

M ntréal



**45th meeting of the International Narcotics Research Conference
Montreal, Quebec, Canada
July 13-18, 2014**

Program and abstracts

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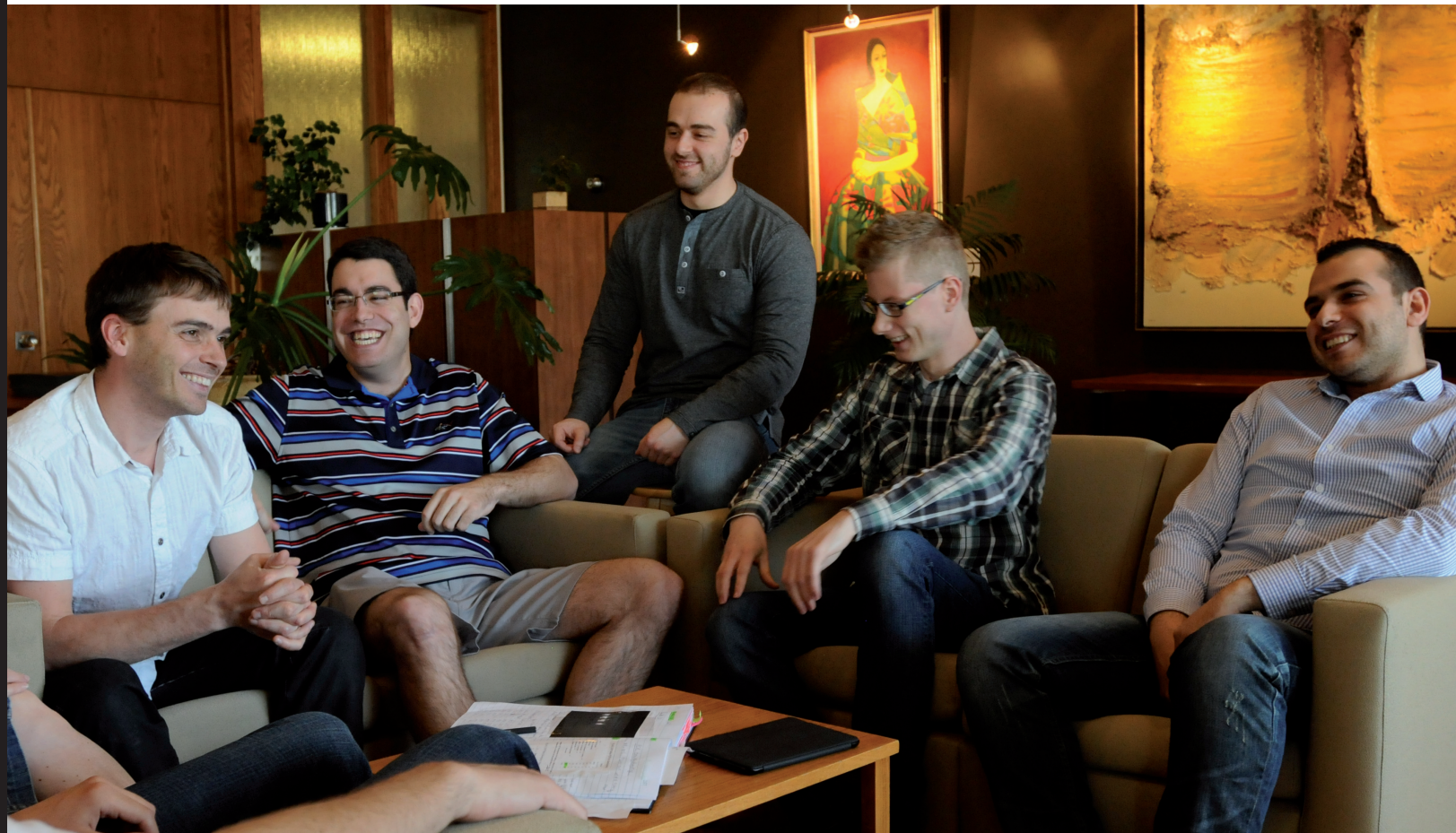
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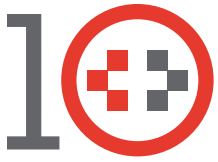
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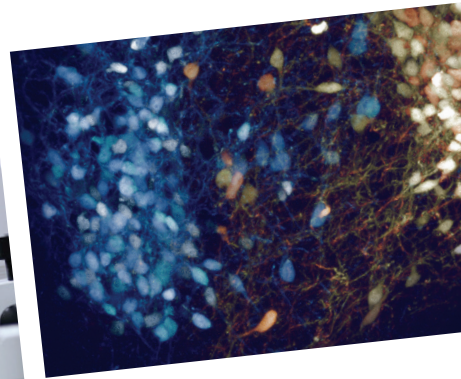
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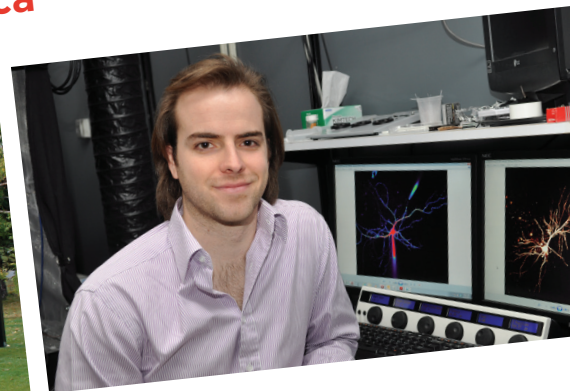
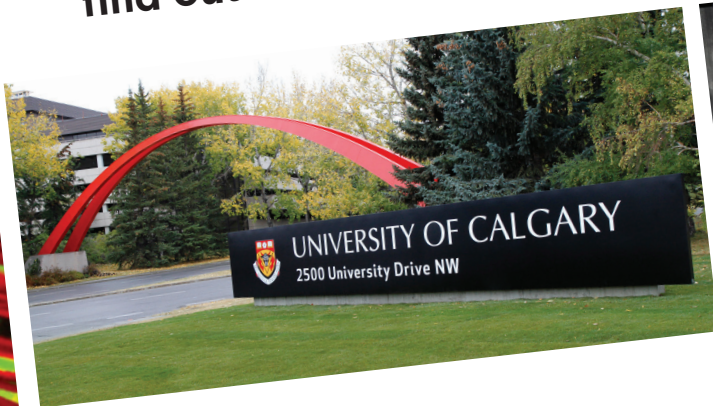
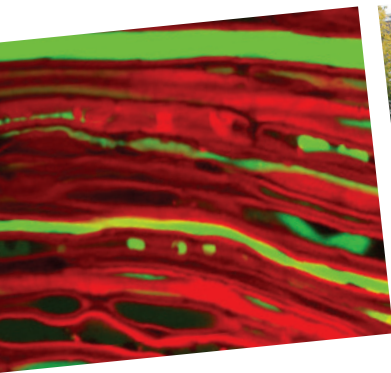
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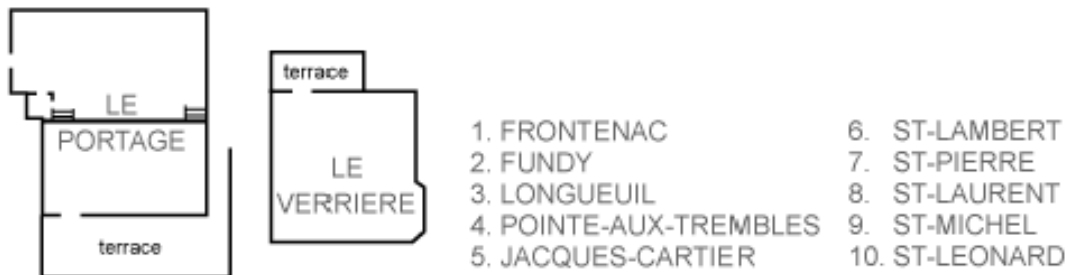
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- 13. CÔTE ST LUC
- 14. WESTMOUNT
- 15. OUTREMONT
- 16. VERDUN
- 17. LACHINE
- 18. LASALLE
- 19. Registration Desk

Program

Sunday July 13th

- 15:00-20:00 **Registration**
18:00-20:00 **Welcome reception** (Le Portage)

Monday July 14th

- 8:00-15:00 **Registration**
6:30-8:00 **Breakfast provided** (Fontaine A)

7:50-8:00 **Welcoming Remarks** (Louis Gendron and John Traynor)

Plenary lecture #1 (Westmount)

8:00-8:45 **Roger Sunahara** (University of Michigan)
“Structural and functional characterization of the receptor-G protein cooperativity”

Symposium #1 (Westmount)

RECEPTOR CRYSTAL STRUCTURE AND NOVEL OPIOID CHEMISTRY

Chair: **Peter Schiller** (Institut de Recherche Clinique de Montréal)

Discussion leader: **Ivy Carroll** (Research Triangle Institute)

8:45-9:10 **Gustavo Fenalti** (Scripps Research Institute)
“Molecular Control of Opioid Receptor Signaling”

9:10-9:35 **Nurulain Zaveri** (Astraea Therapeutics)
“Which way is up? Rethinking Nociceptin Ligand Design and SAR From Nociceptin Opioid Receptor Crystal Structure and Active-State Homology Model comparisons”

9:35-10:00 **Philip Portoghese** (University of Minnesota)
“MOR heteromers as targets for treatment of inflammatory and neuropathic pain”

10:00-10:20 **Nutritional break**

10:20-10:45 **Stephen Husbands** (University of Bath)
“Relapse prevention: targeting multiple receptors”

Hot Topics

10:45-10:55 -D. Tourwé
“Synthesis and biological evaluation of a compact, conformationally constrained bifunctional opioid agonist-neurokinin-1 antagonist”

10:55-11:05 -K.E. Livingston
“Probing Allosteric Modulation of the Mu Opioid Receptor”

11:05-11:15 -P.D. Mosier
“Analogues of salvinorin A bearing sulfur-containing substituents at position C2 are high-affinity kappa-opioid receptor partial agonists with a potentially novel binding mode”

- 11:15-11:25 -A.A. Harland
 "Synthesis, Optimization, and Evaluation of Mixed-Efficacy μ -Opioid Receptor Agonists/ δ -Opioid Receptor Antagonists: A Balancing Act"
- 11:25-11:35 -R.D. Howells
 "Novel small molecule triazoles modeled from naltrindole potently inhibit the proliferation of human multiple myeloma cells"
- 11:35-12:00 **Discussion period**
- 12:00-13:00 **Lunch provided** (Fontaine A)
- Symposium #2 (Westmount)
REVISITING THE THERAPEUTIC POTENTIAL OF DELTA OPIOID RECEPTOR LIGANDS
 Chair: **Junzo Kamei** (Hoshi University)
 Discussion leader: **Emily Jutkiewicz** (University of Michigan)
- 13:00-13:25 **Gregory Scherrer** (Stanford University School of Medicine)
 "Delta Opioid Receptor Function in Skin: Controlling Pain Where it Starts"
- 13:25-13:50 **Jon Violin** (Trevena Inc.)
 "Biased ligands at mu and delta opioid receptors: targeting selective signalling to develop improved therapeutics"
- 13:50-14:15 **Manojkumar Puthenveedu** (Carnegie Mellon University)
 "Increasing delta opioid receptor bioavailability"
- 14:15-14:40 **Amynah Pradhan** (University of Illinois at Chicago)
 "The Potential of Delta Opioid Receptor Agonists for the Treatment of Migraine"
- 14:40-15:00 **Nutritional break**
- Hot Topics
- 15:00-15:10 -M. Bigliardi-Qi
 "Delta Opioid receptors: Important Role In wound healing"
- 15:10-15:20 -A. Saitoh
 "The DOR2 antagonist naltriben abolishes KNT-127-induced anxiolytic-like effects in rats"
- 15:20-15:30 -A.B. Tudashki
 "Ligand ability to evoke $G\alpha_i$ activation and to promote internalization of DOR can predict response duration over a short period"
- 15:30-16:00 **Discussion period**
- 16:00-18:00 **Poster session #1** (odd numbers; Fontaine B-C)

Tuesday July 15th

8:00-15:00 **Registration**
6:30-8:00 **Breakfast provided** (Fontaine A)

Founder's lecture (Westmount)

8:00-9:00 **Brigitte Kieffer** (McGill University) and
Christopher Evans (University of California Los Angeles)
"Cloning: twice in one day"

Symposium #3 (Westmount)

CHANGES IN REWARD CIRCUITRY IN CHRONIC PAIN.

