogenous NAPE to AEA that would suggest there is a signaling change in AEA upon NAPE-PLD deletion<sup>2</sup>. Groups of 6 NAPE-PLD KO mice age 9 months and 2 months from the Palmiter strain and 6 age and sex matched WT mice per group were sacrificed, brains were removed and 8 targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS and over 70 lipids were analyzed for each sample.

Results here highlight those of AEA, 2-AG, NAGly, and prostaglandins; however, many differences were observed in other lipids in these KO mice. In both the 9 and 2 month old NAPE-PLD KO mice, levels of AEA were significantly reduced compared to WT levels in all regions assayed. Importantly, no changes in 2-AG levels in any brain region were observed in the 2 month old NAPE-PLD KO mice compared to WT (though only 6 regions have currently been examined), whereas, there were region specific increases in the 9 month old mice. Likewise, levels of the endogenous AEA metabolite, NAGly, were significantly different in a region specific manner, however, this was demonstrated in both age groups. Levels of PGE<sub>2</sub> increased in all areas of the 2 month old NAPE-PLD KO mice, whereas, most but not all regions showed increases relative to WT in the 9 month old mice. These data showing significant decreases in AEA are at odds with previous data from the Cravatt lab's NAPE-PLD KO mice<sup>1</sup>. Importantly, there are striking differences in the sequence generation between the two strains, which may account for this difference. Namely, the Cravatt strain deletes exon 4 of the NAPE-PLD gene<sup>1</sup>, whereas the Palmiter strain deletes exon 3<sup>2</sup>. Investigating this phenomenon has the potential to clarify understanding of AEA biosynthesis and lead to a more directed manipulation of AEA production, which has significant therapeutic potential.

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## BIDIRECTIONAL MANIPULATIONS OF 2-ARACHIDONOYLGLYCEROL CONTENT MODULATE ANXIETY-LIKE BEHAVIORS

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Stress is a major risk factor for multiple psychiatric disorders including major depression, post-traumatic stress, and anxiety disorders. Therefore, elucidation of novel approaches to mitigate the adverse effects of stress could have broad clinical applications. Disruption of the endocannabinoid (eCB) system has been implicated in various anxiety disorders and converging evidence demonstrates that stress decreases the levels of the eCB ligand, anandamide (AEA), in brain regions implicated in the pathophysiology stress-related disorders. Furthermore, augmentation of AEA content in the amygdala, a key modulator of fear and anxiety behaviors, can mitigate the deleterious effects of stress.

In contrast, the role that the most abundant eCB in the central nervous system, 2-arachidonoylglycerol (2-AG), plays in stress response physiology remains poorly understood. Here we show a transient elevation of 2-AG in the amygdala of mice exposed to foot-shock stress. This response may be an attempt to buffer against stress-induced anxiety, as further augmentation of 2-AG content following foot-shock stress is anxiolytic. Moreover, systemic inhibition of the primary 2-AG degrading enzyme, monoacylglycerol lipase, with JZL-184 reduces stress-induced corticosterone release and reverses stress-induced anxiety behaviors in two preclinical models of anxiety, the novelty induced hypophagia (NIH) and light-dark box (LD) tests. Conversely, genetic deletion of the primary 2-AG synthetic enzyme, diacylglycerol lipase  $\alpha$ , which reduces whole brain 2-AG content without affecting AEA content, results in increased anxiety-like behaviors in the NIH and LD tests. Furthermore, using *ex vivo* slice electrophysiology, we demonstrate that JZL-184 application, which prevents 2-AG metabolism, produces a depression of excitatory field potentials in the central amygdala, which may represent a mechanism underlying the anxiolytic effect of JZL-184 in behavioral tests. These data strongly suggest that augmentation of 2-AG signaling may be an effective strategy for the treatment of anxiety disorders.

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## CANNABIS, ANXIETY, AND PAIN: THE IMPORTANCE OF COPING STYLE

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The positive association between anxiety and pain is well-established. Cannabis-based medicines are noted for both their analgesic and anxiolytic properties, and recent surveys of users of cannabis for therapeutic purposes (CTP) indicate that the majority of patients who use cannabis primarily for pain relief also report cannabis use to alleviate anxiety. However, the extent to which the analgesic effects of cannabis vary according to levels of anxiety has not been determined. In the present study we examined the effects of anxiety on cannabis-induced analgesia among CTP users who identified chronic pain as a primary reason for CTP use. We further examined the extent to which the association between anxiety and cannabis analgesia was moderated by coping style. Among participants who reported CTP use for both pain and anxiety ( $n = 49$ ), those who reported relatively greater cannabis analgesia also reported greater anxiolytic effectiveness for CTP ( $X^2 = 10.17, p < .01$ ). Across all participants ( $n = 68$ ), the perceived analgesic effectiveness of cannabis was not associated with anxiety ( $X^2 < .01, p = .98$ ). However, moderation analyses revealed that the association between anxiety and cannabis analgesia was moderated by the dysfunctional coping styles of *behavioral disengagement* ( $X^2 = 5.86, p < .05$ ) and *self blame* ( $X^2 = 3.90, p < .05$ ). Follow-up analyses of these interactions indicated that anxiety was associated with greater analgesia among those who were less likely to engage in behavioral disengagement ( $X^2 = 4.32, OR = 1.12, p < .05$ ) and with less analgesia among those who were higher in behavioral disengagement ( $X^2 = 3.45, OR = .84, p = .06$ ). Analyses of self blame coping exhibited a similar but less pronounced pattern of association, with a positive association between anxiety and analgesia at lower self blame ( $X^2 = 3.83, OR = 1.13, p = .05$ ), and no relationship among those more prone to self blame ( $X^2 = 2.33, OR = .95, p = .12$ ).

Our findings help to elucidate the role of anxiety in cannabis-induced analgesia. First, we found that perceived analgesic and anxiolytic effectiveness of cannabis covaried positively among patients who use CTP to treat both pain and anxiety. Second, we found that cannabis was perceived to be a more effective analgesic among higher anxiety individuals who refrain from dysfunctional coping, and less effective among higher anxiety individuals who engage in higher levels of dysfunctional coping. These findings suggest that anxiety may play a role in the analgesic effectiveness of cannabis, and also point to meaningful heterogeneity among those who use cannabis for pain relief. These findings highlight the importance of individual differences in affective and cognitive functioning in understanding the complex relationship between cannabis and pain relief.

## **ENDOGENOUS CB1 ALLOSTERIC ENHANCER BALANCES AGE-RELATED COGNITIVE ALTERATIONS**

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Allosteric binding sites at cannabinoid CB1 receptors were suggested 6 years ago, after experiments with synthetic molecules. Recently, we reported an endogenous molecule (lipoxin A4, LXA4) that enhances endocannabinoid signaling through allosteric modulation of these receptors in the CNS (Pamplona et al PNAS 2012). This molecule is a "first-in-class" and its discovery opens the possibility for future fine-tuning allosteric modulation of endocannabinoid signaling. Interestingly, LXA4 is also an important anti-inflammatory molecule, actually a resolvin, whose levels decrease during the aging process. Hence, we decided to investigate the potential role of LXA4 in a number of endocannabinoid-related CNS functions. Therefore, here we report that endogenous LXA4 affects mouse anxiety-like behavior and cognition in aged animals, and that absence of the LXA4-synthesizing enzyme 5-LO induces cognitive deficits in 5-LO KO mice (Leo et al PLoS One 2013). Moreover, LXA4 protects mice against the cognitive deficit induced by i.c.v. injection of beta-amyloid 1-40 peptide, a hallmark of Alzheimer's Disease. The reduction of LXA4 observed in 5-LO KO mice, as well as in aged mice contributes to the generation of cognitive deficits. As LXA4 levels decrease after 60+ years in humans, we are now pursuing the concept that LXA4 could be an important biomarker of brain vulnerability to aging-related cognitive deficit and neurodegenerative diseases in humans. This translational approach is currently being conducted in samples of over 200 patients with healthy aging, diagnosed with mild cognitive impairment or Alzheimer's Disease. LXA4 will be quantified in CSF and plasma samples, compared across these patient groups and plotted against age and cognitive performance. We expect that LXA4 may represent a novel biomarker to help identify individuals at higher risk of developing mild cognitive impairment and/or Alzheimer's disease over time, allowing pre-emptive clinical intervention.

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## $\Delta^9$ -THC RESTORES AGE-RELATED CHANGES IN THE BRAIN

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Aging of the brain is accompanied by a progressive decline in cognitive abilities associated with reduced synaptic densities. This process is accelerated in mice lacking cannabinoid CB1 receptors. We have therefore wondered if an increase of the cannabinoid tone in older animals would protect them against the loss of cognitive functions. Two age groups of animals were therefore treated with 3 mg/kg/day Delta-<sup>9</sup>THC for 28 days through osmotic minipumps. This treatment significantly improved spatial learning in the Morris water maze test as well as object location recognition in the old but not in the young mice. The improved cognitive performance was associated with increased synaptic densities and enhanced expression of synaptic marker proteins. We next investigated the gene expression profile of control and THC treated young and old mice. We found that THC treatment altered the expression of a number of age-related genes in the old but not in the young mice. The CREB and Erk signaling systems were activated in THC treated old animals shown by an enhanced phosphorylation. The beneficial effects of THC on brain ageing was mediated by CB1 receptors, because THC treated CB1 knockout mice failed to show any improvement in learning ability, increase in synaptic densities or change in gene expression.

## A PERIPHERAL ENDOCANNABINOID MECHANISM FOR STRESS-INDUCED AMNESIA

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Memory consolidation is a labile process under the direct impact of emotional experiences, and little is known with regards to the underlying mechanisms. The endocannabinoid system plays an important role in the modulation of both emotions and memory, but the integration of these functions in the memory consolidation processes has not been addressed. This study investigates the involvement of type-1 cannabinoid (CB1) receptors in stress-induced amnesia for a non emotional memory. Using a model for declarative memory in mice, the novel object-recognition test, we found that stress and the arousal state of the animal determines the consolidation outcome in this memory. Such a memory trace was obliterated under physical or psychological acute stress conditions, or corticosterone administration. These amnesic-like effects of stress and corticosterone were not observed after pharmacological (both rimonabant and the peripherally acting CB1 receptor antagonist AM6545) or genetic blockade of CB1 receptors. According to this behavioral data, the c-FOS activation in different brain regions produced after the shock was also blocked by the CB1 antagonism.