Chair: **Howard Fields** (University of California San Francisco)

Discussion leader: **Catherine M. Cahill** (University of California Irvine)

9:00-9:25 **Marco L. Loggia** (Harvard Medical School)
"Disrupted brain circuitry for pain-related reward/punishment in fibromyalgia"

9:25-9:50 **Lucia Hipolito** (Columbia University)
"Chronic pain increases opioid self-administration through an accumbal dopaminergic mechanism"

9:50-10:10 **Nutritional break**

10:10-10:35 **Anna Taylor** (University of California Irvine)
"Neuropathic Pain Modulates Dopaminergic Circuitry: Role for Microglial Activation"

10:35-11:00 **Zhizhong Pan** (University of Texas)
"Pain facilitates response to opioid reward via a shared epigenetic pathway"

Hot Topics

11:00-11:10 -S.D. Comer
"Abuse liability of oxycodone and morphine in buprenorphine-maintained participants with or without chronic pain"

11:10-11:20 -A. Bendixsen
"The Use of Opioids in the Treatment of Young People with Chronic Non-Malignant Pain at a Multidisciplinary Pain Clinic in Denmark"

11:20-11:30 -I. Gomes
"Analysis of Morphine Regulated Striatal Synaptic Networks using Proteomics and Network Analysis"

11:30-12:00 **Discussion period**

12:00-13:00 **Lunch provided** (Fontaine A)

12:00-13:00 **Lunch (Executive committee)** (Salon Fundy)

Symposium #4 (Westmount)

DISSECTING THE FUNCTIONS OF NOCICEPTIN/ORPHANIN FQ RECEPTORS

Chair: **Michael Bruchas** (Washington University)

Discussion leader: **Larry Toll** (Torrey Pines Institute)

- 13:00-13:25 **Raül Andero Galí** (Emory University)
"Amygdala-dependent fear is regulated by opioid receptor-like 1 in mice and humans with PTSD"
- 13:25-13:50 **Kelly Standifer** (University of Oklahoma College of Pharmacy)
"Neuroinflammatory actions of N/OFQ following traumatic stress"
- 13:50-14:15 **Thomas Jhou** (Medical University of South Carolina)
"Nociceptin/Orphanin-FQ in the rostromedial tegmental nucleus"
- 14:15-14:40 **Robert Innis** (National Institutes of Mental Health)
"Imaging of nociceptin/orphanin FQ peptide (NOP) receptors in human brain and whole-body using a novel positron emission tomography radioligand, [¹¹C]NOP-1A"
- 14:40-15:00 **Nutritional break**
- Hot Topics
- 15:00-15:10 -A. Ozawa
"EGFP-NOP Mice: Location in Spinal Cord and DRG"
- 15:10-15:20 -S.M. Spangler
"Dissecting Nociceptin Receptor Modulation of Reward"
- 15:20-15:30 -Y. Zhang
"Effect of the nociception/orphanin FQ peptide receptor antagonist JTC-801 on cytokine expression following exposure to the single prolonged stress model of PTSD"
- 15:30-16:00 **Discussion period**
- 16:00-18:00 **Poster session #2** (even numbers; Fontaine B-C)

Wednesday July 16th

- 8:00-12:00 **Registration**
- 6:30-8:00 **Breakfast provided** (Fontaine A)
- Plenary lecture #2 (Westmount)
- 8:00-8:45 **Antonello Bonci** (NIDA intramural program director)
"Optogenetic approaches to study synaptic plasticity and substance abuse"
- Symposium #5 (Westmount)
- DYSPHORIA AND AVERSION IN ADDICTION AND CHRONIC PAIN**
Chair: **Chris Evans** (University of California Los Angeles)
Discussion leader: **Charles Chavkin** (University of Washington)
- 8:45-9:10 **Julie Kauer** (Brown University)
"Acute stress activates kappa opioid receptors and triggers persistent synaptic changes in the VTA"
- 9:10-9:35 **Joel Schlosburg** (Scripps Research Institute, San Diego)
"Dynorphin-kappa opioid receptor activation regulates the dynamics of drug-taking in the progression towards addiction"
- 9:35-10:00 **Brendan Walker** (Washington State University)
"Quiescent kappa-opioid receptors in the medial prefrontal cortex: the rollercoaster of cognitive control"

- 10:00-10:20 **Nutritional break**
- Hot Topics
- 10:20-10:30 -A. Riegel
"The Functional Rewiring of Cortical Synapses in a Translational Model of Neuropathic Pain"
- 10:30-10:40 -L.G. Rosen
"Opiate exposure state controls a molecular switch in opiate reward memory formation in the basolateral amygdala-prefrontal cortical pathway"
- 10:40-10:50 -A. Lesniak
"Opioid receptor expression in mice with persistent, long-term neuropathic pain"
- 10:50-11:00 -S. Schattauer
"Ligand-directed signaling at kappa opioid receptors: differential mechanisms of JNK MAPK activation by U50,488 and norBNI"
- 11:10-11:20 -G.R. Matyas
"Development of a Vaccine against Heroin"
- 11:20-11:45 **Discussion period**
- 12:00-13:30 **Data Blitz (lunch boxes provided)**

Thursday July 17th

- 8:00-15:00 **Registration**
- 6:30-8:00 **Breakfast provided** (Fontaine A)
- Plenary lecture #3 (Westmount)
- 8:00-8:45 **Yves De Koninck** (Université Laval)
"Expanding your toolbox; new approaches to probe the brain with light"
- Symposium #6 (Westmount)
(Sponsored by the CIHR's Institute of Neuroscience, Mental Health and Addiction)
- WORKSHOP ON NEW TECHNOLOGIES IN NEUROSCIENCE AND DRUG ADDICTION**
- Chair: **Fred Nyberg** (Uppsala University)
Discussion leader: **Jose Moron-Concepcion** (Columbia University)
- 8:45-9:10 **Maxime Descoteaux** (Université de Sherbrooke)
"Diffusion MRI: imaging the wiring of the brain"
- 9:10-9:35 **Philippe Seguela** (McGill University)
"Optogenetic control of pain pathways *in vivo*"
- 9:35-10:00 **Sotiris Masmanidis** (University of California Los Angeles)
"Large-scale electrophysiology and virtual reality for reward circuit research"
- 10:00-10:20 **Nutritional break**
- Hot Topics
- 10:20-10:30 -K. Skold
"Post-sampling changes in opioid peptide levels and the effect of tissue stabilization"

- 10:30-10:40 -A.K. Fakira
"NR2B-mediated changes in hippocampal spine morphology following morphine conditioned place preference"
- 10:40-10:50 -M.J. Schmidt
"Spatiotemporal Control of Mu-Opioid Signaling and Behavior"

10:50-11:15 **Discussion period**

Symposium #7 (Westmount)

DISSECTING OPIOID NEURAL CIRCUITS

11:15-12:00 **YOUNG INVESTIGATOR AWARD SYMPOSIUM**

Chair: **John Traynor** (University of Michigan)

Discussion leader: **Elyssa Margolis** (University of California San Francisco)

Michael Bruchas (Washington University)

"Spatiotemporal Control of Opioid Systems in Reward and Aversion"

12:00-13:00 **Lunch provided** (Fontaine A)

13:00-13:25 **Thomas Kash** (University of North Carolina)

"Dynorphin controls the gain of an amygdalar anxiety circuit"

13:25-13:50 **Matthew Banghart** (Harvard University)

"A photochemical approach to peptidergic neuromodulation"

Hot Topics

13:50-14:00 -E.N. Bobeck

"Endosomal Endothelin Converting Enzyme-2: A regulator of opioid receptor trafficking"

14:00-14:10 -K.J. Tonsfeldt

"Extrasynaptic GABAA receptors in the periaqueductal gray are involved in descending pain modulation and chronic inflammatory pain-induced plasticity"

14:10-14:20 -L. Kotecki

"GIRK channel activation in midbrain GABA neurons is not required for opioid-induced locomotor stimulation"

14:20-14:45 **Discussion period**

14:45-15:00 **Nutritional break**

Symposium #8 (Westmount)

GENETIC AND EPIGENETIC REGULATION IN ADDICTION AND CHRONIC PAIN

Chair: **Laura Stone** (McGill University)

Discussion leader: **Mary Jeanne Kreek** (The Rockefeller University)

15:00-15:25 **Luda Diatchenko** (McGill University)

"Expansion of mu-opioid receptor gene locus: new functional variants"

15:25-15:50 **Pamela Kennedy** (University of California Los Angeles)

"Chromatin Cross-Talk in the Nucleus Accumbens: Implications for Cocaine-Induced Behavioral and Molecular Adaptations"

15:50-16:15 **Marcello Wood** (University of California Irvine)

"The role of HDAC3 in acquisition and extinction of drug-seeking behavior"

Hot Topics

- 16:15-16:25 -O. Levran
“Dopaminergic system gene polymorphisms and heroin addiction: further support for a protective effect of casein kinase 1e (CSNK1E) variants”
- 16:25-16:35 -J.S. Wieskopf
“Broad spectrum analgesic efficacy of IBNtxA is mediated by exon 11-associated splice variants of the mu-opioid receptor gene”
- 16:35-16:55 ***Discussion period***
- 16:55-17:45 ***Business meeting*** (Westmount)

Shuttles to the Banquet dinner leave between 18:15 and 18:30 from the Hilton

19:00-late ***Banquet Dinner***

Friday July 18th

End of the Conference and Departure

INRC 2014 Awardees

Founder's lecture

Dr Brigitte Kieffer



B. L. Kieffer is Professor at McGill University and at the Université de Strasbourg France. Her team uses mouse genetic approaches to tackle the role of opioid receptors in brain physiology and disorders, and to search for novel genes involved in psychiatric disorders. She has developed and shared exquisite genetic tools worldwide, and has developed innovative research lines with strong impact in neuroscience and biomedical research. Her work has important implications for the development of treatment of pain, drug abuse and emotional disorders. She is part of the Center for Opioid Receptors and Drugs of Abuse or CSORDA.

Pr. Kieffer is recipient of the Jules Martin (French Academy of Science, 2001) and the Lounsbery (French and US Academies of Science, 2004) Awards, and has become an EMBO Member in 2009. In 2012 she received the Lamonica Award of Neurology (French Academy of Science) and was nominated Chevalier de la Légion d'honneur. In December 2013 she was elected as a member of the French Academy of Sciences. She started as the Scientific Director of the Douglas Hospital Research Centre, affiliated to McGill University in January 2014.

Dr Christopher Evans



Christopher Evans received his Ph.D. from Imperial College London, conducting his thesis research on endorphins and enkephalins, at the Medical Research Council Institute in Mill Hill. After a postdoctoral fellowship at Stanford University, he joined the UCLA faculty in the Department of Psychiatry and Biobehavioral Science. His research accomplishments have included identification of a number of novel endogenous opioid peptides and the cloning of the first opioid receptor.

Dr. Evans is currently Director of the UCLA Brain Research Institute and the Stefan Hatos Professor directing the Shirley and Stefan Hatos Center for Neuropharmacology in the UCLA Semel Institute. He is also director of a NIH-funded center – The Center for Opioid Receptors and Drugs of Abuse or CSORDA with the broad aim of understanding the action of opioid drugs such as morphine and heroin at the molecular, cellular and behavioral levels.

Young Investigator Award

Dr Michael Bruchas



Michael Bruchas's research training is in GPCR pharmacology and neuroscience. His graduate work focused on adrenergic receptors while his post-doctoral work was in the department of pharmacology, at the University of Washington, Seattle, in Charles Chavkin's laboratory. Here he studied opioid receptor biased signaling in behavior using mouse genetics and behavioral approaches. His laboratory at Washington University (St. Louis) investigates interactions between stress, GPCRs, neuropeptides, and neural circuits in affective behavior.

Recent efforts by his group have focused on developing tools for wireless optogenetics and optical GPCRs for examining signaling pathways and behavior in vivo. Using a variety of genetic, pharmacological, engineering, and optogenetic approaches, he will discuss recent efforts by his team to dissect the role of opioid peptides and receptor signalling in models of affective behavior.

Dr Brigitte Kieffer, Founder's Award, INRC 2014



When a scientist receives an honour it is usually for a single major contribution to advancing the field, but for Brigitte Kieffer I would identify three major contributions to opioid science which have been world-leading and have had a very significant impact on both INRC and on the wider scientific community. Firstly, her cloning of the delta opioid receptor (published in PNAS), secondly the development and characterisation of the mu opioid receptor knockout mouse (published in Nature) and thirdly the development and characterisation of the first conditional opioid receptor knockout mouse, with a fascinating behavioural phenotype that will be published shortly. In between there have been many other significant publications, including really elegant work on the role of opioids in emotional responses (in Nature Genetics) and in maternal attachment (in Science).

Brigitte first came to an INRC meeting in 1990 at Noordwijkerhout, and at that point several laboratories had spent nearly a decade trying to clone the first opioid receptor. In the 1980's I listened to a lot of talks on the cloning of the opioid receptor that really had nothing to say! I felt sorry for a string of PhD students who had to write a thesis with precious little to show for their heroic efforts. So when Brigitte Kieffer and Chris Evans independently succeeded in cloning the first opioid receptor in 1992 these were landmark publications. Chris published in Science and Brigitte published in PNAS, though I know she originally submitted the manuscript to Nature. The editors foolishly said "it was just the cloning of another G-protein coupled receptor" and rejected the manuscript. This paper is now cited over 850 times and I am sure the editors of Nature are kicking themselves. Nature did not make the same mistake twice and when Brigitte submitted her phenotypic characterisation of the first opioid receptor knockout mouse in December 1996 the referee and editors reports were glowing. The citation count for this paper is now approaching four figures. She presented this seminal work at the INRC meeting in Long Beach earlier that year; a clearcut deletion of the mu opioid receptor and a remarkable loss of key behaviours that this receptor is involved in. I recall there was stunned silence in the room, as INRCers listened to this major field advance. Avram Goldstein who was standing next to me remarked "this is almost too good to be true". But it was one of those set of experiments that we would all love to be involved with; I was privileged to be one of the collaborators on this work. Sometimes, experiments don't work out as you had planned. Brigitte had set out to make a delta opioid receptor knockout mouse, and succeeded first in making a mu knockout – the kappa gene knockout came next (again a first) and the delta knockout last. Sometimes the direction of science is rather serendipitous.