Using several cell type-specific conditional CB1 receptor knockout mouse lines, we found that CB1 receptors expressed in noradrenergic dopamine  $\beta$ -hydroxylase-expressing neurons (DBH-CB1) are crucial for this stress-induced amnesia, whereas CB1 receptors in other brain neuronal populations were not involved in this response. Interestingly, conditional mice lacking DBH-CB1 (DBH-CB1-KO) did not increase plasma corticosterone levels induced by the shock compared to wild-type animals.

Finally, the removal of the adrenal glands prevented the amnesic-like effect of stress and the pharmacological noradrenergic modulation shows that both alpha- and beta-adrenergic receptors are involved in this stress-induced amnesia.

In summary, peripheral noradrenergic transmission determines the consolidation of non-emotional memories and this function is under the direct control of peripheral CB1 receptors. The elucidation of this mechanism opens novel therapeutic approaches for the treatment of memory- and stress-related disorders through peripherally acting drugs for CB1 cannabinoid receptors.

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## **PERSISTENT MICROGLIA ACTIVATION WITHIN THE PREFRONTAL CORTEX CONTRIBUTES TO THE DEVELOPMENT OF THE DEPRESSIVE/PSYCHOTIC-LIKE PHENOTYPE INDUCED BY ADOLESCENT THC EXPOSURE IN RATS**

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Emerging evidence suggests that microglia might play a crucial role in brain development, regulating synaptic maturation and function, possibly suggesting that deficits in microglia function may contribute to synaptic abnormalities seen in some neurodevelopmental disorders.

In the present study we investigated whether the depressive-like phenotype induced by adolescent delta-9-tetrahydrocannabinol (THC) exposure in adult female rats (Rubino et al. 2008, 2009; Realini et al. 2011; Zamberletti et al. 2013) was associated with changes in microglia activation within the prefrontal cortex.

To this aim, chronic THC (or vehicle) treatment was performed between PND 35 to 45 and ionized calcium-binding adaptor molecule 1 (Iba1) expression, a marker of activated microglia, was monitored during THC treatment as well as 24 hours (PND 46), 15 days (PND 60) and 30 days (PND 75) after discontinuing it in the prefrontal cortex (PFC). The effect of chronic THC treatment on microglia was also morphologically determined by using Iba1 immunofluorescence.

Adolescent THC treatment significantly increased Iba1 expression from PND 39 to PND 46 and they were still enhanced at PND 75, suggesting the presence of persistent microglia activation following adolescent THC exposure. This hypothesis was supported by morphological studies revealing that in THC-treated rats glial cells were maintained in a chronic activated state (amoeboid morphology) till adulthood.

In order to assess whether microglia activation could play a role in determining the depressive/psychotic-like phenotype observed in adult THC-exposed animals, we inhibited microglia activation by co-administering Ibudilast, a non-selective phosphodiesterase inhibitor, concomitantly to THC treatment and animals were then submitted to behavioral testing in adulthood.

Intriguingly, co-treatment with Ibudilast and THC during adolescence prevented the development of some of the symptoms associated with adolescent THC exposure, thus suggesting that microglia activation could contribute to determine some of the behavioral alterations observed in adult THC-treated rats.

As a whole, the present findings demonstrate that the behavioral phenotype induced by adolescent THC exposure is associated with persistent microglia activation within the PFC that contribute to the development of some signs of THC-induced depressive/psychotic-like phenotype.

Ongoing studies are aimed at elucidating the neurobiological consequences of microglia activation in terms of cytokine release and CB2 receptor expression.

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## **A MEDICAL ETHNOGRAPHIC REPORT OF CANNABIS USE IN PEDIATRIC INTRACTABLE EPILEPSY (IE) PATIENTS**

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Parents of children with severe and untreatable diagnoses are desperate for relief for their child and family systems. Because inefficacy and/or side effects of multiple drug cocktails do not provide cure or relief, some are turning to alternatives, such as CBD-rich Cannabis extractions. Due to US Federal restrictions, these patients and their families do not have access to products of traditional research processes. This report intends to describe demographics, diagnoses, current drug regimens and consequences of a population that has chosen to self-administer Cannabis as a potential therapeutic agent.

A retrospective chart review of thirteen patients was combined with a mixed-methods approach that included parent interviews and correspondence, a parent forum, and analytical data that was collated to describe general quality of life, dosing regimens and unintended consequences. Eligibility criteria included recommendation for Cannabis use in WA State under RCW 69.51 and diagnosis of intractable epilepsy by a neurologist. Additional diagnoses included transient tic disorder, autistic spectrum, and self-injury behavior. Information on demographics, quality of life, changes in seizure frequency, cognitive function, behavioral effects, co-administration with other anti-epileptic drugs (AEDs), analytical data on plant potency and constituents, preparation of Cannabis and dose (mg/kg) were collated and analyzed. Charts of thirteen pediatric patients revealed a patient age range of 2- 21. Parents sought medical help from 2-19 doctors and children had been prescribed 2-6 pharmaceutical drugs. Thirty-five adverse effects of current AEDs were self-reported with the most common of ataxia, anger/rage, behavioral problems including anger and aggression, and loss of appetite. The most common alternative therapies were herbal and nutritional supplements. About half of the parents felt they had support of their neurologist for oral Cannabis supplementation in the form of a plant concentrate diluted with oil. Qualitative changes reported by the parents included reduction of seizure frequency and severity, positive behavioral changes, improved sleep, and general improvement in quality of life for the child, parents and family units.

There is a trend amongst parents of pediatric patients with IE to seek alternative treatments. The families here independently navigated dosing a Cannabis preparation and the downward titration of AEDs, largely without the support of their medical provider. They reported positive support from behavioral interventionists and social and educational workers. This report indicates the need to enable prospective research on the potential therapeutic use of CBD-rich Cannabis preparations for pediatric patients with IE. Further, there is a need to investigate potential drug interactions and appropriate, safe supervised withdrawal from AEDs.

## EXOME SEQUENCING OF FAMILIAL ATRIAL FIBRILLATION INFORMS POSITIVE TREATMENT WITH CANNABIDIOL

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Exome sequencing uncovered multiple non-synonymous variants in several calcium channel genes in a pedigree with multi-generational lone paroxysmal atrial fibrillation (AF). Calcium channel genes, which encode pore-forming subunits of ion channels, are genetically hypervariable. This variability complicates the identification and interpretation of causative variants in individuals with mid-life expression of disease. Exome sequencing was performed on a family of six to prioritize variants identified in cardiac genes that tracked with the atrial fibrillation phenotypes. Based on the affected individual's clinical presentation, we hypothesized that five non-synonymous common and novel variants in two calcium channel genes, *RYR2* and *CACNA1C*, were the candidate causative variants, hypothetically contributing to the phenotype in a polygenic manner. Published mutations in these genes have been reported to cause sudden cardiac death. Animal model studies have suggested a link between atrial arrhythmias and dysfunctional calcium handling<sup>1</sup>. Cannabidiol is known to regulate intracellular calcium homeostasis and in theory presents a possible therapy for response in this unique case<sup>2</sup>.

The patient failed conventional therapy with beta blockers and calcium channel blockers secondary to symptomatic bradycardia. Treatment with the antiepileptic drug cannabidiol (CBD), a non-psychoactive phytocannabinoid derivative of cannabis, was initiated by the patient. Arrhythmia occurrence was documented with ambulatory monitoring via the Alive Cor ECG monitor and patient reported symptoms. Atrial fibrillation abated with oral mucosal use of CBD at dose of 75mg/day. In the proband, atrial fibrillation, frequency and severity correlated with cannabidiol dose, with atrial fibrillation returning with doses below 25mg/3xday. This case presents a private scenario where variants in opposing calcium channels (cell membrane and sarcoplasmic reticulum) were unresponsive to non-specific calcium channel blockers but appeared to be responsive to CBD.

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## EPIGENETIC REGULATION OF THE ENDOCANNABINOID SYSTEM IN HUMAN PSYCHIATRIC DISORDERS

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Alterations of the endocannabinoid system (ECS) have been associated with psychotic disorders (particularly schizophrenia), and thus they are getting more and more attention as potential diagnostic markers and/or therapeutic targets of innovative drugs.

Here, we investigated changes in DNA methylation of the major components of the ECS (*i.e.*, CB<sub>1</sub>, CB<sub>2</sub>, GPR55 and TRPV1 receptors, and NAPE-PLD, FAAH, DAGL and MAGL metabolic enzymes) in peripheral blood mononuclear cells (PBMCs) of patients suffering from major depressive disorder (MDD) or bipolar disease of type I (BDI) and type II (BDII). PBMCs were chosen because they are easily accessible cells, that possess the same machinery for epigenetic regulation as neuronal cells, and are known to mirror defects associated with neurological diseases.

We found a selective down-regulation of CB<sub>1</sub> mRNA expression in PBMCs from MDD patients only, compared to healthy controls. Such a down-regulation was not due to altered DNA methylation and correlated with histone deacetylase 1 (HDAC1) and HDAC10 expression, but not with that of HDAC5 or HDAC7. Interestingly, down-regulation of CB<sub>1</sub> was paralleled by a similar down-regulation of mitogen-activated protein kinase phosphatase-1 (MKP-1), that is a key control point in the neurobiology of depression.

Taken together, we suggest that CB<sub>1</sub> might regulate gene expression through HDAC1 and HDAC10, controlling MKP-1-dependent signal transduction.

Understanding the impact of ECS epigenetic regulation on human psychiatric disorders appears of great value for the possible design of more specific epigenetic drugs, also because those currently approved by FDA, although promising, have both a non-specific target and a genome-wide effect.