There is a third landmark discovery to come and I suspect in her Founders lecture she might talk about her work in developing a conditional deletion of an opioid receptor to enable us to identify the role of these receptors in specific regions in the brain. This work and the previous development of knockout mice, have taken many years and stoic perseverance to come to fruition. It reflects Brigitte's critical attention to detail, a characteristic I have seen when writing both grant applications and research publications with her. She has very many successful collaborations in both Europe and worldwide, and in looking at the author lists of her papers you might think she collaborates with everyone. This is not true – she chooses her collaborators very carefully. I remember her telling me when she cloned the delta opioid receptor that she gave the clones to everyone who asked; but far too many of those that asked did not do anything useful with them! So when the opioid knockouts were developed she took a much more cautious approach, and it has delivered over 200 high quality publications.

Brigitte is a person who is in love with her science and her success has been recognised by four national prizes from both French and US academies of science. She was appointed as Director of the world-leading IGBMC in Strasbourg in 2012 where most of her opioid research has been carried out, but she always said that she must not allow leadership and management to compromise the important science she still had to do. So this year she moved to be scientific director of the Douglas Institute at McGill University in Canada – there is more to come, investigating opioid involvement in emotion.

For me, it has been an honour to collaborate with Brigitte for 20 years. There has been some great science and great friendship. In the early 1980's you would have found Hans Kosterlitz (one of the founders of INRC) in the bar late at night at INRC meetings, working hard and playing hard. From 1992, you would find Chris Evans and Brigitte Kieffer (and me!) there too. As I said, great science and great friendship; it is what INRC is all about.

*Professor Ian Kitchen
University of Surrey, UK
April 2014*

Dr Christopher Evans, Founder's Lecture Award, INRC 2014



When I was asked by the organizers of this year INRC to write a short text to acknowledge Chris Evans' contribution to our society, I was wondering where has the time gone. It seems like yesterday that Chris and I first met, at the 1981 Kyoto meeting, when INRC was at its first golden age. It was at the time when endogenous opioid peptides were being identified and the society experienced a large influx of scientists whose research focuses deviated from the traditional approaches used by the anatomists, biochemists, medical chemists, pharmacologists and physiologists that populated the society at that time. Chris is one of those scientists who joined INRC during that time and his scientific footprints since then have been tremendous. His thesis training was as a peptide chemist with Derek Smyth at MRC England. Chris then went to Stanford for a postdoctoral position with Jack Barchas where, in close collaboration with Eckard Weber, he isolated several new opioid peptides, including metorphamide, and developed antibody probes for studying the different processing products of proenkephalin, prodynorphin and proopioidmelanocortin.

Everyone in the society knows that Chris was instrumental in the cloning of the opioid receptors. His initial report on the successful cloning of δ -opioid receptor by expression cloning in 1992 has been cited >1000 times. Together with Brigitte Kieffer, who independently reported the cloning of δ -opioid receptor at the same time, they launched INRC into its second golden age. Subsequent successful attempts to clone other opioid receptors and the Orphanin F/Q receptor are based on the two cDNA sequences reported by Chris and Brigitte. But few know that before his successful attempt, Chris was involved in a failed attempt to clone the receptor by expression cloning. I was one member on the team that failed. To his credit and persistency, an ingenious method of synthesizing ^{125}I -labeled peptide for high affinity binding and autoradiography to identify clones, and a library generated in collaboration with Rob Edwards that eliminated secondary mRNA structure thus enabling complete reverse transcription of the receptor mRNA, Chris and his talented technician Duane Keith, were successful while many of us failed in cloning the receptor. For this singular achievement, Chris' contribution to the society is unparalleled.

In addition to the cloning of the receptor, another significant contribution that alters the course of INRC is Chris' work on biased agonism. Together with Mark von Zastrow, based on my initial observations that morphine is a partial agonist in the δ -opioid receptor and will not internalize the receptor, Chris demonstrated unequivocally that biased agonism exists in the opioid receptor systems. Chris' initial reports on the differential regulation of receptor trafficking laid the foundation for biased agonism, probable mechanisms for differential opiate tolerance and addiction, and probable drug development that one day might eliminate the many side-effects of opiate analgesics, the holy grail of opioid receptor research.

Chris' contribution to the society is not limited to his scientific accomplishments. Chris has been an active participant in yearly INRC meetings, has served on the Executive Committee, and local organizing committees. But I think the other major impact that Chris has on INRC is his mantra for collaborative research and out of the box ideas. I am sure many of us have benefited from Chris' generosity in providing reagents and clones. Our studies on the opioid genes transcription and generation of the μ -opioid receptor knockout mice would not be possible if not for his initial willingness to share the yet to be reported δ -opioid receptor cDNA clone. Sometimes, I still have problems comprehending his ideas, seemingly come out from the right field, to paraphrase an American baseball idiom. A case and point is his continued enthusiasm and efforts on the phylogenetic studies of the opioid receptors and peptides. I guess one of these days, another high impact study will come out of Chris' group that will change the course of how we think about the biological functions of opioids.

What I have attempted does not detail all Chris' contributions to INRC. What I have summarized is Chris' contributions that change the course of INRC, contributions that rank with those of INRC founders, such as Avram Goldstein, Eric Simon and Hans Kosterlitz. For these contributions, Chris definitely deserves the honor of being one of this year Founder's Lecture speakers. I am honored to have the opportunity to write this tribute and called Chris as my friend and collaborator.

*Professor Ping-Yee Law
University of Minnesota, USA
May 2014*

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ORAL PRESENTATIONS

Allosteric modulation of the orthosteric binding site by G proteins: mechanism of high affinity agonist binding

Roger K. Sunahara

Department of Pharmacology, University of Michigan Medical School

Recent advances in the structural biology of G protein-coupled receptors (GPCRs) have unraveled many intricacies of ligand binding. Our recent efforts to elucidate the crystal structure of the β 2-adrenergic receptor (β 2AR), in a complex with the stimulatory G protein, Gs, have provided insight into how GPCR activation leads to GDP release, the principle step that precedes GTP binding and G protein activation. The structural data also reveal a possible mechanism through which G proteins in turn allosterically modulate hormone binding. These and more recent high resolution structural data using β 2AR-selective camelid antibodies (nanobodies) suggest that occupation of the binding site for the G protein C-terminus stabilizes high affinity agonist binding. The conformation changes results in the formation of a cap over the orthosteric site, located at the extracellular face of the receptor, slowing ligand dissociation. Here we provide functional evidence to suggest that this ‘closed’ conformation garners high affinity agonist-binding properties through slowing the dissociation rates of ligands bound at the orthosteric site. Moreover, coupling of a nucleotide-free G protein or nanobody to a ligand-free, or empty receptor, can also stabilize the ‘closed’ conformation and physically prevent a ligand’s access to the hormone binding site. Taken together these structural changes and the slower observed ligand dissociation rates account for the G protein-dependent high affinity binding properties of agonists.

Optogenetic approaches to understanding synaptic plasticity and substance use disorders.

Antonello Bonci, MD

National Institute on Drug Abuse

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.

Expanding your toolbox; new approaches to probe the brain with light

Yves De Koninck

Université Laval and Institut universitaire en santé mentale de Québec, Canada

The future of neuroscience lies in our ability to assess, in a context sensitive manner, how each component of the enormously complex CNS integrates, processes and transfers neurochemical information. Although colossal advances in understanding cell signalling events have been made using ex vivo preparations, the highly reactive and plastic CNS imposes in vivo studies to assess their relevance to normal function and pathology. Thus, the true enabling discovery technologies will be those that bridge single cell molecular signalling studies with whole animal physiological and behavioural assessments.

The recent advent of photoactivatable proteins to generate novel sensors and actuators open new arrays of possibilities on this front. Yet, harnessing their full potential remains limited by properties of light such as diffraction, absorption and scattering which restrict resolution and depth of observation/intervention. Thus, our ability to probe and control cellular and molecular events across the length and time scales (from subcellular compartments to neuronal networks; from milliseconds to days) in vivo hinges on the development of novel techniques to deliver light and measure events with extreme sensitivity and precision.

I will describe recent techniques developed to undertake these challenges. At one end of the spectrum, fluorescence fluctuation approaches to conduct quantitative analysis of molecular interactions in situ at previously unachievable resolutions. At the other end of the spectrum, optical approaches to enable single cell signalling and electrophysiology studies in deep brain structures. I will also describe means to detecting direct molecular constituents, such as lipids, without any label or contrast agent, which is critical for translation to human studies. I will finally discuss challenges that remain to develop methods to probe and control neuronal activity non-invasively during behavior.

This work was funded by the Canadian Institutes of Health Research, the Pfizer-Fonds Recherche Québec – Santé Innovation Fund and the Natural Science and Engineering Research Council of Canada.

Cloning: Twice in One Day

Brigitte Kieffer and Christopher Evans

Douglas Research Centre, McGill University, Canada; UCLA, USA

Cloning of opioid receptors was a significant and frustrating blockade for the progress of INRC research from the mid 80's till the final cloning of the delta opioid receptor in 1992. There were many approaches attempted; some based on receptor purification, others on subtractive cloning or G-protein coupled receptor homology cloning. However, expression cloning won the day with two groups, one in Strasbourg, led by Brigitte Kieffer, and one in Los Angeles, led by Chris Evans, successfully cloning the delta opioid receptor from NG108-15 cells using very similar strategies and over the same time period. Papers were submitted in the summer of 1992 and the delta opioid receptor sequence known by all by the winter of 1992.

As a result of cloning the delta opioid receptor, sequences of the kappa and mu opioid receptors were quickly cloned or discovered by homology. A fourth receptor, the Opioid Receptor-Like (ORL) Receptor was also discovered by several homology cloners and a new GPCR system revealed. The cloning of the opioid receptor family opened many new exciting possibilities for opioid research. These included; the identification of the selectivity and structure activities of the opioid receptor family, the ability to tag and follow receptor trafficking within live cells both in vitro and in vivo, and the ability to knockout and knock-in opioid receptors to determine receptor participation in innate and drug-modified behaviors. The cloning clearly pushed the opioid field to another era of discovery but was just one piece of the puzzle.

The Holy Grail remains as to whether separating analgesic from addictive mu opiates can be achieved, and ligand-biased signaling at these receptors may hold promise. Current questions in the field also include evaluation of the true therapeutic potential for delta agonists or kappa antagonists in the treatment of mood disorders. Finally, the complexity of endogenous opioid physiology, including receptor signaling and within-system interactions throughout the broad opioidergic neural networks, will surely maintain this fascinating system at the center of neuroscience research.

Spatiotemporal Control of Opioid Systems in Reward and Aversion

Ream Al-Hasani, Jordan G. McCall, Gunchul Shin, Sung-Il Park, Jenny M. Wong, Omar S. Mabrouk, Nicole A. Capik, Gavin Schmitz, Blessan Sebastian, Daniel Y. Hong, Michael J. Krashes, Bradford B. Lowell, Thomas L. Kash, Robert T. Kennedy, John Rogers, Michael R. Bruchas

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Stress responses are largely controlled by specific neurotransmitters and their receptors in the central nervous system. Many of these signals are conveyed through activation of both neuropeptide (i.e. CRF and Opioid) and monoamine (norepinephrine, dopamine, serotonin) receptor systems. These receptors are seven transmembrane spanning G-protein coupled receptors (GPCR) and they can stimulate a variety of signaling cascades following neurotransmitter/neuropeptide release. Neuropeptide and monoamine circuits are engaged by stress, and elicit a complex array of behavioral responses relevant to anxiety, addiction, and depression. Two neuropeptide systems of major interest in stress responsivity include dynorphin opioid peptides and CRF. These systems and circuits have classically been studied using pharmacological approaches, in vivo and in vitro electrophysiology and biochemical methods. Here we will describe recent advances in optogenetic technology including development and implementation of cellular scale wireless optogenetic devices for in vivo behavioral measures. In addition, we report divergence of behavioral responses using optical control of discrete brain region subnuclei containing dynorphin expressing neurons. We find that optical control of this neuropeptide system in select regions results in differences in reward and aversion behavior. Finally, we will also discuss recent advances in controlling various monoamine and peptide GPCR signaling pathways with optogenetic strategies and how these technologies reveal novel insights into neuromodulator function in stress-induced affective behavior.

Molecular Control of δ -Opioid Receptor Signaling

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Opioids represent widely prescribed and abused medications, although their signal transduction mechanisms are not well understood. Here we present the 1.8Å high-resolution crystal structure of the human δ -opioid receptor (δ -OR), revealing the presence and fundamental role of a sodium ion mediating allosteric control of receptor functional selectivity and constitutive activity. The distinctive δ -OR sodium ion site architecture is centrally located in a polar interaction network in the 7-transmembrane bundle core, with the sodium ion stabilizing a reduced agonist affinity state, and thereby modulating signal transduction. Site-directed mutagenesis and functional studies reveal that changing the allosteric sodium site residue Asn131 to Ala or Val augments constitutive arrestin-ergic signaling. Mutation of Asp95Ala, Asn310Ala, and Asn314Ala transform classical δ -opioid antagonists like naltrindole into potent β -arrestin-biased agonists. The data establish the molecular basis for allosteric sodium ion control in opioid signaling, revealing that sodium-coordinating residues act as “efficacy-switches” at a prototypic G protein-coupled receptor.

Which way is up? Rethinking Nociceptin Ligand Design and SAR from Nociceptin Receptor Crystal Structure and Active-State Homology Model comparisons

NURULAIN T. ZAVERI, Ph.D.