## GENETIC DIFFERENCES IN THE CB1/CNR1 GENE MODULATE FEAR EXTINCTION IN HUMANS

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The endocannabinoid system plays an important role in the regulation of anxiety behavior. A large amount of animal research shows the ability of the endocannabinoid system to regulate both cue-specific and context-specific fear responses, through the modulation of cannabinoid receptor 1 (CB1) activation [1-2]. Enhancing CB1 receptor activation with CB1 receptor agonists or anandamide re-uptake inhibitors increases extinction of fear [1-2]. Decreasing CB1 receptor neurotransmission, either through pharmacological blockade or genetic deletion of the CB1 receptor, diminishes the extinction of fear [1-3]. Whether endocannabinoids play a role in acute fear relief, long-term habituation or relearning is still subject of debate. Taken together, animal literature strongly suggests that the endocannabinoid system holds great promise for development of pharmacotherapy in human anxiety disorders. Fear extinction is used as a human laboratory for exposure-based psychotherapy. However, research on the role of the CB1 receptor in the regulation of fear extinction in humans is still scarce. Recently, a study showed that administration of  $\Delta$  9-THC enhanced fear extinction [4]. Another study reported similar results using cannabidiol [5]. Additional research is needed to bridge the gap between preclinical and human research.

In a previous study from our research group we found that genetic variability in the human CB1 receptor gene may underlie individual differences in fear extinction [6]. Healthy human volunteers (N = 150) underwent a fear conditioning and extinction procedure in a virtual reality environment. Fear-potentiated startle of the eye-blink reflex was recorded to assess fear-conditioned responding. Subjects were genotyped for a polymorphism located in the promoter region (rs2180619) of the CNR1 gene. Importantly, homozygote A/A carriers displayed a complete lack of fear extinction, whereas G-allele carriers displayed robust extinction of conditioned fear. Furthermore, failure to extinguish fear resulted in a higher conditioned fear at the end of the experiment in the homozygote A/A group when compared to the G-allele carriers. In parallel to preclinical literature, no effects of rs2180619 genotype were found on the acquisition and expression of conditioned fear. These findings suggest that deficient fear extinction in A/A carriers of rs2180619, may predispose to developing anxiety disorders. Because candidate gene studies often use small samples and have relatively small effects-sizes, resulting in an increased risk for false-positives, we aim to replicate and extend on our previous findings. Therefore we have performed a replication study in a larger sample (N = 204) and included a testing session 1 week later to evaluate whether rs2180619 exerts long-lasting effects on fear extinction. Results of the ongoing analyses will be presented at ISCR2014.

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## A QUESTION OF RANK: USING DNA BARCODES TO CLASSIFY CANNABIS SATIVA AND CANNABIS INDICA

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**INTRODUCTION:** The botanical taxa *C. sativa* (European fiber plants) and *C. indica* (Asian drug plants) should not be confused with the folk taxonomy of “sativa” and “indica” used in the medical cannabis world. Botanical taxa have rank: species > sub-species > variety. Rank allocation is vital (*e.g.*, endangered species laws concern species, not subspecies, as do drug laws), but notoriously subjective, exemplified by *C. sativa* and *C. indica*, arbitrarily segregated at the rank of species, or subspecies, or variety. Animal taxonomists propose using a “DNA barcode” to allocate rank. They employ the *COI* gene sequence, and a “barcode gap” (2.7% difference between two *COI* sequences), as the threshold for separating animals at the rank of species. No barcode gap separating plant species has been proposed; botanists recommend combining two or more sequences as a potential barcode. Candidates include *rbcL*, *matK*, *trnH-psbA*, *trnL-trnF*, and *ITS*.

**METHODS:** Five sequences (*rbcL*, *matK*, *trnH-psbA*, *trnL-trnF*, *ITS*) were obtained from *C. sativa* and *C. indica*. Pairwise alignments of each sequence were made with BLAST. We quantified the difference between aligned sequences by tallying the number of nucleotide non-identities as a percentage of the total alignment. These calculations were repeated with a pair of very different species (apples and oranges), then with five pairs of related species (tomato and potato, hops and Japanese hops, buckwheat and bitter buck-wheat, trema and Jamaican trema, Asian ginseng and Himalayan ginseng). Many plant species, unlike animal species, can hybridize. So we repeated the process with four closely related species that can hybridize (radish and charlock, white poplar and black cottonwood, Asian rice and African rice, upland cotton and Pima cotton). Lastly we made five pairwise comparisons between plants classified at the rank of subspecies or variety (long-grain rice and short-grain rice, cabbage and broccoli, melon and wild melon, calamus and American calamus, Chinese tea and Assam tea). All sequences were obtained from GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)).

**RESULTS:** Apples and oranges (different genera) differed by  $18.1 \pm 2.87\%$  (mean of five sequences). The mean barcode gap of five related species was  $3.0 \pm 0.292\%$ . The mean barcode gap of four closely related that can hybridize was  $1.0 \pm 0.134\%$ . The mean barcode gap of five subspecies or varieties was  $0.43 \pm 0.01\%$ . *C. sativa* and *C. indica* differed by  $0.41 \pm 0.26\%$ .

**DISCUSSION:** We calculated a barcode gap between non-hybridizing plant species that nearly equals the animal *COI* barcode gap. The barcode gap between *C. sativa* and *C. indica* is similar to that of other pairs of plants at the rank of subspecies or variety. Contrary to recent taxonomic studies, *C. sativa* and *C. indica* should not be considered different species. We propose rank allocation as *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica*.

## OREXIN/ENDOCANNABINOID/LEPTIN INTERACTION AFFECTS HYPOTHALAMIC TAU PHOSPHORYLATION BY GLYCOGEN SYNTHASE KINASE-3BETA ACTIVATION

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Synaptic plasticity in the hypothalamus is coordinated by hormonal signals responding to the physiological energy status. This mechanism is strictly regulated, among other things, by the fine balance between phosphorylated/unphosphorylated proteins of axonal microtubules. Tau is a microtubule-associated protein (MAP) mainly expressed in neurons and its hyperphosphorylation decreases affinity and binding to microtubules with consequent axonal retraction, swelling and inhibition of fast transport. Glycogen synthase kinase-3 beta (GSK-3 $\beta$ ) is an ubiquitous serine/threonine kinase that regulates numerous cellular functions and neuronal architecture, including Tau phosphorylation. Unlike other protein kinases, GSK-3 $\beta$  is constitutively active under resting conditions and is inactivated by extracellular signals like leptin through phosphorylation of its Ser-9 residue via Akt (Cross et al., 1995). However, GSK-3 $\beta$  activity is further increased by phosphorylation of Tyr-216 mediated by lysophosphatidic acid (LPA), with subsequent phosphorylation of Tau via tyrosine kinase Pyk2 (Sayas et al., 2006). LPA is a bioactive lipid mediator, which can be both biosynthetic precursor of, or produced by, the endocannabinoid 2-AG (Nakane et al., 2002).

By comparing adult leptin defective *ob/ob* mice to littermates we have previously found, in the arcuate nucleus (ARC) of the hypothalamus: (i) the elevation of orexin-A/hypocretin-1 (OX-A) trafficking and release; (ii) a ~4-fold increase of 2-AG levels as a possible consequence of OX-A receptor-1 (OX-AR) over-activation; (iii) a significant increase of pTau/Tau ratio, which is reversed 60 min after acute i.p. leptin injection (Cristino et al., 2013; Cristino et al., in preparation). On this basis, we hypothesized that the massive increase of hypothalamic 2-AG levels known to occur in *ob/ob* or fasted mice (Di Marzo et al., 2001) as a result of leptin deficiency, might lead to a strong increase of LPA, with subsequent hyperphosphorylation of Tau, axonal destabilization of pre-*pro*melanocortin (POMC) neurons of the ARC and reduction of the food-intake inhibitory activity that these neurons exert. To test this hypothesis we examined the effects of leptin and orexin-A both on the amounts of LPA [with main focus on 2-AG-derived *sn*-1-lyso-2-arachidonoyl-PA] and on the two opposing pathways of GSK-3 $\beta$  phosphorylation, via Akt or Pyk2, after in vivo injection of leptin (i.p., 5 mg/kg), orexin-A (i.p., 40 mg/kg) and the OX-AR antagonist, SB334867 (i.p., 30 mg/kg). Neuronal primary cultures from the ARC of wild-type P0 mice were also used to replicate in vitro the same experiments.

Our data suggest that, in *ob/ob* mice, Pyk2-mediated GSK-3 $\beta$  phosphorylation constitutively contributes to Tau instability (i.e. Tau hyperphosphorylation) in anorexigenic POMC neurons, in manner prevented by leptin or SB-334867A treatment. Conversely, in wild-type fed mice, PI3K/Akt-mediated GSK-3 $\beta$  phosphorylation constitutively contributes to Tau stability in these neurons, in a manner prevented by short term (12 hr) food deprivation or OX-A injection. This set of data was confirmed in vitro by time lapse study of elongation/retraction of neuronal processes from primary POMC-expressing neurons incubated with leptin or OX-A or LPA, alone or in the presence of the respective receptor antagonists. Finally, by immunofluorescence, we found significant CB1/OX-AR co-expression in the ARC due, at least in part, to the formation of CB1/OX-AR heteromers, as assessed by immunoblots, coimmunoprecipitation and FRET analysis.

We suggest that, in *ob/ob* mice, elevated 2-AG levels, due to impaired leptin and enhanced OX-A signaling, together with these two latter alterations, determine, via either LPA- or CB1-mediated mechanisms (or both) profound changes in hypothalamic connectivity through GSK-3 $\beta$ -mediated Tau phosphorylation in POMC neurons, contributing to feeding disinhibition.

## CANNABINOID RECEPTOR 2 DEFICIENCY ALTERS NEUROINFLAMMATION IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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The endocannabinoid system (eCS) encompasses two G-protein-coupled receptors, the cannabinoid receptor 1 (CB1), the cannabinoid receptor 2 (CB2), as well as their ligands and their respective synthesizing and degrading enzymes. While the CB1 is expressed primarily in the brain, the CB2 is mainly detected in immune cells. As various studies suggest a role of CB2 receptors in the modulation of microglia activity, which may be relevant to Alzheimer's disease (AD), we wanted to investigate the role of the CB2 receptor in a mouse model of AD. In AD microglia are attracted by deposits of accumulated amyloid- $\beta$  (A $\beta$ ) peptides and exhibit an activated state involving enhanced proliferation, increased expression of cell surface markers and production of chemokines and cytokines. Thus, we investigated the role of the CB2 receptor in activation of microglia cells *in vitro* as well as in the inflammatory process in an *in vivo* model in Alzheimer's disease (APP/PS1xCB2<sup>-/-</sup> mice). CB2<sup>-/-</sup> microglia showed a reduced expression of cell surface markers such as ICAM and CD40 as well as a decreased release of chemokines and cytokines, e.g. CCL2, IL-6 and TNF- $\alpha$  as compared to wild-type microglia. Absence of the CB2 receptor, however, did not result in a difference in A $\beta$  phagocytosis in neonatal microglia.

APP/PS1xCB2<sup>-/-</sup> mice showed reduced numbers of microglia cells as well as infiltrating macrophages and lowered expression levels of pro-inflammatory chemokines and cytokines. Subsequently, the amount of soluble A $\beta$  40/42 was diminished in APP/PS1xCB2<sup>-/-</sup> in middle-aged (9 months) but not in aged (14 months) mice. Interestingly, this cutback in neuroinflammation did not affect learning and memory abilities in APP/PS1xCB2<sup>-/-</sup> mice.

Taken together, we show that CB2<sup>-/-</sup> microglia have a limited capacity to respond to pro-inflammatory stimuli, whereas the phagocytosis capacity is not influenced. The knockout of the CB2 receptor in an AD mouse model led to a significant reduction in AD-linked neuroinflammation, which did not influence A $\beta$  plaque load or cognitive impairment. This suggests a functional impact of the CB2 in neuroinflammatory responses associated with Alzheimer's disease but independent of influencing A $\beta$  pathology and cognitive impairment.

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## SELECTIVE ACTIVATION OF CB2Rs ELIMINATES VTA DOPAMINE NEURONAL BURSTING FIRING IN RODENTS

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Recently, the “peripheral cannabinoid type 2 receptors (CB2Rs)” have been detected in various brain areas, suggesting that CB2Rs are also expressed in the central nervous system and they may participate in the modulation of neuronal functions under both physiological and pathological conditions. Our previous study demonstrated that the activation of central CB2Rs significantly reduced animal cocaine seeking behavior, which indicates an important role played by CB2Rs in mesolimbic circuit for drug addiction. It is well known that drug addictive behavioral is highly correlated with VTA dopamine (DA) neuronal firing activity, especially bursting firing. However, whether central CB2Rs modulate VTA DA neuronal bursting firing is unknown. We hypothesize that the selective activation of functional CB2Rs in the VTA eliminates VTA DA neuronal bursting firing through the enhanced small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (SK). In the present study, we test our hypothesis using *in vivo* and *in vitro* electrophysiological approaches. In anesthetized mice, we performed extracellular single unit recording and found that systemic injection of CB2Rs agonist (JWH 133, i.p., 10 mg/kg) moderately reduced VTA DA neuronal firing rate but dramatically reduced bursting firing fraction in WT, but not CB2R KO, mice. In WT mice, the JWH133-induced inhibition in DA neuronal firing can be prevented or reversed by injection of CB2Rs antagonist AM630 (i.p. 10 mg/kg), suggesting that systemic JWH133 alters VTA DA neuronal bursting firing through the activation of CB2Rs. In VTA DA neurons in slice, bath-applied JWH133 (1 μm) significantly enhanced the amplitude of apamin-sensitive after-hyperpolarization, suggesting that the altered SK conductance may underlie JWH133-induced reduction of neuronal bursting firing. To elucidate this possible mechanism, we induced DA neuronal bursting firing with NMDA (30 μM) in VTA slices both in rats and mice. We found that JWH133 significantly inhibited NMDA-induced bursting firing represented as a decrease in amplitude of the inter-burst hyperpolarization potential. Importantly, this inhibition can be blocked by either AM630, or a SK selective blocker NS8593. Furthermore, JWH133 also enhanced SK current amplitude in DA neurons of VTA slices. Taken together, our results suggest that the selective activation of VTA CB2Rs significantly eliminates the bursting firing of VTA DA neurons, which is mediated through enhanced amplitude of SK currents. Therefore, our findings provide directly experimental evidence to improve our understanding, for the first time, of how CB2Rs play a critical role in drug addiction and DA associated diseases.

## CHRONIC CANNABINOID CB<sub>2</sub> AGONIST REVERSES PACLITAXEL NEUROPATHY WITHOUT TOLERANCE, CB<sub>1</sub>-MEDIATED WITHDRAWAL OR SIDE EFFECTS

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Mixed cannabinoid CB<sub>1</sub>/CB<sub>2</sub> agonists, such as  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), can produce tolerance, dependence, and unwanted CB<sub>1</sub>-mediated central nervous system side effects following chronic administration. Whether the beneficial effects of repeated systemic administration of a CB<sub>2</sub>-preferring agonist involves CB<sub>1</sub> receptors or produces unwanted CB<sub>1</sub>-mediated side effects is unknown. We evaluated the anti-allodynic efficacy, possible tolerance, and cannabimimetic side effects of repeated dosing with a CB<sub>2</sub>-preferring agonist (AM1710), in comparison to  $\Delta^9$ -THC, in a model of chemotherapy-induced neuropathy produced by paclitaxel using CB<sub>1</sub>KO, CB<sub>2</sub>KO, and WT mice. We also investigated the site and mechanism of action of AM1710. Paclitaxel-induced mechanical and cold allodynia developed equivalently in CB<sub>1</sub>KO, CB<sub>2</sub>KO, and WT mice. Both AM1710 and  $\Delta^9$ -THC suppressed established paclitaxel-induced allodynia in WT mice. Unlike  $\Delta^9$ -THC, chronic AM1710 did not engage CB<sub>1</sub> activity or produce antinociception tolerance, CB<sub>1</sub>-mediated cannabinoid withdrawal, hypothermia, or motor dysfunction. The anti-allodynic efficacy of systemic AM1710 was absent in CB<sub>2</sub>KO mice or WT mice receiving the CB<sub>2</sub> antagonist AM630, administered either systemically or intrathecally. Intrathecal AM1710 also attenuated paclitaxel-induced allodynia in WT but not CB<sub>2</sub>KO mice, suggesting a role for spinal CB<sub>2</sub> receptors in AM1710 anti-allodynic efficacy. Finally, both acute and chronic treatment with AM1710 decreased expression of tumor necrosis factor alpha and monocyte chemoattractant protein-1 mRNAs in the lumbar spinal cord of paclitaxel-treated WT mice. Our results highlight the therapeutic potential of CB<sub>2</sub> agonists for managing chemotherapy-induced allodynia with a favorable therapeutic ratio marked by sustained efficacy and absence of tolerance, CB<sub>1</sub>-mediated withdrawal or side effects.

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## **THE COMBINATION OF AMITRIPTYLINE WITH FAAH OR MGL INHIBITORS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY IS CB<sub>2</sub> MEDIATED**

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Cisplatin, a platinum-derived chemotherapeutic agent, produces mechanical and cold allodynia in rodents that is reminiscent of chemotherapy-induced neuropathy observed in humans. Enzymes such as fatty-acid amide hydrolase (e.g. FAAH) and monoacylglycerol lipase (MGL) break down the body's own endocannabinoid ligands (e.g. anandamide) and 2-arachidonoyl glycerol. They represent targets for analgesic drug development. We compared antinociceptive effects of FAAH (URB597, URB937) and MGL (JZL184) inhibitors on mechanical and cold allodynia induced by cisplatin treatment. Anti-allodynic efficacy of each endocannabinoid modulator was compared with agents used clinically to treat neuropathy (i.e. the tricyclic antidepressant amitriptyline). Groups received intraperitoneal (i.p.) injections of either amitriptyline, URB597, URB937, JZL184 or vehicle. FAAH (URB597, URB937) and MGL (JZL184) inhibitors administered alone at lower dose partially reversed mechanical and cold allodynia. Pharmacological specificity was assessed by coadministering amitriptyline with FAAH or MGL modulator with either a CB<sub>1</sub> (AM251 3 mg/kg) or CB<sub>2</sub> (AM630 3 mg/kg) antagonist. Interestingly, the combination of FAAH or MGL inhibitors with amitriptyline, reversed cisplatin-evoked mechanical and cold allodynia to pre-cisplatin levels. Moreover, this reversal of mechanical and cold allodynia by the combination of amitriptyline with FAAH or MGL inhibitors is CB<sub>2</sub> mediated. However, RT-PCR analysis demonstrate no changes in FAAH, MGL, CB<sub>1</sub> or CB<sub>2</sub> mRNA levels following chronic administration of amitriptyline, JZL184, URB937 or their combination. Our results suggest that both FAAH and MGL inhibitors in combination with amitriptyline attenuate mechanical and cold allodynia in a model of chemotherapy-induced peripheral neuropathy. Moreover, the combination of amitriptyline with FAAH or MGL inhibitors is mediated by cannabinoid receptors 2. Our studies suggest that the endocannabinoid system combined with amitriptyline represent a promising target for suppressing chemotherapy-induced neuropathic pain.

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## TAMOXIFEN IS AN ALLOSTERIC MODULATOR OF THE CB2 CANNABINOID RECEPTOR

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Recently, we have reported that several second- and third-generation selective estrogen receptor modulators (SERMs) are inverse agonists for the CB2 cannabinoid receptor. The purpose of the current study was to investigate the pharmacology of tamoxifen, a first-generation SERM and three of its metabolites, 4-hydroxytamoxifen, endoxifen, and N-desmethyltamoxifen, at the CB2 cannabinoid receptor.