Astraea Therapeutics

The antagonist-bound crystal structure of the nociceptin opioid receptor (NOP) was recently reported along with those of the other opioid receptors bound to opioid antagonists. Crystal structures of the active-state opioid receptor GPCRs bound to opioid agonists still remain to be determined. We recently reported the first homology model of the 'active-state' of the nociceptin receptor (Daga and Zaveri, 2012). Using this NOP 'active-state' homology model for docking NOP agonists and for molecular dynamics simulations was used to understand NOP ligand structure-activity relationships and agonist-induced receptor activation. However, comparing binding poses of NOP antagonists in the NOP crystal structure to the binding pose of NOP agonists revealed some interesting differences, which may be important to consider during lead optimization of NOP receptor ligands. These insights have assisted our structure-based drug design efforts for novel nociceptin ligand discovery. Grant Support: R01DA014026 and R01DA027811.

MMG22 selectively targets a putative MOR-mGluR5 heteromer for treatment of chronic inflammatory pain

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The management of chronic pain with clinically employed analgesics is problematic due to development of tolerance and other adverse effects. As an approach to this problem, a bivalent ligand (MMG22) containing both mu agonist and mGluR5 antagonist pharmacophores recently was reported (PNAS USA, 2013:110, 11595) to produce potent antinociception via a putative MOR-mGluR5 heteromer in mice with LPS-induced acute inflammatory pain. Here we present studies of MMG22 in a chronic inflammatory pain model in mice implanted with fibrosarcoma cells. As intrathecal MMG22 exhibited 250,000-times greater potency than a mixture of mu opioid agonist (M19) and mGluR5 antagonist (MG20) monovalent ligands, the data are consistent with interaction of MMG22 with the protomers of a putative MOR-mGluR5 heteromer. Since a 572-fold potency increase of MMG22 observed over a period of 21 days after implantation of fibrosarcoma cells in mice mirrored the time-course increase of hyperalgesia, we investigated the possibility that activated NMDAR might be inhibited via a MMG22-occupied heteromer. After inhibition of NMDAR-mediated hyperalgesia with MK801, the potency of MMG22 was reduced over a thousand-fold and was similar to that of control mice and TLR4 KO mice. These results suggest that the NMDAR is inhibited through physical association with a MMG22-occupied MOR-mGluR5 heteromer via allosteric interaction with the mGluR5 protomer. Presumably, the potent antinociception of MMG22 is due to a combination of NMDAR inhibition and activation of the MOR protomer. There was no significant difference in the ED50 of morphine between control and LPS-pretreated mice in the presence or absence of MK801, supporting the crucial role of inhibited mGluR5 protomer in blocking activated NMDAR. Given the high potency, long duration (<24 hrs) of action, and absence of tolerance, MMG22 has potential as an analgesic for chronic inflammatory pain.

This project was supported by NIH grant DA030316.

Potential Relapse Prevention Agents Targetting Multiple Opioid Receptors

Stephen Husbands

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Current treatments for drug addiction are only marginally effective; 70% of treated addicts relapse within the first year following treatment, while the majority of drug addicts take more than one addictive drug at any one time (poly-drug abuse).

Buprenorphine and mu opioid (MOP) receptor antagonists have been combined and shown, in preclinical and early clinical studies, to be effective as a treatment of cocaine, alcohol and opiate abuse (particularly relapse prevention) and also against major depressive disorder. The objective of this project has been to mimic these combinations by developing single chemical entities that have potent kappa and delta opioid (KOP, DOP) receptor antagonist and measurable nociceptin/ORL-1 (NOP) receptor partial agonist activity coupled with substantially lower efficacy at MOP receptors than displayed by buprenorphine. Lead compounds have been identified within the C7-methyl orvinol series. One such compound, BU10119, has been confirmed as an opioid antagonist in vivo, exhibiting no antinociceptive activity in the acetic acid writhing assay, while in the warm water tail withdrawal (50oC) assay BU10119 (10mg/kg sc) completely suppressed the antinociceptive activity of the KOP receptor agonist EKC, and was not surmountable up to 10mg/kg EKC (approx 100-times the ED50 dose of EKC) indicating very potent KOP antagonism. Antagonism lasted for 24h, indicating an extended, but not overly long, duration of action. In the acetic acid writhing assay BU10119 (1 mg/kg, sc) completely prevented the effect of 1 mg/kg morphine (sc), confirming MOP antagonism. In an assay for hyperalgesia, which responds to very low opioid agonist activity and NOP agonism there was a significant effect of BU10119 that was blocked by the NOP antagonist J113397 but not blocked by the MOP antagonist naltrexone, confirming NOP receptor agonism in vivo. Importantly, this compound which has MOP, KOP and DOP antagonist activity and NOP partial agonist activity is effective at blocking reinstatement of cocaine seeking in rats induced by the pharmacological stressor yohimbine; a model of stress-induced relapse to cocaine.

Supported by NIDA Grant DA07315

Delta Opioid Receptor Function in Skin: Controlling Pain Where it Starts

Gregory Scherrer

Stanford University

The delta opioid receptor (DOR) has long been considered a promising target for the treatment of chronic pain. Specifically, numerous studies indicate that DOR agonists can reduce tactile hypersensitivity in rodent models of neuropathic or inflammatory pain. However, how and where DOR operates along neural circuits to control pain has remained unclear. Here I will provide evidence that the skin is a major locus of DOR-mediated modulation of nociception. Early during embryonic development discrete populations of large-diameter somatosensory neurons of the dorsal root ganglia (DRG) start to express DOR. These neurons, in which DOR expression is maintained during adulthood, will give rise to myelinated mechanosensory fibers that innervate the glabrous and hairy skin. Additionally DOR is expressed by cutaneous unmyelinated mechanonociceptors that co-express the G protein-coupled receptor MrgprD. Recordings using an ex vivo somatosensory system preparation demonstrated that DOR-expressing DRG neurons respond to mechanical stimulation of the skin. In these mechanosensory neurons, DOR is present at the plasma membrane and trafficked along both central and peripheral axons. DOR-positive peripheral axons terminate either as free nerve endings that meander through the epidermis to innervate the stratum granulosum, as seen for MrgprD-positive fibers, or innervate differentiated structures in the skin, including Merkel cell touch domes, hair follicles or Meissner corpuscles, to form touch-detecting organs. I will also discuss the function of DOR in these peripheral terminals and how DOR regulates neuronal excitability in skin. Financial support: National Institute on Drug Abuse grant DA031777.

Biased ligands at mu and delta opioid receptors: targeting selective signalling to develop improved therapeutics

Jonathan Violin

Trevena Inc.

On-target adverse effects have limited the clinical utility of drugs targeting opioid receptors. "Biased ligands" stabilize subsets of receptor conformations compared to classical agonists and antagonists, and may offer the possibility of dissecting beneficial and adverse pharmacology at the same receptor.

TRV130 is a G protein-biased ligand at the mu opioid receptor: it stimulates G protein coupling with potency and efficacy similar to strong opioid analgesics, but stimulates markedly reduced coupling to beta-arrestins. This profile translates to an improved therapeutic index in preclinical models of pain and opioid-related adverse events. Early clinical trials of TRV130 suggest that this profile may deliver enhanced analgesia with improved safety and tolerability compared to morphine. These results support the concept that biased ligands can deliver differentiated pharmacology at opioid receptors.

Similarly, emerging data suggests that biased ligands targeting the delta opioid receptor may be able to reduce the on-target seizure liability that has hindered drug discovery targeting selective delta opioid agonists. Preclinical studies of beta-arrestin knockout mice and G protein-biased ligands suggests that analgesic, anti-depressant, and anti-Parkinsonian benefits of delta opioid receptor activation can be engaged without engaging seizure liability.

Further discovery and characterization of biased ligands may uncover new opportunities to deliver differentiated therapeutics with improved efficacy, safety and tolerability.

Conflict of interest: Dr. Violin is an employee of Trevena Inc, a pharmaceutical company developing some of the compounds that will be described in this presentation.

Increasing delta opioid receptor bioavailability

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The delta opioid receptor (DOR) is an attractive candidate as an alternate target for pain management. However, DOR agonists are not very effective *in vivo*, although they are efficient at initiating signaling *in vitro*. One potential explanation for this lower efficacy is that DOR is predominantly retained in intracellular compartments in neurons, therefore not being available at the cell surface to bind ligands. This leads to the simple hypothesis that release of this retention will increase surface delivery of DOR, and, therefore, higher efficacy of DOR agonists. We have developed a tractable model using PC12 cells and primary sensory neuronal cell cultures to test this hypothesis at a molecular level. I will present our work on the molecular mechanisms and functional relevance of the retention and regulated surface delivery of DOR. This work is supported by internal funds from CMU.

Delta Opioid Receptor Agonists for the Treatment of Migraine

Amynah A Pradhan

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Migraine is an extraordinarily common brain disorder for which therapeutic options continue to be limited. The aim of this study was to use new and established preclinical mouse models to examine the effects of delta opioid receptor agonists on basic mechanisms of migraine. The nitric oxide donor, nitroglycerin, is a consistent migraine trigger in humans, and was shown to evoke both acute mechanical and thermal hyperalgesia in mice. Chronic intermittent administration of nitroglycerin resulted in a progressive and sustained hyperalgesia, as well as conditioned place aversion. Several delta opioid receptor agonists, including SNC80, significantly reduced nitroglycerin-evoked hyperalgesia. SNC80 also abolished nitroglycerin-induced conditioned place aversion, suggesting that delta opioid receptor activation may also alleviate the negative emotional state associated with migraine. In addition, we found that SNC80 significantly attenuated cortical spreading depression, a model of migraine aura that is considered useful in predicting the efficacy of migraine preventive therapies. We have also used conditional knockout mice to determine the different roles of central vs. peripheral delta opioid receptors in mediating the migraine-relieving effects of delta agonists. Together, our data demonstrates that delta opioid receptor agonists modulate multiple basic mechanisms of migraine, and indicate that delta opioid receptors are a promising therapeutic target for this disorder.

This research was supported by NIH-NIDA Grants DA05010 and DA031243, the Shirley and Stefan Hatos Research Foundation, and the Department of Psychiatry UIC.

A.Pradhan has a contract with Trevena.

Disrupted Brain Circuitry for Pain-Related Reward/Punishment in Fibromyalgia

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While patients suffering from fibromyalgia (FM) are known to exhibit hyperalgesia, the central mechanisms contributing to this altered pain processing are not fully understood. In this study we investigate potential dysregulation of the neural circuitry underlying cognitive and hedonic aspects of the subjective experience of pain such as anticipation of pain and of pain relief.

fMRI was performed on 31 FM patients and 14 controls while they received cuff pressure pain stimuli on their leg, calibrated to elicit a pain rating of 50/100. During the scan, subjects also received visual cues informing them of impending pain onset (pain anticipation) and pain offset (relief anticipation).

Patients exhibited less robust activations during both anticipation of pain and anticipation of relief within regions commonly thought to be involved in sensory, affective, cognitive and pain-modulatory processes. In healthy controls, direct searches and region-of-interest analyses in the ventral tegmental area (VTA) revealed a pattern of activity compatible with the encoding of punishment: activation during pain anticipation and pain stimulation, but deactivation during relief anticipation. In FM patients, however, VTA activity during pain and anticipation (of both pain and relief) periods was dramatically reduced or abolished.

FM patients exhibit disrupted brain responses to reward/punishment. The VTA is a source for reward-linked dopaminergic/GABAergic neurotransmission in the brain and our observations are compatible with reports of altered dopaminergic/GABAergic neurotransmission in FM. Reduced reward/punishment signaling in FM may relate to the augmented central processing of pain and reduced efficacy of opioid treatments in these patients.

Chronic pain increases opioid self-administration through an accumbal dopaminergic mechanism

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Prescription opioid use is at an all-time high, and abuse of this class of medications has skyrocketed in the past two decades. Here we investigated the relationship between chronic pain and opioid self-administration. We found a striking rightward shift in the dose response curve for heroin self-administration in the presence of chronic pain with a concomitant alteration of dopamine release in the nucleus accumbens. Together, these results identify a previously unknown connection between chronic pain and loss of dopaminergic signaling in opioid self-administration that induces opioid intake at very high doses, which may act to promote opioid dose escalation and perhaps abuse.

This work was supported by NIH, NIDA grants DA027460 (JAM), DA035144 (MRB), and by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number UL1 RR024156.

Neuropathic Pain Modulates Dopaminergic Circuitry: Role for Microglial Activation

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UC Irvine, UC Los Angeles, Laval University

The use of opioids for the treatment of non-cancer chronic pain is controversial because of suboptimal analgesia and risk of iatrogenic addiction. The mesolimbic dopamine system, consisting of dopaminergic cell bodies in the ventral tegmental area (VTA) projecting to the ventral striatum, is of particular interest in pain research because of its overlapping function in the negative affective state of pain, opioid reward, and analgesia. In this study, we examine how chronic pain alters signaling in the mesolimbic dopamine system and the effect this has on the reinforcing and analgesic properties of opioids.

A neuropathic pain condition was modeled in C57bl/6 male mice via a loose ligation of the left sciatic nerve with a polyethylene cuff (chronic constriction injury; CCI). Ventral striatal dopamine release was measured via microdialysis. After behavioral testing, brains were isolated and prepared for molecular and chemical analysis.

Systemic morphine two weeks after nerve injury failed to stimulate dopamine release in the ventral striatum. Nerve-injury also led to a disruption in the chloride (Cl⁻) gradient in GABAergic neurons in the VTA, caused by a decrease in K⁺/Cl⁻ co-transporter function. This disrupted Cl⁻ gradient in GABA neurons led to an increased inhibition on dopaminergic neurons as measured by lowered cFOS activation in dopamine cell bodies stimulated by systemic morphine in nerve-injured animals. GABAergic KCC2 function could be restored by interfering with BDNF signaling or by chronic treatment with the microglial inhibitor, minocycline. This treatment also recovered morphine-stimulated dopamine release in the ventral striatum in nerve-injured animals.