Using human CB2 stably expressed in HEK293 cells, our experiments demonstrated that at concentrations up to 1  $\mu$ M, tamoxifen and two of its metabolites (endoxifen and N-desmethyltamoxifen) failed to either modulate cAMP accumulation or compete for specific [<sup>3</sup>H]CP-55,940 binding. In contrast, 4-hydroxytamoxifen enhanced forskolin-stimulated cAMP accumulation and competed for specific [<sup>3</sup>H]CP-55,940 binding in a concentration-dependant manner. Furthermore, pretreatment of HEK293 cells stably expressing human CB2 with 4-hydroxytamoxifen caused a rightward, parallel shift of the concentration-response curves of the cannabinoid agonists HU-210, CP-55,940, and WIN55,212-2. However, pretreatment with endoxifen and N-desmethyltamoxifen did not alter the concentration-response curves of these cannabinoid agonists. Most importantly, we discovered for the first time that pretreatment with tamoxifen resulted in an enhancement of CP-55,940 efficacy to inhibit forskolin-stimulated cAMP accumulation without altering the efficacy of the cannabinoid agonists HU-210 and WIN-55,212-2. These data demonstrated that tamoxifen exhibits a positive allosterism on CP-55,940 efficacy. The allosteric effect of tamoxifen on CB2 is specific for CP-55,940 since the efficacy of the other two cannabinoid agonists was not altered by the pretreatment of tamoxifen. In addition, the allosteric nature for the effects of tamoxifen was further validated by [<sup>3</sup>H]CP-55,940 dissociation kinetic studies as there was a significant decrease in each of the slow and fast radioligand dissociation rates in the presence of tamoxifen.

In recent years, several synthetic compounds have been shown as allosteric modulators for the CB1 cannabinoid receptor. However, to our knowledge, there have been no allosteric modulators reported for the CB2 cannabinoid receptor. In this study, by identifying tamoxifen as the first allosteric modulator for CB2, we have provided the first piece of evidence indicating the existence of an allosteric site on the CB2 cannabinoid receptor. Furthermore, we have demonstrated a possible novel mechanism of action for tamoxifen, a well-known, first-generation SERM.

## TARGETING CB<sub>2</sub> RECEPTOR AS A NEW PHOTOTHERAPY APPROACH

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The success of targeted cancer therapy largely relies upon the selection of target and development of efficient therapeutic agents that specifically bind to the target. Cannabinoid CB<sub>2</sub> receptor (CB<sub>2</sub>R) is considered as an attractive target for cancer treatment. Under healthy conditions, high CB<sub>2</sub>R expression is only present in immune cells. However, CB<sub>2</sub>R expression is up-regulated in many types of cancers, such as prostate, skin, liver, brain, colon and breast cancer.

In the current study, we chose CB<sub>2</sub>R as a new target for phototherapy treatment and developed a new CB<sub>2</sub>R-targeted photosensitizer, IR700DX-mbc94. We found that phototherapy treatment using IR700DX-mbc94 greatly inhibited the growth of CB<sub>2</sub>R positive tumors, but not CB<sub>2</sub>R negative tumors. In addition, phototherapy treatment with non-targeted IR700DX did not show significant therapeutic effect. Similarly, treatment with IR700DX-mbc94 without light irradiation or light irradiation without the photosensitizer showed no tumor-inhibitory effect. Taken together, IR700DX-mbc94 is a promising phototherapy agent with high target-specificity. Moreover, CB<sub>2</sub>R appears to have great potential as a phototherapeutic target for cancer treatment.

**Acknowledgements:** We thank Dr. Nephi Stella at the University of Washington for providing DBT cells and technical advice. This work was supported by the startup fund provided by the Department of Radiology, University of Pittsburgh. This project used the UPCI imaging facilities supported, in part, by award P30CA047904. We also thank the Grant of Shanghai Science and Technology (12DZ1940606 and 12ZR1439900) and Grant of Shanghai Municipal Health Bureau (20124195) for supporting N.J.'s work. We also thank the National Institutes of Health National Institute on Drug Abuse (NIH NIDA) for grant R01DA025612 (to X.-Q.X.).

## PEPTIDE ENDOCANNABINOIDS (PEPCANS) ARE PAMs OF CB2 RECEPTORS AND INVOLVED IN THE INNATE IMMUNE RESPONSE

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Peptide endocannabinoids (Pepcans) are N-terminally extended “hemopressin peptides” endogenously present in different tissues, such as brain, with RVD-hemopressin (Pepcan12) being the major representative. We have previously shown that Pepcans are negative allosteric modulators (NAMs) at CB1 receptors (Bauer et al. J. Biol. Chem. 2012, 287, 36944-67). During receptor profiling and binding studies, we realized that unlike hemopressin, these peptides do not only bind to CB1 receptors, but also CB2 receptors. Using different *in vitro* assays, we show that Pepcans exert significant positive allosteric modulation (PAM) at CB2 receptors in the low nanomolar concentration range, both at the level of receptor binding and function (cAMP, GTPgammaS). Using our in house generated Pepcan mAb we measured levels of Pepcans in normal and inflamed peripheral tissues. To address the role of Pepcan12 during inflammation we studied its effect in macrophage polarization and differentiation, processes in which CB2 receptors play a role. We show that during constitutive CB2 receptor activation (likely produced by the 2-AG autocrine tone), Pepcan-12, which showed the most potent effect, already at low nanomolar concentrations modulates the shift from M1 to M2. Moreover, Pepcan12 inhibits osteoclastogenesis using stimulated primary human monocytes and controls endotoxin resistance in a model using human whole blood. As expected for a PAM at CB2 receptors, Pepcan12 acts synergistically with endocannabinoids in macrophage polarization in a CB2 receptor-dependent manner, though effects via CB1 receptors cannot be excluded.

Our data point to a prominent role of Pepcan-12 as endogenous modulator of CB2 receptors and in the innate immune response. To our knowledge, Pepcan-12 is the first endogenous PAM reported. Overall, our mechanistic studies with Pepcan12 point toward a key modulatory role of this peptide in the endocannabinoid system as it is a NAM for CB1 and PAM for CB2, thus an ideal physiological modulator of cannabinoid receptors in the periphery. Given the explicit aggregation behaviour of Pepcans *in vitro*, we have elaborated a protocol for the handling of these peptides. Future studies will address the site of production and receptor binding site of these peptides.

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## REVERSING THC-INDUCED IMPAIRMENT OF VERBAL MEMORY IN HEALTHY HUMANS

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One of the most often reported cognitive deficits of acute THC administration is an impaired recall of previously learned information. The aim of the present study was to determine whether THC-induced memory impairment in humans is mediated via glutamatergic or cholinergic pathways. Fifteen occasional cannabis users participated in a double blind, placebo-controlled, 6-way cross-over study. On separate test days, subjects received combinations of pre-treatment (placebo, vardenafil 20 mg or rivastigmine 3 mg) and treatment (placebo or 150 µg THC /kg bodyweight). Cognitive tests were administered immediately after inhalation of treatment was finished and included measures of memory (Visual Verbal Learning Task; Prospective Memory Test; Sternberg Memory Test), perceptual-motor control (Critical tracking task), attention (Divided Attention Task) and motor impulsivity (Stop signal task). The results of this study demonstrate that subjects under the influence of THC were impaired in all memory tasks, critical tracking, divided attention and the stop signal task. Pre-treatment with rivastigmine attenuated the effect of THC on delayed recall and non-significantly on immediate recall. When THC was given in combination with vardenafil, there were no significant interactions in any of the tasks. The present data therefore suggest that acetylcholine plays an important role in the THC-induced memory impairment, whereas for glutamate this has not been demonstrated in this study.

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**SELECTIVE STIMULATION OF CANNABINOID TYPE 2 RECEPTORS (CB<sub>2</sub>) IN MONOCYTES PREVENTS THEIR ENGAGEMENT OF BRAIN ENDOTHELIUM PROTECTING BLOOD BRAIN BARRIER**

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CB<sub>2</sub> is highly expressed in immune cells and stimulation decreases inflammatory responses. We tested the hypothesis that selective CB<sub>2</sub> activation in primary human monocytes diminishes their ability to engage the brain endothelium and migrate across the blood brain barrier (BBB). In an *in vitro* BBB model, CB<sub>2</sub> activation in monocytes substantially decreased adhesion to and migration across monolayers of primary human brain microvascular endothelial cells (BMVEC) and attenuated BBB injury. CB<sub>2</sub> stimulation in monocytes downregulated active forms of integrins, LFA-1 and VLA-4. Cells treated with CB<sub>2</sub> agonists showed increased levels of inhibitory sites of the actin binding proteins, cofilin and VASP, upstream regulators of conformational integrin changes. Activated by relevant stimuli, small GTPases Rac1 and RhoA were suppressed by CB<sub>2</sub> agonists in monocytes paralleling decreased formation of lamellipodia that play a key role in monocyte migration. Intravital videomicroscopy was used to quantify adhesion of leukocytes to cortical vessels in LPS-induced neuroinflammation, following injection of *ex vivo* CB<sub>2</sub>-activated leukocytes into mice; CB<sub>2</sub> agonists markedly decreased adhesion of *ex vivo* labeled cells *in vivo*. These results indicate that selective CB<sub>2</sub> activation in leukocytes decreases key steps in monocyte-BBB engagement suppressing inflammatory leukocyte responses and preventing neuroinflammation. Development of selective, specific, nontoxic and efficacious CB<sub>2</sub> agonists with improved pharmacologic properties will provide new treatment for neuroinflammatory disorders.

Funding: supported by NIH grant AA015913

## CB<sub>2</sub> AGONISTS: HOW SELECTIVE ARE THEY?

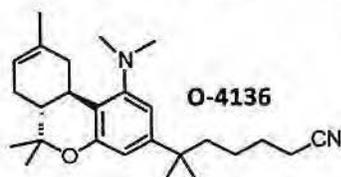
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Of the two identified cannabinoid receptors, the CB<sub>2</sub> receptor has received far less research attention than the CB<sub>1</sub> receptor. Until recently, CB<sub>2</sub> receptors were believed to be confined to the periphery, but recent research suggests that they may also be located in the CNS, albeit their numbers and distribution may not as extensive as CB<sub>1</sub> receptors (Atwood & Mackie, 2010). Several structural motifs have served as templates for the development of selective CB<sub>2</sub> agonists that may be used to investigate these possible CNS effects. In the present study, we evaluated a series of C-1 amino and aminoalkyl substituted dibenzopyrans in a battery of in vitro and in vivo assays. Results for one of these compounds (O-4136) are presented here.

In CP55,940 displacement assays, O-4136 showed 86-fold selectivity for the CB<sub>2</sub> vs CB<sub>1</sub> receptor (CB<sub>2</sub> K<sub>i</sub>=29±3.8 nM; CB<sub>1</sub> K<sub>i</sub>=2491±404 nM). Further, its receptor activation profile in [<sup>35</sup>S]GTPγS mirrored its binding profile, with no activation of CB<sub>1</sub> receptors and an E<sub>max</sub>=99% (compared to E<sub>max</sub>=109% for CP55,940) for the CB<sub>2</sub> receptor (EC<sub>50</sub>=43±8 nM). In vivo, O-4136 produced characteristic cannabinoid agonist effects in the tetrad in mice and these effects were attenuated by rimonabant. In



mice trained to discriminate THC from vehicle, O-4136 (30 mg/kg) partially substituted (~70%) without suppression of responding. This partial substitution was reversed by rimonabant, but not by the CB<sub>2</sub> antagonist SR144528.

Based upon in vitro results, O-4136 appears to be a classic CB<sub>2</sub>-selective agonist. In contrast, in vivo results suggest that it may also have CB<sub>1</sub>-receptor mediated effects, despite its low affinity for this receptor. Previous findings have suggested that CB<sub>2</sub> receptor ligands demonstrate functional selectivity in which signaling pathways they activate, with WIN55,212-2 showing the most selectivity and CP55,940 showing the least (Atwood et al., 2012). While these prior results point to the need to include diverse structural motifs in investigation of CB<sub>2</sub> receptor functioning, the present results emphasize the need for evaluation of putative selective ligands in both in vitro and in vivo assays. Hence, in investigation of the selectivity of CB<sub>2</sub> ligands, “how selective is it?” might be a less pertinent question than “*how* is it selective?”

**Acknowledgements:** Funded by National Institutes of Health National Institute on Drug Abuse Grant DA-003672.

Atwood and Mackie. (2010). *Br J Pharmacol*, **160**: 467-79.

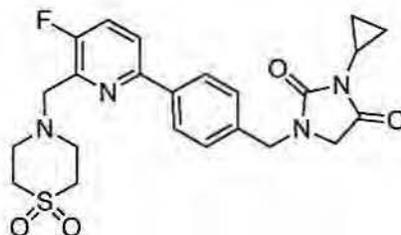
Atwood et al. (2012). *Mol Pharmacol*, **81**: 250-63.

## PERIPHERALLY RESTRICTED, SELECTIVE CANNABINOID CB<sub>2</sub> RECEPTOR AGONIST LEI-101 PREVENTS CISPLATIN-INDUCED NEPHROPATHY

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Recently, we have identified LEI-101 as a novel, peripherally restricted cannabinoid CB<sub>2</sub> receptor agonist. LEI-101 is a potent, selective and orally bioavailable CB<sub>2</sub> receptor agonist (hCB<sub>2</sub> pEC<sub>50</sub> (cAMP) = 8.0; hCB<sub>1</sub> pEC<sub>50</sub> < 5; F<sub>po</sub> = 100%), which was active in a rat spinal nerve ligation model of neuropathic pain [1]. Here, we present the further characterization of this compound in both *in vitro* and *in vivo* models. LEI-101 is ~100-fold more selective in a CB<sub>2</sub> than in a CB<sub>1</sub> binding assay, and does not display any activity on hFAAH, MAGL, NAPE-PLD and DAGL. In a clinically relevant murine model of nephropathy (induced by the widely used antineoplastic drug cisplatin), in which the tubular injury is largely dependent on inflammation and oxidative/nitrative stress [2], we found that LEI-101 dose-dependently ameliorated kidney dysfunction and morphological damage. At 3 or 10 mg/kg (po) LEI-101 largely prevented cisplatin-induced increases in serum creatinine and blood urea nitrogen levels, improved renal histopathological injury, and attenuated oxidative/nitrative stress and inflammation in the kidney. These protective effects were absent in CB<sub>2</sub> KO mice, which is indicative of a CB<sub>2</sub>-mediated effect. In addition, LEI-101 up to a dose of 60 mg/kg (po) did not exert any effects in the mouse tetrad assay of cannabimimetic activity. These results suggest that peripherally restricted CB<sub>2</sub> agonist LEI-101 has a good therapeutic potential in kidney and other diseases that are associated with inflammation and oxidative stress.



**LEI-101**

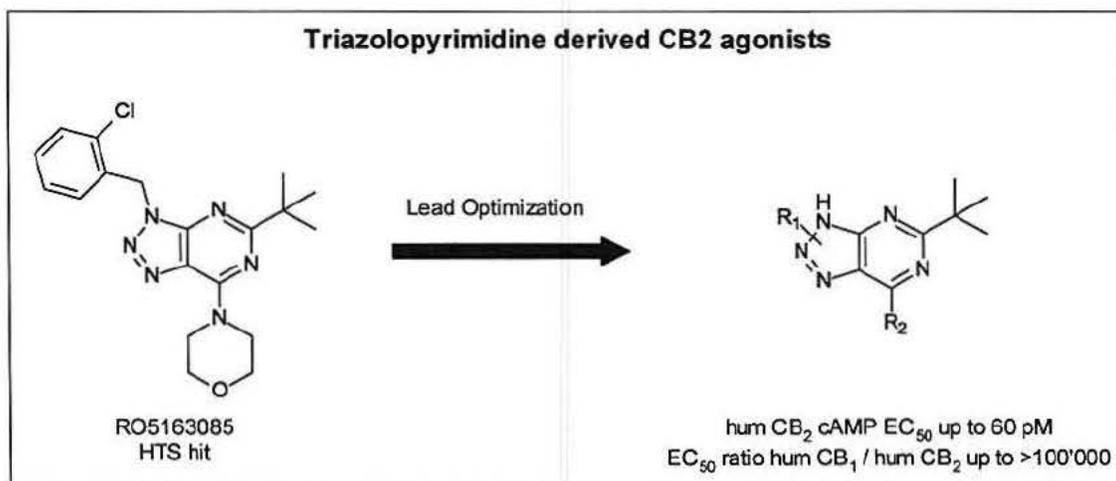
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[2] Mukhopadhyay et al., Free Radic. Biol. Med. 2010, 48, 457-467.

## TRIAZOLOPYRIMIDINES – A NOVEL CLASS OF HIGHLY POTENT, HIGHLY SELECTIVE AND IN VIVO ACTIVE CB<sub>2</sub> AGONISTS

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CB<sub>2</sub> mediated *in vivo* efficacy is often highlighted using CB<sub>2</sub> agonists which are not very selective against the CB<sub>1</sub> receptor subtype. Therefore, it is difficult to unequivocally assign the pharmacodynamics effects to CB<sub>2</sub> rather than CB<sub>1</sub> activation. We generated novel triazolopyrimidine derived CB<sub>2</sub> ligands highly selective against the CB<sub>1</sub> receptor. Starting from the potent high throughput screening hit RO5163085, which exhibited over 2'000 fold selectivity against the human CB<sub>1</sub> receptor in a cAMP functional assay, lead optimization provided a series of structurally novel 1- and 2-substituted triazolopyrimidines that stimulate CB<sub>2</sub> receptors with potencies of up to 60 pM.



Details of their structure activity relationship for CB<sub>2</sub> and CB<sub>1</sub> binding and functional assays will be the subject of this communication. Additionally the physicochemical properties including solubility, membrane permeation and lipophilicity as well as metabolic stability and cytochrome P450 inhibition potential were optimized. Advanced compounds combined high *in vitro* potency with favorable early ADME properties and have been profiled in *in vivo* pharmacokinetic and efficacy studies. Selected compounds such as RO6871304 were found to protect mouse kidneys from ischemia reperfusion injury. After a period of 25 min ischemia @ 37 °C and 24 h reperfusion (n=6 per group) 10 mg/kg RO6871304 p.o. produced a statistically significant improvement of kidney function as measured by plasma creatinine levels (51% improvement; p<0.03). Moreover, the efficacy of RO6871304 was reflected in the reduction of relevant plasma biomarkers of kidney injury (NGAL 75%, p<0.001; osteopontin 85%, p<0.001). In addition, RO6871304 significantly reduced fibrosis in a rat unilateral ureter obstruction model (UUO) as measured by a 39% reduction in collagen I deposition 8 d after UUO (n=6, p=0.02), thereby suggesting that CB<sub>2</sub> agonists might have beneficial effects in both acute and chronic kidney disease. These data suggest that CB<sub>2</sub> activation mediates protective effects in the kidney.

## “WEED AGAINST ZIT?” – EXPLORATION OF THE MECHANISMS OF THE COMPLEX ANTI-ACNE ACTIONS OF CANNABIDIOL

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We have previously shown that the non-psychotropic phytocannabinoid cannabidiol (CBD) quantitatively and qualitatively normalized the “pro-acne agents” (e.g. arachidonic acid) induced lipogenesis of human SZ95 sebocytes, via the activation of transient receptor potential vanilloid-4 (TRPV4) ion channels. Moreover, we have also demonstrated that CBD exerted a unique “trinity of cellular anti-acne actions”; indeed, besides its lipostatic action, it suppressed proliferation of the sebocytes (both *in vitro* and *ex vivo* in full thickness human skin organ culture) and showed remarkable, universal anti-inflammatory effects. However, the exact mechanism-of-action of the above findings remained unclear. Therefore, in our current study, we aimed at exploring the intracellular “anti-acne” signaling pathways coupled to CBD.