These results point to a global dysregulation in mesolimbic reward processing in chronic pain states that may undermine the analgesic and hedonic attributes of opioids. Interfering with BDNF signaling or blocking microglial activation may represent a novel therapeutic target to ameliorate the analgesic effectiveness of opioids in chronic pain states.

This work is supported by NIH, CIHR and The UCLA Hatos Center. The authors declare no conflict of interest.

Pain facilitates response to opioid reward via a shared epigenetic pathway

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In an animal model of chronic pain, we show that pain facilitates the acquisition of behaviors of opioid reward. The pain effect is mediated by pain- and opioid-mediated upregulation of the transcription regulator MeCP2 in the central nucleus of the amygdala (CeA) that integrates emotional responses. The downstream effects of MeCP2 upregulation include repression of the histone dimethyltransferase G9a and activation of BDNF function. These findings suggest a shared epigenetic regulation pathway of MeCP2–G9a–BDNF in CeA regulation of emotional responses to pain and opioid reward.

Supported by NIH National Institute on Drug Abuse grants DA023069 and DA027541. The authors declare no financial conflict of interest.

Amygdala-dependent fear is regulated by Oprl1 in mice and humans with PTSD

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Emory University

The amygdala-dependent molecular mechanisms driving the onset and persistence of posttraumatic stress disorder (PTSD) are poorly understood. Recent observational studies have suggested that opioid analgesia in the aftermath of trauma may decrease the development of PTSD. Using a mouse model of dysregulated fear, we found altered expression within the amygdala of the *Oprl1* gene (opioid receptor-like 1), which encodes the amygdala nociceptin (NOP)/orphanin FQ receptor (NOP-R). Systemic and central amygdala infusion of SR-8993, a new highly selective NOP-R agonist, impaired fear memory consolidation. In humans, a single-nucleotide polymorphism (SNP) within *OPRL1* is associated with a self-reported history of childhood trauma and PTSD symptoms ($n = 1847$) after a traumatic event. This SNP is also associated with physiological startle measures of fear discrimination and magnetic resonance imaging analysis of amygdala-insula functional connectivity. Together, these data suggest that *Oprl1* is associated with amygdala function, fear processing, and PTSD symptoms. Further, our data suggest that activation of the *Oprl1*/NOP receptor may interfere with fear memory consolidation, with implications for prevention of PTSD after a traumatic event.

NEUROINFLAMMATORY ACTIONS OF N/OFFQ FOLLOWING TRAUMATIC STRESS

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Post-traumatic stress disorder (PTSD) frequently shares co-morbidity with chronic pain and negatively impacts the course of treatment for both disorders. We recently reported that persistent mechanical allodynia and thermal hyperalgesia were produced in an animal model of PTSD, single prolonged stress (SPS), in the absence of additional physical injury. The appearance of allodynia was preceded by elevated TNF α in serum, and followed by elevated nociceptin/orphanin FQ (N/OFFQ) in the CSF at day 9 and in various brain regions associated with pain and emotional processing between days 9-28. In addition, levels of the pro-inflammatory cytokine, IL-6, or its mRNA were elevated in spinal cord and amygdala during the same time period. The neuropeptide, N/OFFQ, has immunomodulatory actions in addition to modulating nociceptive sensitivity, anxiety and many other responses. When rats received daily injections of the NOP receptor antagonist JTC-801 on Day 7 of SPS, JTC-801 treatment reversed allodynia and hyperalgesia, and returned N/OFFQ and IL-6 levels back to baseline by day 21.

To better understand how N/OFFQ modulates cytokine expression, cytokine mRNA levels in U937 human monocyte cells were assessed by quantitative PCR (qPCR). N/OFFQ-induced up-regulation of IL-6 mRNA was blocked by NOP receptor antagonists and inhibitors of NF κ B, p38 and Akt signaling. It was not blocked by inhibitors of PKC or ERK MAP kinase. This suggests potential mechanisms by which N/OFFQ increases IL-6. Our findings suggest that N/OFFQ may maintain chronic pain states by stimulating IL-6 up-regulation during chronic stress and that this up-regulation may contribute to allodynia and hyperalgesia in the PTSD model.

Animal protocol was approved by the OUHSC IACUC committee & U.S. Army Research and Materiel Command ACURO office, and complied with the Animal Welfare Act and adhered to the principles described in the Guide for Care and Use of Laboratory Animals. This study was supported by the Department of the Army DMRDP W81XWH-11-2-0077 and the Oklahoma Center for the Advancement of Science and Technology. No conflict exists.

Nociceptin/Orphanin-FQ and opioid mechanisms involving the rostromedial tegmental nucleus

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The rostromedial tegmental nucleus (RMTg) was identified in 2009 as a midbrain GABAergic region that encodes negative reward prediction errors, projects intensely to midbrain dopamine neurons, and receives major inputs from the lateral habenula, frontal cortex, and other regions involved in motivated behavior. Subsequent studies showed the RMTg to be a critical modulator of aversive behavior, and of some physiological and motivational effects of mu opioids. We now report that RMTg neurons in both rats and transgenic mice express mRNA for prepronociceptin (PNOC), the precursor to the peptide nociceptin (also called orphanin FQ), as well as nocicstatin. The predicted function of PNOC in these neurons is somewhat different from its apparent roles in other brain regions, adding to an already diverse view of PNOC roles in motivated behavior.

This work is supported by 1R03DA034431 and 1R01DA037327. No financial conflicts of interest are declared.

Imaging of nociceptin/orphanin FQ peptide (NOP) receptors in human brain and whole-body using a novel positron emission tomography radioligand, [11C]NOP-1A

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Introduction: Nociceptin/orphanin FQ peptide (NOP) receptor is a new member of opioid receptor that may be linked to pathophysiology of pain and other neuropsychiatric disorders. To image NOP receptor in vivo, we developed a new positron emission tomography (PET) ligand, [11C]NOP-1A, that has high affinity ($K_i = 0.15$ nM) at NOP and high binding (50 - 70% specific) in monkey brain. Next, we assessed the ability of [11C]NOP-1A to image and quantify NOP receptors for the first time in human brain and estimate its radiation safety profile in humans.

Methods: After i.v. injection of [11C]NOP-1A, two hour dynamic PET scans were performed in healthy subjects for brain ($n = 7$), brain test and retest in same subject ($n = 11$), and whole-body imaging ($n = 9$). During brain scans, arterial blood was sampled serially to measure [11C]NOP-1A concentrations in plasma. From brain scans, total distribution volume (VT; a measure of receptor density) was determined by kinetic modeling. Whole-body scans provided radioactivity biodistribution and radiation exposure estimates (effective dose).

Results: [11C]NOP-1A showed high radioactivity uptake (5–7 peak SUV) in brain. VT values (mL/cm³) were high, ranged from 10.1 in temporal cortex to 5.6 in cerebellum, well identified (1.1% SE), and stable after 70 min scan time indicating minimal entry of radiometabolites in brain. The retest variability and reliability of VT were moderately good averaging 12% and 0.46 for large brain regions. In whole-body, the radioactivity distributed in NOP receptor expressing peripheral organs - such as heart, pancreas and spleen. The average effective dose was 4.3 μ Sv/MBq, which is safe and similar to other 11C-labeled radioligands.

Conclusion: [11C]NOP-1A is an excellent PET ligand to study changes in NOP receptor density or receptor occupancy by novel drugs in living subjects.

Disclosures: Study was supported by NIMH IRP and by CRADA with Eli Lilly & Co. Authors declare no conflicts of interest.

Blocking kappa opioid receptors, even after stress, rescues LTPGABA and prevents reinstatement of cocaine seeking

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Stress can be an important precipitating factor that drives escalation or relapse of drug-seeking behavior, and dopaminergic neurons in the ventral tegmental area (VTA) are an important site of convergence of drugs and stress. We previously identified a long-term potentiation of GABAergic synapses onto these neurons (LTPGABA). Multiple drugs of abuse and stress block or inhibit LTPGABA (Nugent et al, Nature, 2007; Niehaus et al, Eur J Neurosci 2010), suggesting that this common mechanism may play a role in addiction and stress-related diseases. Our recent work shows that the block of LTPGABA by stress requires glucocorticoid (GR, Niehaus et al 2010) and kappa opioid receptors (KOR, Graziane et al, Neuron, 2013), however, it remains unknown how long this lasts. As previously shown, LTP of GABAergic synapses onto dopamine neurons in VTA slices can be triggered by application of a nitric oxide donor (part of the LTP signaling cascade) (IPSC amplitude, $155 \pm 28\%$ of baseline values). After a five-minute cold water swim stress, LTPGABA is blocked for at least five days and returns to normal levels by ten days. Thus, stress causes long-lasting, but not permanent, alterations in plasticity of VTA GABAergic synapses. Remarkably, administration of the KOR antagonist nor-BNI 2 hours ($131 \pm 7\%$ of baseline) or 24 hours ($138 \pm 15\%$ of baseline) after stress also rescues LTPGABA and preliminary data suggest rescue even at 4 days after stress. In parallel, post-stress administration of nor-BNI (2 hours after stress) also prevents reinstatement of cocaine self-administration. Our results show that a single exposure to acute stress can cause days-long changes in the reward circuitry mediated by KORs rescued by a KOR antagonist. No conflicts of interest. This research was supported by NIH grants DA011289 (JAK), MH019118 (NMG and AMP), AA007459 (AMP), DA033372 (LAB), DA15214 (RCP), DA22339 (RCP), DA33641 (RCP), DA18678 (RCP) and a NARSAD Young Investigator Award (AMP).

Dynorphin-kappa opioid receptor activation regulates the dynamics of drug-taking in the progression towards addiction

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In an effort to understand the neurobiology, and potential therapeutic targets, involved in drug addiction, identifying underlying differences between non-dependent use and escalating drug dependence is critical. Increasing efforts are focusing on the negative reinforcing properties of drug withdrawal and activation of brain stress/dysphoric systems, which oppose the positive reinforcing/euphoric effects of drugs of abuse. Key amongst these systems is the dynorphin-kappa opioid receptor system, which has established roles in decreasing dopamine response to drugs of abuse, as well as stress-induced drug relapse.

Using an extended-access model of drug self-administration (6-23 hr/session), we have demonstrated that a single systemic injection of the long-acting kappa opioid antagonist, nor-BNI, has powerful effectiveness in suppressing the progressive escalation and increased motivation for drug infusions typical in the transition to dependent drug-taking. Though to varying degrees, nor-BNI significantly blunts the increased drug-seeking in self-administration of heroin, methamphetamine, cocaine, and nicotine. This inhibition of drug escalation is paralleled by concurrent decreases in progressive ratio breakpoints for drug infusion, indicating that nor-BNI prevents enhancement of drug reinforcement efficacy. Evidence also suggests that increasing drug intake produces increased prodynorphin content in key brain regions involved in reward (e.g. nucleus accumbens) and stress (e.g. central nucleus of the amygdala), which may guide points of intervention for preventing continued progression of the addiction cycle. However, the specific pattern of prodynorphin dysregulation appears to be drug-specific. Using intracranial infusions of nor-BNI, or virally-mediated prodynorphin shRNA knockdown, we can replicate systemic effects into these same identified regions. Together, the evidence suggests dynorphin-kappa receptor overactivation is a key mediator in excessive drug-seeking and taking.

Research supported by DA004043. Training for JES & TWT provided by AA007456 and DA033726, respectively. The authors declare no conflicts of interest with this research.

Quiescent kappa-opioid receptors in the medial prefrontal cortex: the rollercoaster of cognitive control

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Understanding the neurobiological basis of impulsive-like behavior is critically important for the treatment of numerous neuropsychiatric disorders. Impulsivity declines with age, but is a hallmark symptom of numerous neuropsychiatric disorders, including addictive disorders such as alcohol dependence. Utilizing a combination of neurobehavioral techniques and agonist-stimulated GTP γ S assays, we demonstrate that central kappa-opioid receptor (KOR) stimulation induces an impulsive phenotype in rodents with greater effects in younger animals than those that are older. Additional analyses identified that within the medial prefrontal cortex (mPFC), KORs show increased function in younger compared to older Wistar rats. Furthermore, mPFC KOR function declined with the maturity to the extent of becoming 'silent' in older rats. However, following chronic alcohol exposure, KOR function in the mPFC increased significantly and may account for the cognitive deficits commonly observed in alcoholics. In support of this hypothesis, site-specific administration of a KOR agonist into the mPFC was shown to induce working memory deficits. The present data highlight the role of the mPFC KORs as an endogenous impulse/cognitive control mechanism and are could be highly relevant in the clinical management of impulse/cognitive control deficits commonly observed in many neuropsychiatric disorders. Disclosures: Support for this research was provided in part by R01AA020394-01 from the National Institute on Alcohol Abuse and Alcoholism and the WSU Alcohol and Drug Abuse Research Program according to the State of Washington Initiative No. 171. BMW is a consultant for H. Lundbeck A/S, Denmark.

Diffusion MRI : imaging the wiring of the brain

Maxime Descoteaux

Sherbrooke Connectivity Imaging Lab (SCIL), Computer Science, Université de Sherbrooke

In this talk, diffusion MRI (dMRI) will be introduced, from its basics to its many applications for human brain mapping. We will see how one can go from raw diffusion data to full brain white matter structural connectivity. This computerized process is called tractography. In particular, we will see how diffusion tractography is currently at the heart of connectomics studies and how it can be used for research and clinical applications. Connectomics is at the heart of modern neurosciences, neuroimaging and neuroinformatics.

Optogenetic control of pain pathways in vivo

Philippe Séguéla

Montreal Neurological Institute- McGill University

Nociceptors are specialized primary sensory neurons activated by noxious stimuli. Their selective activation and/or silencing can control pain perception. Using a conditional genetic strategy, we generated transgenic rodent models in which pain is either optically evoked by activation of excitatory channelrhodopsin-2 in nociceptors or inhibited by silencing nociceptor activity using hyperpolarizing opsins. Our approach consists of expressing the proton pump archeorhodopsin (ArchT) fused to EGFP in the Nav1.8+ nociceptors, using the Nav1.8-Cre driver line. Cellular distribution of the ArchT-EGFP actuator was assessed in fluorescence in DRG, trigeminal ganglia, sciatic nerve, glabrous skin and dorsal horn of the spinal cord, and showed a selective expression of ArchT in nociceptive somata and distal processes. Strong labeling both in spinal laminae I and II and in glabrous skin demonstrated an efficient trafficking of ArchT from cell soma to central terminals and peripheral targets. Electrophysiological recordings confirmed significant outward photocurrents and hyperpolarizations in ArchT-expressing cultured DRG neurons in response to yellow light (589 nm) stimulation. These light-evoked inhibitions were sufficiently large to block electrically- as well as chemically-induced action potentials. Analgesic effects of transdermal illumination with yellow light were observed in freely moving transgenic mice. A complete behavioral analysis is currently in progress as sensory phenotyping is conducted under several acute and chronic pain conditions. Our results validate the development of constitutive transgenic models in which the function of genetically-defined neuronal populations in somatosensory pathways can be optically and non-invasively controlled with high spatiotemporal resolution. This technology allows the selective interrogation of candidate cellular targets in search of genetic or pharmacological treatment of chronic pain conditions resistant to opioids.

Large-scale electrophysiology and virtual reality for reward circuit research

Sotiris Masmanidis

University of California, Los Angeles

I will introduce a recently developed technology, nanofabricated silicon-based probes, for recording large-scale neural activity across multiple brain areas. This technology routinely allows simultaneous recording of over one hundred neurons from the basal ganglia and related brain areas. Additionally, I will discuss the development of a virtual conditioned place preference assay relying on olfactory cues, and some preliminary results in combining these techniques to understand the network-level mechanisms of reward-based learning and addiction.

Kappa Opioid Receptor signaling controls the gain of an amygdalar anxiety circuit

Thomas Kash, Nicole Crowley

University of North Carolina Chapel Hill

A large body of behavioral evidence indicates that activation of kappa opioid receptors (KOR) increases anxiety-like behavior. However, to date the circuit that mediates KOR induced anxiety has yet to be identified. Using a combination of electrophysiological and optogenetic techniques we found that activation of KOR inhibits glutamate release in the bed nucleus of the stria terminalis (BNST) and prevents the anxiolytic phenotype seen with activation of basolateral amygdala (BLA) to BNST projections. In addition, optically-evoked dynorphin-induced heterosynaptic plasticity of glutamate inputs in the BNST is demonstrated. These results demonstrate a local dynorphin-dependent, neuropeptidergic mechanism of inhibiting an anxiolytic pathway, providing a discrete therapeutic target for anxiety treatment

A photochemical approach to peptidergic neuromodulation

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The mammalian striatum is rich in the opioid neuropeptides enkephalin and dynorphin, as well as the tachykinin Substance P. Despite their abundance, little is known about their role in modulating striatal activity. The inability to deliver these relatively large and hydrophobic molecules with precision in brain tissue has limited quantitation of their actions and hindered studies into the kinetics of peptidergic transmission. The use of photoactivatable or ‘caged’ signaling molecules is widespread in neuroscience due to the spatiotemporal precision afforded by light. To facilitate our studies into peptidergic transmission in the striatum, we have developed caged opioid, tachykinin and cholecystokinin neuropeptides and a caged analog of the opioid antagonist naloxone. Photochemical release of opioid peptides during electrophysiological experiments from striatal brain slices revealed two temporal domains of opioid signaling that are specific to distinct targets within the striatal microcircuit. Furthermore, we have successfully integrated optogenetic tools with our photochemical reagents using dual-color one-photon excitation to study peptidergic modulation of genetically identified synapses in the striatum. These tools should be generally useful to the opioid research community.

This work was supported by the Helen Hay Whitney Foundation (MRB), the Charles A. King Trust/Bank of America Co-Trustee (MRB), the National Institute on Drug Abuse (K99DA034648 to MRB; RO1DA08163 to JTW), the Canadian Institute of Health Research (SQN), the National Institute of Mental Health (RO1MH085418 & R01MH100568 to BLS), and the Howard Hughes Medical Institute (BLS).

Mu-opioid receptor gene locus: new functional variants

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Opioids are the most widely used analgesics to treat moderate to severe pain. However, the analgesic efficacy of opioids is compromised by wide range of optimal dose response and side-effects such as tolerance, dependence and hyperalgesia (OIH). The primary target for clinically used opioids is the μ -opioid receptor (MOR). Genetic variability within human OPRM1 gene locus, coding for MOR receptor, has been proposed to explain variation in responses to opioids, with the primary focus on the non-synonymous change A118G. Through the association study design we recently identified new functional genetic variant within OPRM1 gene locus, SNP rs563649, that led to the cloning and characterization of new human MOR isoform MOR-1K. MOR-1K isoform codes for 6 transmembrane (TM) MOR variant, instead of 7TM structure that is characteristic for GPCR, and shows substantial functional differences in comparison with major isoform.

Supported by NIH/NIDA grant # 5R41DA032293-02;

Conflict of interest: Shareholder and Consultant for Algynomics Inc

Chromatin Cross-Talk in the Nucleus Accumbens: Implications for Cocaine-Induced Behavioral and Molecular Adaptations

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Repeated exposure to cocaine results in increased histone acetylation and decreased histone methylation in the nucleus accumbens (NAc), both of which promote drug-induced alterations in gene expression. Histone deacetylases (HDACs) are a family of enzymes that regulate the acetylation of histone tails. We show that local knockdown of HDAC1 from the NAc of floxed HDAC1 mice blocks cocaine-induced behavioral plasticity. We further show that pharmacological inhibition of class I HDACs in the NAc increases global levels of histone acetylation (H3K9ac and H3K14ac), but also induces repressive histone methylation (H3K9me2), and blocks cocaine-induced molecular and behavioral adaptations. Repeated exposure to cocaine increased expression of many GABAA receptor subunit genes in the NAc and increased the frequency of inhibitory post-synaptic currents on medium spiny neurons. These effects of cocaine were blocked by HDAC inhibition which was correlated with a prominent increase in repressive histone methylation at the promoters of GABAA receptor subunit genes. Our findings suggest a novel mechanism by which prolonged and selective HDAC inhibition can alter behavioral and molecular adaptations to cocaine.

Kennedy: Canadian Institutes for Health Research (ref 90086)

Eric Nestler: NIDA and NIH

The role of HDAC3 in acquisition and extinction of drug-seeking behavior

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University of California Irvine

Repeated use of drugs of abuse causes persistent alterations in gene expression responsible for the long-term behavioral and structural changes in central reward pathways. Recently, it has been suggested that epigenetic mechanisms are responsible, in part, for these drug-induced changes in gene expression. Epigenetic regulation of gene expression may provide transient and potentially stable conditions, which in turn may ultimately participate in the molecular mechanisms required for neuronal changes subserving long-lasting changes in drug-seeking behavior. Our research is focused on understanding the role of chromatin modifying enzymes in the acquisition and extinction of context-drug associated memory formation. In particular, we examine how the histone acetyltransferase CREB-binding protein (CBP) and the histone deacetylase 3 (HDAC3) are pivotally involved in regulating histone acetylation required for transcription underlying context-cocaine associated memory formation using the conditioned place preference (CPP) paradigm. One exciting result of this research is that HDAC inhibition after establishing a CPP significantly facilitates extinction of drug-seeking behavior in a manner that is refractive to reinstatement. Thus, understanding chromatin modifying mechanisms that establish and maintain drug-dependent plasticity changes may lead to a better understanding of substance abuse disorders as well as novel approaches for treatment.

This research is currently supported by NIDA.

POSTER PRESENTATIONS

No. 1 - Structural and functional interaction between 6TM MOR isoform and β 2-adrenoreceptors

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Opioids are the most widely used analgesics to treat moderate to severe pain. However, the analgesic efficacy of opioids is compromised by side-effects such as tolerance, dependence and hyperalgesia (OIH). The primary target for opioids is the 7 transmembrane (7TM) μ -opioid receptor (MOR). 6TM, an alternatively spliced isoform of MOR, lacks the first TM and extracellular domains and has been associated with high pain sensitivity and poor response to morphine (Shabalina et al., 2009). Two independent groups recently showed that, 6TM is not localized at the cell membrane, instead is retained in the intracellular compartments (Gris et al., 2010; Majumdar et al., 2011). Current literature hypothesizes that 6TM localization and functional activities could be strongly linked to a chaperone or a specific GPCR partner. It was also suggested the β 2-adrenoreceptor (AR) could be such co-factor (Liang et al., 2007; Hudson et al., 2010). To investigate this important possible link between 6TM MOR and AR we screened three AR subtypes (β 1-, β 2- and β 3-ARs) for their ability to traffic 6TM to the cell surface. We expressed 6TM alone or in combination with three AR subtypes and we screened for their ability to alter 6TM localization in human cells. We observed that, when expressed alone, 6TM was mainly present in the intracellular compartments and colocalized with marker for ER but not with the marker for plasma membrane. When coexpressed with β 2-ARs, 6TM was translocated from ER compartments to the plasma membrane. However, upon coexpression with β 1- or β 3- AR, 6TM retained its intracellular localization, suggesting that β 2 could be the only subtype of AR responsible for 6TM alterations. To further investigate the mechanism of action, we demonstrate that coexpression of 6TM with β 2-ARs in neuronal Be(2)C cells increases the percentage of cells with positive Ca^{2+} response upon treatment by a selective ligand for 6TM - 10 μ M IBNtxA (Majumdar et al., 2011), compared to the cells transfected with 6TM alone. Our data strongly suggest that β 2-AR could be a structural and functional partner of 6TM MOR.

No. 2 - Synthesis and evaluation of mixed efficacy mu opioid receptor (MOR), delta opioid receptor (DOR) ligands

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Although opioid analgesics represent the gold standard for the treatment of acute and chronic pain, their usage is often accompanied by undesirable side effects such as the development of dependence and tolerance. A considerable amount of research has thus been done to find a potent analgesic that does not display these negative attributes. In general, clinically used opioids such as morphine evoke both the desired and undesired effects through activation of the mu opioid receptor (MOR). Numerous reports have indicated that the undesired MOR-related side effects may be ameliorated by concomitant ligand interaction with the delta opioid receptor (DOR). Our current research is focused on the development of small molecule opioid ligands that display a mixed MOR/DOR efficacy profile. Here, we describe a diverse series of structural modifications to opioid peptidomimetic 1, which employs a tetrahydroquinoline (THQ) scaffold and is modeled on a series of cyclic tetrapeptide opioid agonists. Compound 1 is currently the only small molecule MOR agonist/DOR antagonist reported to cross the blood brain barrier, and is approximately equipotent to morphine in the mouse warm water tail withdrawal assay following intraperitoneal administration. Structural modifications focused on increasing the metabolic half life of compound 1 will be presented, based on the results of metabolite identification studies.

We also describe a series of 4-substituted piperidine and piperazine compounds based on THQ 2, a compound that shows balanced, low nanomolar binding affinity for MOR and DOR. We have shown that by changing the length and flexibility profile of the side chain in this position, binding affinity is improved at both receptors by a significant degree. Furthermore, several of the compounds in this series also display good efficacy at MOR, while simultaneously displaying DOR antagonism.

No. 3 - The fungal collybolides as a new class of sesquiterpenes that selectively interact with the kappa opioid receptor

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Selective kappa opioid receptor agonists and antagonists have potential as therapeutics for the treatment of psychiatric and neurological disorders, as well as obesity. Most kappa receptor ligands are nitrogenous compounds with tertiary amine function. An exception to this is salvinorin A, a non-nitrogenous diterpene extracted from the hallucinogenic Mexican mint *Salvia divinorum* that exhibits potent and highly selective agonistic activity at kappa opioid receptors. The structure of salvinorin A has a furyl- δ -lactone motif; this motif is also present in collybolides, sesquiterpenes extracted from the fungus *Collybia maculata*. We therefore examined whether collybolide and its sesquiterpene diastereoisomer, 9-epi-collybolide function as opioid receptor ligands. Competition binding assays as well as functional GTP γ S binding and cAMP assays carried out with human embryonic kidney cells expressing either mu, delta or kappa opioid receptors show that both compounds are highly selective kappa opioid receptor agonists. Interestingly, collybolides display a biphasic binding and signaling profile with a transition at very low ligand concentration (EC₅₀ >10-12 M) that is reported for the first time and likely originates from a receptor population functionally different from the major population associated with the transition at higher ligand concentration. Interestingly, 7-epi-collybolide, a hemisynthetic epimer at the level of the furan group, also exhibits a partial agonistic activity at higher concentrations. Taken together, these studies show that collybolides represent a novel class of selective kappa opioid receptor agonists that could be developed as therapeutics in the treatment of drug addiction, mood disorders or pain.