First, we showed that, similar to the lipostatic effect, anti-proliferative action of CBD was mediated via the activation of TRPV4, whereas TRPV4-antagonism was unable to abolish the anti-inflammatory effect. In order to identify the signaling pathway(s) underlying the beneficial anti-acne actions, genome-wide microarray analyses were performed. These analyses (followed by confirmatory RT-qPCRs) identified TRPV4-dependent alterations in the expressions of lipid synthesis (down-regulation of nuclear receptor interacting protein-1 [NRIP1; positive regulator of the lipid synthesis in adipocytes]; and up-regulation of Rho GTPase activating protein-9 [ARHGAP9; endogenous inhibitor of the pro-lipogenic ERK-signaling]) and proliferation-related genes (down-regulation of Ki67), as well as TRPV4-independent regulation of various “immune” genes (e.g. up-regulation of tribbles homolog 3 [TRIB3; an inhibitor of the pro-inflammatory P65-NFκB pathway]). Next, we investigated the putative causative role of these newly identified target genes and the related signaling pathways in mediating the various anti-acne modalities of CBD. We found that the lipostatic activity was mediated by a TRPV4-dependent interference with the “pro-lipogenic” ERK1/2 mitogen activated protein kinase pathway and the down-regulation of NRIP1. Importantly, silencing of NRIP1 mimicked the lipostatic effect of CBD. On the other hand, the anti-inflammatory action was found to be mediated by the A2a adenosine receptor-dependent up-regulation of TRIB3 and the subsequent inhibition of the pro-inflammatory P65-NFκB-signaling.

Taken together, our results demonstrate that CBD exerts its complex anti-acne effects (lipostatic, anti-proliferative and anti-inflammatory actions) on human sebocytes via two parallel signaling pathways, i.e. via the TRPV4→[Ca<sup>2+</sup>]<sub>ic</sub>↑→ARHGAP9↑ and NRIP1↓ (lipostatic/anti-proliferative effects) and the A2a→cAMP→TRIB3↓NFκB (anti-inflammatory effects) pathways. Therefore, CBD, and possibly other modulators of the identified signaling pathways, might be powerful, novel tools in the future treatment of acne vulgaris.

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## CANNABINOID AND TERPENOID PROFILING OF CANNABIS IN CALIFORNIA AND WASHINGTON

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While already well into the thousands the current number of different *Cannabis Sativa L.* strains presently available in legal markets is continuously on the rise. Consumers seeking medical relief would like to see consistency in a cultivar while some recreational users may continuously seek out novel and uniquely flavored varieties. All consumers are interested in the potential physiological impacts of each unique varietal. Numerous cultivars have received critical acclaim for their potency or general physiological impacts through international awards and press publications which popularized a specific name or national media outlets and documentaries singling out a cultivar for its' high cannabidiol content. Colorful names such as "OG Kush," "Lemon Haze," "Jack Herer," and "Charlotte's Web" have been used to represent a unique varietal. With popularity of a name comes the greater potential for its abuse by those simply seeking to capitalize on a transaction involving the cultivar.

UPLC-UV based cannabinoid and GC-FID based terpenoid chemical profiling methods coupled to principle component analysis has led us to the current understanding that there is considerable lack of standardization of cultivation methods with a concurrent lack of uniformity of chemotypic profiles across some popularized cultivar names. We have observed non-distinct chemotype profiles for "indica" and "sativa" designations, despite those terms medicinally being touted as specifically delivering a more sedative or more stimulating physiological effect respectively. Ultimately a specifically named cultivar at one dispensary is not necessarily the same product in the package at another dispensary simply because it possesses the same name. This may also be the case even week to week at the same dispensary, lending towards many different reports of the physiological impacts for a specific name and unfortunately considerable numbers of frustrated patients seeking to find relief with a specific varietal.

The latest results of our combined cannabinoid and terpenoid chemical profiling efforts across both California and Washington will be presented to provide an overview of the naming, current classifications and representative chemical profiles of the numerous cannabis cultivars available in these markets.

## **“SMELLS LIKE GOOD WEED”: THC LEVELS IN CANNABIS MAY BE PREDICTED BY MONOTERPENE HEADSPACE CONCENTRATIONS**

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The characteristic odour of cannabis is due primarily to a mixture of terpenes, with substantial variance between cannabis strains in terpene concentrations leading to markedly different odours between plants. Many consumers use smell to predict the potency of cannabis despite  $\Delta^9$ -tetrahydrocannabinol (THC) and other phytocannabinoids being odourless. This link between odour and potency is plausible given common biosynthetic precursors for terpenes and phytocannabinoids within the plant, and previous research that shows a correlation between total terpene and phytocannabinoid levels (Fischedick et al., *Phytochemistry* 71 (2010) 2058-2073). To our knowledge, however, the association between the levels of terpenes as they occur naturally in the headspace of cannabis plant material and specific phytocannabinoids has never been directly examined. In the present study, we therefore analysed 40 cannabis samples for THC content using our previously published HPLC methods (Swift et al., *PLoS One* 8 (2013) e70052) and volatile terpenes using dynamic headspace sampling coupled with GC-MS.

Exploratory factor analyses revealed a three factor solution best described the data accounting for 74.88% of the variance in the terpene set. Several monoterpenes loaded strongly onto THC content, including  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene,  $\beta$ -linalool and D-limonene (loadings between 0.5 and 0.9). Conversely sesquiterpenes such as  $\beta$ -caryophyllene and humulene loaded onto their own factors separate from THC. Regression analysis reveals that the monoterpene factor significantly predicts THC content ( $F_{1,21}=8.64$ ,  $p=0.008$ ), whereas the other two factors did not ( $p=0.4$  and  $0.2$ ). Mean sample THC content (computed as THC + THC-A content) was 16.7% (SD=10.04, Range: 0.95–39.76%).

These results may reflect the fact that monoterpenes and THC have common biosynthetic precursors in the plastidial deoxyxylulose phosphate/methyl-erythritol phosphate pathway. Sesquiterpenes, on the other hand, may rely on the cytosolic mevalonate pathway. Since monoterpenes strongly influence the odour of cannabis, these results lend some support to the seemingly common consumer strategy of using odour to predict potency. This can be verified in future human psychophysical tests. Our approach might also provide an analytical method to approximate plant potency from sample headspace without further sample preparation or destruction. Interestingly, given emerging evidence of potentiation of some phytocannabinoid effects by terpenes, monoterpenes may become particularly influential when their levels rise along with increasing THC content.

## PROTECTIVE EFFECT OF THE PHYTOCANNABINOID BETA-CARYOPHYLLENE IN LIVER ISCHEMIA REPERFUSION INJURY

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Liver transplantation is the ultimate therapy for end-stage liver disease. The availability of donor liver is severely limited, major approaches are underway to extend the donor criteria. Warm ischemia time is an independent risk factor for transplant dysfunction, effective novel strategies are needed to protect the liver against ischemia reperfusion injury. As a proof-of-concept, we have previously shown that activation of cannabinoid CB2 receptors in the liver protects against ischemia-reperfusion (I/R) injury. We have demonstrated the therapeutic protection for several synthetic CB2 agonists. Beta-caryophyllene (BCP) is a plant-derived natural non-toxic and potent CB2 receptor agonist that has shown an array of therapeutic effects in preclinical studies. In this study we assessed effects of systemic application of BCP in a clinically relevant rodent model of hepatic I/R injury. Hepatic I/R injury was induced by 60 min ischemia and followed by 2, 6 or 24h reperfusion *in vivo*.

BCP given as pretreatment before the induction of I/R, attenuated hepatic injury (measured by serum alanine aminotransferase and aspartate aminotransferase levels), decreased oxidative stress markers (tissue protein carbonyl adducts, 4-hydroxy-2-nonenal), inflammatory markers (chemokines such as CCL3 and CXCL2, TNF- $\alpha$ , intercellular adhesion molecule 1 (CD54) mRNA levels) and hepatocyte apoptosis (caspase 3/7 activity and DNA fragmentation). Histological evaluation revealed reduced tissue neutrophil infiltration. Protective effects of BCP against liver injury were still present when the compound was given at the onset of reperfusion.

In summary, hepatic I/R is associated with CB2 receptor activation and BCP treatment successfully decreased tissue injury and inflammation in our *in vivo* model. BCP treatment offers a novel therapeutic strategy against warm ischemia in liver and might be useful to preserve donor livers in the clinical setting.

## EFFECT OF BETA-CARYOPHYLLENE ON PHENCYCLIDINE-INDUCED BEHAVIOURAL CHANGES

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Core evidence from laboratory and clinical research points to the involvement of the endocannabinoid system in the development of schizophrenia. Specifically, recent studies have highlighted a role for the cannabinoid CB<sub>2</sub> receptor in schizophrenia. In this study the effect of beta-caryophyllene, a CB<sub>2</sub> receptor-selective agonist, was investigated in a mouse model for schizophrenia. Phencyclidine (PCP; 5 mg/kg), an NMDA antagonist, was injected after birth to Sabra mice. The effect of beta-caryophyllene (10 mg/kg; 3 times a week for 14 days) on locomotor activity was studied in the open field test. Compared with the control group, PCP significantly decreased the exploration activity and the number of rears ( $p < 0.004$ ). Treatment with beta-caryophyllene significantly reversed the effect of PCP on rearing ( $p < 0.008$  vs. PCP-treated). Thus, following treatment with beta-caryophyllene the rearing activity of PCP-induced mice was not significantly different from that of the control group. The CB<sub>2</sub> receptor antagonist/inverse-agonist AM630 (10 mg/kg) inhibited the effect of beta-caryophyllene on rearing activity and significantly reversed the effect of beta-caryophyllene on ambulation ( $p < 0.04$ ). These results further support our previous study with HU-308 and suggest that under certain conditions, such as the inhibition of the NMDA receptors, there is a role for the CB<sub>2</sub> receptor in the modulation of motor activity.