This work was supported by NIH grants DA008863, DA019521, and NS026880 to L.A.D.

No. 4 - Chronic Morphine Treatment Alters Cannabinoid Modulation of GABAergic Synaptic Transmission in the Periaqueductal Grey

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Cannabinoids and opioids produce antinociception by modulating GABAergic synaptic transmission in a descending analgesic pathway from the midbrain periaqueductal grey (PAG). While chronic opioid treatment produces opioid tolerance, it has recently been shown to enhance cannabinoid-induced antinociception within the PAG. This study examined the effect of chronic opioid treatment on opioid and cannabinoid modulation of GABAergic synaptic transmission in the PAG. Midbrain PAG slices were prepared from untreated animals, and animals that had undergone chronic morphine (4x 5 mg/kg), or saline pre-treatment over 2 days. Whole cell voltage-clamp recordings were made from neurons within the ventrolateral PAG. In slices from untreated animals, the pan-cannabinoid receptor agonist WIN55212 and the opioid agonist DAMGO reduced the amplitude of electrically evoked GABA_A receptor-mediated inhibitory postsynaptic currents (IPSCs) in PAG neurons, with EC₅₀s of 30 and 100 nM, respectively. The inhibition of evoked IPSCs produced by WIN55212 (30 nM) and DAMGO (100 nM) was similar in PAG neurons from morphine and saline treated animals. However, when eIPSCs were recorded in the presence of the CB1 antagonist AM251, DAMGO-mediated inhibition of the current was significantly greater in the morphine-treated neurons. Finally, AM251 increased the frequency of spontaneous miniature IPSCs in PAG neurons from chronic morphine, but not saline treated animals. These results indicate that while the potency of cannabinoid agonist-induced inhibition of GABAergic synaptic transmission is unaffected by chronic morphine treatment, there is an enhancement of endocannabinoid-mediated tonic inhibition. Thus, endocannabinoid modulation of synaptic transmission should be an important consideration in the development of novel opioid analgesic therapies.

Research was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number F32DA034464.

No. 5 - EGFP-NOP Mice: Location in Spinal Cord and DRG

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NOP receptors and N/OFQ are found throughout the brain, in the spinal cord, dorsal root ganglia, and a variety of peripheral tissues. To better characterize the localization of the receptor, immunohistochemical experiments were conducted on mice in which the NOP receptor is replaced by an active NOP-eGFP receptor C-terminal fusion protein. After perfusion and fixation, sections from dorsal root ganglia and spinal cord were stained with anti-GFP antibodies, as well as with antibodies to NF200, CGRP, PKC γ , and with the isolectin IB4. In the DRG, NOP-eGFP receptors are expressed on large, medium, and relatively small NF200+ (myelinated) cells that are mostly CGRP-, suggesting they are not the peptidergic A δ nociceptors. This suggests that the NOP-eGFP receptors might be on myelinated low-threshold mechanosensory neurons, including down-hair afferents. Down-hair afferents are the most sensitive mechano-sensitive somatosensory neurons and contribute to touch perception. Down-hair afferents have myelinated axons and relatively small diameter cell bodies, and are NF200+. These cells contain neither mu nor delta receptors. They project to the ventral border of inner lamina II of the spinal cord where PKC γ + spinal neurons are located. There are also a number of small cells that are NOP-eGFP+, NF200-, CGRP+: these are likely the typical peptidergic nociceptive C-fibers, all of which co-express mu receptors. In the spinal cord, NOP-eGFP receptors are highly expressed in laminae I-III, colocalizing with PKC γ (ventral border of lamina II inner) as well as with IB4 (dorsal border of lamina II inner), but appear to be expressed ventrally throughout the spinal cord. In most of the cells visualized, whether they be from the spinal cord, DRG, or brain, NOP receptors appear to be located throughout the cell, rather than being localized to the plasma membrane. In summary, our immunohistochemical characterization reveals information regarding the cell types containing NOP receptors in DRG and the laminar location in the spinal cord, information that will be useful in understanding the mechanism by which NOP receptors alter a nociceptive response.

This work was funded by NIH

No. 6 - The DOR2 antagonist naltriben abolishes KNT-127-induced anxiolytic-like effects in rats.

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Objective: We previously reported that the delta opioid receptor (DOP) agonists KNT-127 produce potent anxiolytic-like effects in animal models. Here, we report on the roles of DOR subtypes (DOR1 and DOR2) play in mediating KNT-127-induced anxiolytic-like effects.

Methods: Male Wistar rats were used for behavioral experiments. The anxiolytic-like effect was evaluated using the elevated-plus maze test. As reference, the antinociceptive effects were evaluated using the formalin test. KNT-127 was administered 30 min before the test session. The DOR1-selective antagonist 7-benzylidenenaltrexone (BNTX) and the DOR2-selective antagonist naltriben (NTB) were administered 30 min before KNT-127 treatment.

Results: Pretreatment with NTB completely abolished KNT-127-induced anxiolytic-like effects in rats. By contrast, BNTX produced no effect at a dose that completely blocked the antinociceptive effects of KNT-127.

Conclusion: We clearly demonstrated that the DOR2-selective antagonist, but not the DOR1-selective antagonist, abolishes the anxiolytic-like effects of the DOR agonist KNT-127, suggesting different roles of these DOR subtypes in anxiety. We propose that DOR2-selective agonists would be good candidates for future development of anxiolytic drugs.

Financial Disclosures: The authors report no biomedical financial interests or potential conflicts of interest. This research was supported by research grants from an Intramural Research Grant (24-2) for Neurological and Psychiatric Disorders of NCNP.

No. 7 - NR2B-mediated changes in hippocampal spine morphology following morphine conditioned place preference

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The hippocampus plays a critical role in the development of opiate preference and cravings triggered by environmental cues. The non-medical use of prescription drugs occurs in 13% of young adults and 1.7% of the overall population. Moreover, relapse to opiate addiction occurs at alarming rates which signifies the need for improved therapeutics. To understand the mechanisms that control the formation and retrieval of drug associated memories we investigate how morphine exposure in a novel context modifies hippocampal function. Following morphine conditioned place preference (CPP), levels of the NR2B NMDA receptor subunit are increased in post-synaptic density fractions of the hippocampus which persists after extinction of morphine CPP. This may be important for cue dependent relapse as hippocampal infusion of the NR2B antagonist ifenprodil blocks morphine induced reinstatement. Furthermore, hippocampal LTP is impaired after morphine CPP and extinction, indicating that long lasting changes in synaptic function occur after repeated morphine exposure. To investigate this, we are examining dendritic spines on CA1 pyramidal neurons of which there are three main varieties; thin, stubby and mushroom. The formation and retraction of these spines is controlled by Rho family GTPases by regulation of actin polymerization. Preliminary data suggests that the density of thin spines is decreased following morphine CPP. Interestingly, it is known that increased RhoA activity can decrease the number of thin spines and that RhoA regulators p250GAP and kalirin-7 both form direct interactions with the NR2B subunit. Future studies using a combination of 2-photon and confocal imaging will determine how NR2B-RhoA signaling is involved in alterations of spine morphology after morphine CPP. Altogether, our data highlight new mechanisms underlying context-dependent drug seeking behavior and may lead to development new therapeutic approaches to preventing relapse.

NIH grant R01 DA025036

No. 8 - Prolonged activation of Toll-like receptor 4 (TLR4) down-regulates NOPr expression in human glioblastoma cells, hampering nociceptin ability to counteract TLR4-mediated elevation of [Ca²⁺]_i and induction of NF-κB

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INTRODUCTION: The bidirectional interaction between neurons and neighboring glia contributes to the onset and maintenance of chronic pain. Toll-like receptor 4 (TLR4) is a key glial activator inducing the release of cytokines and chemokines. The endogenous peptide nociceptin/orphanin FQ (N/OFQ) and its receptor (NOPr) are involved in pain transmission and inflammatory response. Interestingly, NOPr is expressed in glia and may be activated by N/OFQ released by other neuronal or immune cells, possibly playing a role in the glial adaptive responses to the altered activity of nociceptive neurons in chronic pain. We aimed to investigate any cross-talk between TLR4- and NOPr-mediated intracellular signaling in a model of human glia exposed to *E. coli* lipopolysaccharide (LPS), a TLR4 agonist.

MEHTODS: Experiments were carried out in U87-MG glioblastoma cells after acute (0-24h) or prolonged (72h) exposure to LPS (0.05–10ng/ml) and/or N/OFQ (1μM). Interleukin-1β (IL-1β) mRNA levels were detected by qPCR; LPS-induced elevation of [Ca²⁺]_i and transcriptional activation of NF-κB reporter gene were detected by calcium imaging and dual reporter gene assays, respectively. LPS-induced changes of NOPr transcripts and density of NOPr binding sites were investigated by qPCR and receptor binding assays.

RESULTS: An acute activation of TLR4 by LPS induces a significant increase of IL1-β mRNA that is blocked by the simultaneous exposure to N/OFQ, which is also able to counteract LPS-induced increase of [Ca²⁺]_i and NF-κB activation. The selective NOPr antagonist [Nphe(1)]-Nociceptin (1-13)-NH₂ prevents the inhibitory effect of N/OFQ. Interestingly, a prolonged activation of TLR4 by LPS significantly down-regulates NOPr expression through transcriptional processes requiring the activation of p38MAPK. In these conditions, N/OFQ-mediated inhibition of LPS-induced increase of [Ca²⁺]_i and NF-κB activation is absent. These findings suggest a relevant role played by N/OFQ/NOPr system, balancing glial activation after an acute exposure to LPS; this action is lost after a prolonged exposure to LPS that mimics activation of TLR4 observed in chronic pain.

Funded by FARB2012, RFO2012, RFO2013

No. 9 - Neuroprotective effects of biphalin in a mouse model of mild traumatic brain injury (mTBI)

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Traumatic brain injury (TBIs) concussion is often a result of traffic accidents, contact sports as well as battlefield or terrorist explosions. TBI is classified based on severity. A mild form of traumatic brain injury (mTBI), sometimes called mild head injury – MHI, often results in the post-concussion syndrome (PCS). Unfortunately, PCS is usually underestimated, because the immediate physical symptoms decrease rapidly and conventional neuroimaging studies of the brains of most mTBI victims often do not express any radiological evidence of brain lesions. However, cognitive impairments persist for weeks, months or even years after the incident. A mouse weight drop model mirrors well the mTBI-induced long-lasting learning and memory impairments observed in humans [1]. Recent results indicate that opioids, especially biphalin show promising anti-neurodegenerative properties [2,3]. Therefore, we decided to assess if an immediate post-injury injection of biphalin provided any benefits in mTBI behavioral impairments. After a systemic administration of biphalin we observed an improvement in spatial and recognition memory in the Morris Water Maze and Novel Object Recognition tests 7 and 30 days post-trauma. Our new data suggest that opioid receptor activation may provide neuroprotection of post-traumatic neurodegeneration processes. Further investigations will be carried out in the development of optimal post-accidental therapeutic time-window for efficacious treatment of mTBI.

Acknowledgements: This work was supported by the Polish National Science Center (NCN), grant no. 2011/03/N/NZ4/02021 for Anna Lesniak. The technical support by Zdzisława Kowalska is highly acknowledged.

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No. 10 - The Use of Opioids in the Treatment of Young People with Chronic Non-Malignant Pain at a Multidisciplinary Pain Clinic in Denmark.

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Background: In Denmark the general opinion among doctors is that as far as possible opioids should be avoided in the treatment of young people with chronic non-malignant pain.

Aim: To study the use of opioids in young patients from 17-28 years of age with chronic non-malignant pain treated by trained pain specialists in a multidisciplinary pain clinic in Denmark with a total of 3000 referrals a year.

Method: A retrospective systematic review of the records of all 60 young patients (45 female and 15 male) treated medically in the clinic during 12 months from 2013-2014.

Results: 52% were in opioid therapy when referred to our clinic. At the end of their therapy 65% of all patients were on opioid treatment, 64% of the women and 66% of the men. In the clinic they were all placed on antidepressants and anticonvulsants before opioids were started. The opioids used when well treated were methadone in 51% of the patients with a mean dose of 18 mg a day (4,5-40mg), buprenorphine in 10% with a mean dose of 0,36 mg a day (0,12-0,6 mg), slow release morphine in 8% with a mean dose of 18 mg a day (15-20mg), slow release tramadol in 26% with a mean dose of 225 mg a day (50-400 mg) and tapentadol in 5% with a mean dose of 350 mg a day (300-400mg).

Conclusion: Despite the view on opioid usage in young people with chronic non-malignant pain and the attempt to avoid addictive drugs, this study shows that opioids are necessary analgesics in young patients too in order to achieve a satisfactory pain relief. Relatively low doses and only long-acting opioids were used. There was no difference between female and male patients with regard to opioid use. Methadone was the preferred opioid because it was well tolerated by most, and because of low level of tolerance development.

No. 11 - Opioid receptor expression in mice with persistent, long-term neuropathic pain

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Introduction

Chronic pain arising from peripheral nerve lesions serves a challenge due to its multifactorial character and consequent resistance to treatment. Despite extensive research there is still not enough knowledge about this phenomenon in the aspect of developing effective pain-relief. There is much controversy around the use of opioids in neuropathic pain. Numerous studies bring forward hypotheses explaining reduced effectiveness of opioid analgesia under neuropathic pain conditions. Among them are the alterations in opioid receptor expression, but studies employing different models of neuropathy demonstrate variable results. A decrease or no change in opioid receptor expression was reported, while others claim that this is a dynamic process changing over time. Therefore, our aim was to study the expression of 3 types of opioid receptors in a mouse model of long-lasting neuropathic pain.