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## **CANNABIDIOL PROVIDES PROTECTION FROM ETHANOL AND AMMONIUM TOXICITY IN A HIPPOCAMPAL MODEL OF HEPATIC ENCEPHALOPATHY**

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Hepatic encephalopathy (HE) is a neuropsychiatric disorder that includes learning deficits and impairment of long-term memory. HE can be caused by chronic and excessive ethanol ingestion along with the accumulation of toxic substances that are normally removed by the liver. The pathogenesis of HE in the central nervous system includes damage to the prefrontal cortex, striatum and the hippocampus, and this pathology is believed to be mediated by the accumulation of free radicals and oxidative stress. In the present study, an *in vitro* model of HE has been utilized to evaluate the protective properties of cannabidiol (CBD), a substance with demonstrated protective properties against oxidative stress. For these studies, embryonic day 18 hippocampal tissue was utilized to prepare dissociated cultures consisting of a mixture of neurons and non-neuronal cells. Fluorescent assays measuring cell death (propidium iodide) and neuronal viability (CFDA) were multiplexed in the same culture well to assess the response of the toxins as well as efficacy and potency of the CBD treatment.

The amounts of ethanol and ammonium acetate required to produce a relevant and reproducible toxic response in the hippocampal cultures were determined. These toxic responses combined with the clinically determined amount of ammonia in stage 4 HE helped establish a working concentration of ammonium acetate (300  $\mu$ M). An ethanol concentration of 30 mM was used to produce toxicity. Concentration-effect studies with CBD indicated EC<sub>50</sub>'s of 1  $\mu$ M for both toxins as estimated with the neuronal viability assay. Slightly increased EC<sub>50</sub>'s for CBD were observed to prevent cell death: 4  $\mu$ M. The EC<sub>90</sub> for these protection assays ranged between 3-10  $\mu$ M. The protective effects of CBD were also measured in cultures receiving combinatorial treatment with ethanol and ammonium acetate revealing EC<sub>90</sub>s of 8-14  $\mu$ M. In all cases, the efficacy levels after CBD treatment brought toxin-induced changes back to control levels. Further exploration of CBD responses in hippocampal cultures indicated a significant decrease in neuronal viability (25  $\pm$  3% of control) at 100  $\mu$ M. These studies suggest that CBD and CBD-like substances may provide significant protective value for the treatment of HE. Based on these results, our future CBD-related analogs will focus on safety as well as protective efficacy and enhancement of drug-like properties.

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## NATURAL PRODUCT-DERIVED CANNABIMIMETICS AS SOURCE OF POLYPHARMACOLOGY IN THE ENDOCANNABINOID SYSTEM

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To generate cannabimimetic agents that exert polypharmacology within the lipid network of the endocannabinoid system (ECS) is becoming a new concept for rational drug design. In the past decade, selective and potent pharmacological compounds have been reported for all known major targets in the ECS (CB1 and CB2 receptors, FAAH and MAGL). In recent years, several research groups have put efforts in studying polypharmacology in the ECS, mainly by modifying pre-existing molecules or by generating new chemical entities. We have approached ECS polypharmacology by starting from the unlimited source of multidiverse natural products. For example, we have investigated several chemical modifications of the widespread plant sesquiterpene  $\beta$ -caryophyllene, already known to be a CB2 receptor-selective agonist showing anti-inflammatory, antifibrotic, and analgesic effects *in vivo*. Structural insights into the pharmacophore of this hydrocarbon, which lacks functional groups other than double bonds, are missing. Our structure-activity study provides evidence for the existence of a well-defined sesquiterpene hydrocarbon binding site in CB2 receptors, highlighting its exquisite sensitivity to modifications of the strained endocyclic double bond of  $\beta$ -caryophyllene. While most changes on this element were detrimental for activity, ring-opening cross metathesis of  $\beta$ -caryophyllene with ethyl acrylate followed by amide functionalization generated a series of new monocyclic amides that not only retained the CB2 receptor functional agonism of  $\beta$ -caryophyllene with similar potency, but also reversibly inhibited FAAH (in the low micromolar range). Intriguingly, further modification of this monocyclic scaffold generated the FAAH and endocannabinoid substrate specific COX-2 dual inhibitors, which are probes with a novel pharmacological profile. This study shows that by removing the conformational constraints induced by the medium-sized ring, and by introducing functional groups in the sesquiterpene hydrocarbon of  $\beta$ -caryophyllene, a new scaffold with pronounced polypharmacological features in the ECS could be generated. We also investigated natural products with the aim of identifying new probes with polypharmacological features. To that aim, a library of derivatives of the lignans magnolol and 4-*O*-methylhonokiol, a selective CB2 mixed-typed biased agonist, was screened on all major ECS targets. Modification of the substituents on the biphenyl scaffold of 4-*O*-methylhonokiol and magnolol led to the identification of selective COX-2 substrate specific inhibitors with submicromolar potency showing selectivity towards endocannabinoid over arachidonic acid oxygenation. The structural and functional repertoire of cannabimimetics and their yet poorly understood intrinsic promiscuity may be exploited to generate novel probes and ultimately more effective drugs. These compounds could serve as probes to guide the *de novo* design of agents that synergistically target different components of the ECS. Moreover, novel probes differentially targeting the intricate network of lipid pathways may be useful to better understand synergy in endocannabinoid function.

## CANNABIDIOL REPEATED TREATMENT INCREASES SURVIVAL AND PROMOTES RESCUE OF COGNITIVE FUNCTION IN A MURINE MODEL OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a severe complication resulting from *Plasmodium falciparum* infection that causes permanent neurological and behavioral deficits after infection resolution by antimalarial drugs. Cannabidiol (CBD) is the major nonpsychotomimetic constituent of *Cannabis sativa* with neuroprotective properties. The present work aimed to determinate if CBD treatment could prevent behavioral changes found in mice infected by *Plasmodium berghei*-ANKA (PbA). Female C57Bl6 mice were infected or not with PbA (10<sup>6</sup> parasitized/0.2 mL PBS i.p.). On day 3 after infection (dpi) all groups received the first injection of CBD (30mg/Kg/day-7 days i.p.) or vehicle. On 5dpi, infected animals started to be treated with artesunate (32mg/kg/day-5 days i.p). Five days after completed clearance of parasitemia, all groups were submitted to the Object Recognition task (OR) and to the Elevated Plus Maze (EPM). After the EPM (18<sup>th</sup> dpi) animals were sacrificed under deep anesthesia, their brains removed and prefrontal cortex (PFC) and hippocampus (HC) dissected for cytokines (IL-2, IL-4, IL-6, IL-10, IL-17, TNF- $\alpha$  and IFN- $\gamma$ ) and neurotrophins (BDNF and NGF) determination.

Cannabidiol treatment increased the survival of PbA-infected mice. After the complete clearance of the parasitemia by artesunate, PbA vehicle treated mice displayed memory deficits in the OR (represented by decrease in the % of new object index) and exhibited an increase in anxiety-like behaviors in the EPM. Also, levels of TNF- $\alpha$  was found increased in HC while levels of IL-6 were found increased in PFC and HC on day 5<sup>th</sup> post-infection but not after parasite clearance. Cannabidiol treatment was able to prevent long lasting anxiogenic and cognitive impairment found and in PbA mice. CBD treatment also increase BDNF expression in the HC after the complete parasite clearance. Our results indicate that CBD could be useful as an adjunctive therapy to prevent brain damage during the course of CM.

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## CANNABIDIOL PROMOTES OLIGODENDROCYTE SURVIVAL AFTER HYPOXIA-ISCHEMIA IN NEWBORN RATS

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**Background:** Hypoxic-ischemic (HI) insults enhance the proliferation of oligodendrocyte (OL) precursors (preOL) in immature brain. Survival of those proliferating preOL, however, is very poor because those cells are particularly sensitive to oxidative stress and inflammation. This leads eventually to hypomyelination, which plays a key role in the genesis of cerebral palsy. We described that cannabidiol (CBD) increases the number of proliferative cells in newborn rat brain after a hypoxic-ischemic (HI) insult. In the present work we aimed to determine how CBD treatment affects OL survival.

**Methods:** Unilateral HI brain damage was induced in newborn Wistar rats (7 day-old: P7) by exposure to hypoxia (10% FiO<sub>2</sub>) for 112 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups received s.c. vehicle (HV, n=18) or CBD 1 mg/kg single dose (HC, n=24). Other pups remained as controls (SHM, n= 16). One (P8), 7 (P14) or 30 (P37) days after HI rats were sacrificed, transcardially perfused with formalin 4% and their brains cut off into coronal slices for Nissl staining for a neuropathological score (NPS; 0=no damage; 5= massive tissue loss) in P8 cortex and for immunohistochemical (IHC) study on the subventricular zone (SVZ) in P8 and P14 rats: Ki67 was used to detect proliferating cells, Olig-2 for glial precursors and SOX-10 for preOL. In P37 GST $\pi$  IHC and Myelin basic protein (MBP) fluorescence in the external capsule was used to quantify the presence of mature OL.

**Results:** HI insult was associated with a decrease of the proliferative response in SVZ 7 days after the HI insult affecting mainly glial precursors. CBD administration prevented that decrease to occur. In particular, CBD administration led to a dramatic increase of preOL proliferation in the following hours after HI. As a result, CBD administration blunted the HI-induced decrease of long-term myelination, preserving mature OL and myelin production.

Group	NPS		Ki67		Olig2		SOX-10		GST $\pi$	MBP
	P14	P8	P14	P8	P14	P8	P14	P37	P37	
SHM	100(10)	100(11)	100(11)	100(8)	100 (9)	100(10)	100(16)	100(28)	51.8(4)	
HV	<i>307(22)</i>	<i>94.6(11)</i>	<i>65.1(10)</i>	81.6(14)	87.4(11)	<i>24.5(4)</i>	156(22)	<i>78(13)</i>	<i>38.5(7)</i>	
HC	<b>217(40)</b>	94.1(6)	<b>95(20)</b>	92.8(5)	<b>106.1(10)</b>	<b>165(50)</b>	172(30)	<b>100(14)</b>	<b>52.1(7)</b>	

Mean (SEM). Results from NPS and IHC are normalized for SHM values (%). MBP fluorescence is normalized for the contralateral (healthy) brain hemisphere (%). *Italic: p<0.05 vs SHM. Bold: p<0.05 vs. HV*

**Conclusions:** CBD administration preserves neuroproliferation, activating preOL and preserving OL maturation after a HI insult, thus preventing HI-induced hypomyelination.

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