Methods

To study opioid receptor expression we utilized a spared nerve injury (SNI) mouse model. Male, NMRI mice were sacrificed after 40 days after nerve ligation or sham surgery and the dorsal and ventral portions of the lumbar and sacral parts of the spinal cord were removed. The relative abundances of opioid receptor mRNAs was quantified in qPCR analysis using specific primer sets.

Results

Transcripts of all 3 opioid receptors were detected in the dorsal horn of the spinal cord ipsilateral to the ligation. But their amount did not differ as compared to the sham or control mice. There was also no alteration in the expression level of either of the receptors.

Conclusion

Lack of alterations in opioid receptor expression in the spinal cord dorsal horn in long-term neuropathy is the first step towards a notion that opioids may be adequate for clinically meaningful analgesia in patients with a long history of neuropathic pain. However, opioid receptor functionality and interaction with other neurotransmitter systems demands further investigation to support this claim.

Acknowledgements

This work was supported by the Berzelii Technology Centre for Neurodiagnostics and Swedish Medical Research Council (Grant 9459).

No. 12 - The Functional Rewiring of Cortical Synapses in a Translational Model of Neuropathic Pain

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Neuropathic pain is a progressive neurological disease and often refractory to therapeutic intervention. The negative affect and emotion associated with chronic pain suggests a rewiring of higher cognitive functions and a dysfunctional cortex. To investigate the cellular mechanisms underlying this behavioral state, we used the spared nerve injury (SNI) model of neuropathic pain in wild-type and transgenic rats to assess structural and neuronal plasticity in the prelimbic cortex. This strategy enabled us to link a persistent (<40d) SNI-induced regional increase in C-Fos activation and increased density of long/thin dendritic spine filaments ballistically loaded with fluorescent DiI, with an overactive release of calcium from the endoplasmic reticulum (ER) and neuronal hyperexcitability. The increased firing measured in brain slices from SNI animals was not attenuated by blockade of NMDA/AMPA receptors or activation of GABA-A/B receptors, indicating an adaptation beyond synaptic inputs. Instead, the hyperexcitability was linked to a failure of intrinsic-inhibition mediated by KCNQ (Kv7) ion channels, a loss of the slow after-hyperpolarization (AHP) and an elimination of inhibitory accommodation (spike-frequency adaptation)—all of which could be restored by blockade of PKA, manipulation of calcium stores or stabilization of KCNQ channels with retigabine. These SNI-induced functional adaptations demonstrated an unexpected specificity to CaMKII-positive, deep cortical (5/6) layer neurons in the prelimbic cortex contralateral to the SNI.

Likewise, the SNI-induced structural changes in spine density and filament length suggestive of new synaptic connections in prelimbic layer 5/6 neurons were not observed in pyramidal neurons in the visual cortex. Taken together these findings demonstrate (1) the complexity of the neuroadaptations during neuropathic pain, and (2) that the enduring hyperexcitability in the cortex during this state often attributed to NMDA-dependent LTP may actually result from an ER calcium-dependent loss of intrinsic inhibition.

This work was supported by NIDA/NIH (DA033342 & DA028811). No conflicts of interest.

No. 13 - Synthesis, Optimization, and Evaluation of Mixed-Efficacy μ -Opioid Receptor Agonists/ δ -Opioid Receptor Antagonists: A Balancing Act

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Several studies have shown that selective δ -opioid receptor (DOR) antagonism in the presence of a μ -opioid receptor (MOR) agonist or administration of a MOR agonist in DOR knockout mice greatly reduces the development of MOR-mediated morphine tolerance and dependence.¹⁻³ Unfortunately, multi-drug regimens and administration of “drug cocktails” are often impractical and difficult to implement in clinical practice due to undesired side effects and decreased patient compliance. With these limitations in mind, our lab is interested in creating and synthesizing a bi-functional ligand, or series of bi-functional ligands, with a mixed-efficacy profile that display MOR agonism/DOR antagonism. Although our original opioid peptidomimetic series displayed the desired profile and validated our tetrahydroquinoline (THQ) scaffold as being a suitable and bioavailable template, the compounds in this original series displayed 10-130-fold preference for MOR over DOR, and many of the compounds still exhibited considerable affinity and efficacy at the κ -opioid receptor (KOR).⁴ In a subsequent generation of compounds, we have explored two different modifications to our original scaffold in attempt to balance the affinity at MOR and DOR while reducing KOR affinity and efficacy. One modification included replacement of a THQ scaffold with a tetrahydronaphthalene (THN) scaffold. However, this change not only unbalanced MOR and DOR affinities by preferentially increasing affinity at MOR, it also disrupted the efficacy profiles at all three receptors. Additionally, we modified the original scaffold through N-acetylation of the nitrogen in the THQ core. This modification not only improved DOR affinity, but also reduced KOR affinity without significantly altering efficacy profiles, thereby creating a bi-functional ligand with a cleaner and more balanced MOR agonist/DOR antagonist profile.

No. 14 - DENKIs (Dual Enkephalins Inhibitors), a pharmacological and therapeutic breakthrough

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Enkephalins are endogenous peptide-ligands of opioid receptors with affinities for the mu (MOR) and delta (DOR) receptors in the range of morphine [1]. They are released by intense stimuli such as pain but their effects are transient (\leq min) due to their rapid degradation by two metallopeptidases, NEP and APN. As early as 1984, we have developed inhibitors named DENKIs (Dual ENkephalinase Inhibitors) increasing concentration and half-life of ENKs, specifically in the painful area, hence inducing a “physiological” analgesia. The most advanced DENKIs are PL37 and PL265 both in clinical trials. DENKI-induced analgesia is first new concept emerging in the clinical treatment of pain since decades [1]. DENKIs have two main indications : i) via i.v., severe acute or chronic pain due to surgery, traumatism or cancer; ii) p.o., severe neuropathic pain, such as direct nerve suffering due to chemical (antitumor drugs), metabolic (diabetes), infectious (shingles) or mechanical (accidental section during surgery) lesions.

Both types of pain are unsatisfactorily treated. Morphine (MO) remains the current treatment, but its side-effects restrain its use. As DENKIs are devoid of MO side effects P37 can be substituted, combined or alternated with MO. Furthermore, the analgesia induced by protected ENKs is amplified by their stronger effects than MO on the emotional status of the suffering patient by DORs recruitment in the whole limbic system of reward. This is far from negligible when treating severe chronic pain.

Current medications for neuropathic pain have been developed for other indications: depression for duloxetine, seizures for pregabalin but are effective in less than half of patients, with total relief in only 30% of responders.

Opiates are sometimes prescribed in neuropathic pain, but two issues limit their use: i) an inhibitory effect on the spino-bulbo-spinal descending pathway, reducing their analgesic effects [1]; ii) need of high doses generating numerous and unacceptable side-effects (tolerance and constipation). As shown, none of these inconvenients were observed with ENKs. Finally, a six-fold synergy has been demonstrated when PL37 and pregabalin are combined.

[1] B.P. ROQUES, M.C. FOURNIE-ZALUSKI and M. WURM, Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nature Rev. Drug Discovery*, 2012, 11, 292-311.

No. 15 - Delta Opioid receptors: Important Role In wound healing

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In addition to its analgesic functions, the peripheral opioid receptor system affects skin homeostasis through effects on cell differentiation, proliferation, apoptosis, migration and adhesion. Our group has shown that delta-opioid receptor (DOR) knockout mice reveal as phenotype an atrophic epidermis and over-expression of keratin 10. In addition, the DOR knockout mice have delayed wound healing in a burn wound model and have on the wound margin epidermal hypertrophy and over-expression of dermal MMP-2 and Collagen IV. This suggests that DOR effects keratinocyte migration and/or proliferation during skin homeostasis.

Recent results from our group reveal that not only keratinocytes, but also human fibroblasts are under control of delta opioid receptors and its ligands. We used an in-vitro wound scratch model using human immortalized keratinocytes (N/TERTS) and primary human fibroblasts and we could observe that the DOR ligands were much more stimulating migration than MOR ligands and that there exists a complicated agonist/antagonist relationship. New results on mice by using topically applied opioid ligands show a significant improvement of wound healing in a full thickness, punch biopsy model. In addition, for the past a few years, it has been our major task to ask what is the molecular consequence of delta opioid receptor activation in keratinocytes and how could that influence skin differentiation, homeostasis and finally wound healing. Our discovery of a transcription factor exclusively expressed in epidermis and acts as a bi-functional transcription factor activating and repression of keratin expression is involved in this important signaling pathway.

no conflict of interests to declare

No. 16 - Effects of alcohol on mu opioid receptor-mediated plasticity at dorsal striatal inputs

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The dorsal striatum is one brain area in which the opioid system is strongly impacted by alcohol use. Alcohol use produces changes in mu opioid receptor (MOR) levels and functionality in dorsal striatum. Alcohol also alters the levels of enkephalins, the endogenous MOR ligands in the striatum. It is likely that these changes will alter MOR-mediated signaling. It is unknown however, what effect alcohol has on MOR-mediated synaptic plasticity in this brain region. We previously showed that MOR mediates long-term depression (mOP-LTD) of excitatory transmission in dorsal striatum and that this plasticity was sensitive to in vivo oxycodone exposure. mOP-LTD is mutually occlusive with endocannabinoid-LTD and endocannabinoid-LTD is impaired by both oxycodone and ethanol exposure. We therefore hypothesized that alcohol would impair mOP-LTD at dorsal striatal inputs similar to oxycodone.

To determine the effects of in vivo oxycodone and ethanol exposure, we used single I.P. injections of oxycodone and a chronic intermittent ethanol exposure (CIE) paradigm using vapor chambers in C57BL/6J mice. Whole-cell patch clamp electrophysiology was used to probe striatal inputs in mouse brain slices for changes to MOR-mediated synaptic plasticity induced by exposure to oxycodone or alcohol.

DAMGO induces mOP-LTD of excitatory transmission in dorsal striatal brain slices. A single in vivo exposure to oxycodone disrupted mOP-LTD for multiple days following exposure. We also found that, similar to oxycodone, 2 weeks of CIE disrupted the ability of DAMGO to induce mOP-LTD in the dorsal striatum of mouse brain slices. Therefore two distinct drugs of abuse cause the same disruption to MOR-mediated plasticity in the dorsal striatum. We are currently using an optogenetic approach to determine whether these effects are broad or restricted to specific striatal inputs.

No conflict of interest. Work is supported by the Division of Intramural Clinical & Biological Research of NIAAA.

No. 17 - Naltrexone, like Nalmefene, is a Kappa opioid receptor partial agonist

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In prior published work from this Laboratory, nalmefene (10 mg) and naloxone (10 mg), both selective mu opioid receptor antagonists, were administered intravenously (IV) to healthy human subjects on separate days. In males, nalmefene caused a modest rise in prolactin levels, whereas naloxone did not, demonstrating the kappa opioid receptor partial agonist properties of nalmefene, as documented in appropriate binding studies. We have now compared nalmefene to naltrexone administered IV to evaluate kappa opioid receptor agonist activity of naltrexone. In total, 13 healthy subjects participated: 5 males and 8 females. Males and females were analyzed separately, since females have higher and more variable basal prolactin levels than males. In males, naltrexone or nalmefene (10 mg, IV) were given on separate study days. The serum prolactin levels area under the curve (AUC; from 0-120 minutes post-antagonist administration) was similar for both drugs. The AUC following naltrexone administration was elevated compared to the AUC for the baseline day (Student's T-test; $p > 0.005$). The AUC following nalmefene administration was similarly elevated compared to baseline (Student's T-test, $p > 0.01$). In females, there was an apparent elevation in serum prolactin levels after naltrexone, as well as after nalmefene administration compared to baseline. However, due to the wide variability both at baseline and after antagonist administration, the apparent changes were not significant. These data demonstrate for the first time that naltrexone is a kappa opioid receptor partial agonist in humans.

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

The authors declare no conflict of interest.

No. 18 - Effects of morphine on GABAergic plasticity on dopamine neurons of the VTA: roles of PKA, AKAP, calcineurin, and GABA-A receptor trafficking

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Morphine treatment modulates the synaptic strength of GABAergic synapses onto dopamine (DA) neurons of the VTA by modulating both long-term potentiation (LTP) and long-term depression (LTD). Morphine inhibits a form of LTP mediated by NO and cGMP at these GABA synapses (Nugent et al, *Nature* 446:1086, 2007). A single exposure to morphine also blocks a recently discovered form of long-term depression (LTD-GABA) in DA neurons of VTA (Dacher & Nugent, *Neuropharmacol.* 61:1166, 2011). In LTD-GABA, the postsynaptic A-kinase-anchoring scaffolding protein 79/150 (AKAP79/150) signaling complex selectively regulates inhibitory synaptic transmission by tethering protein kinase A (PKA) and calcineurin (CaN) to GABAergic synapses (Dacher et al, *J. Neurosci.* 33:2650, 2013). Using immunofluorescence and whole-cell patch clamp recording in rat midbrain slices, we report that post-synaptic DA D2 receptor activation in the VTA induces LTD of GABAergic synapses on DA neurons through CaN activation accompanied by PKA inhibition in a process requiring AKAP79/150 as a bridging signaling molecule. Activation of CaN through AKAP induces a clathrin-mediated loss of GABA-A receptors in the synapse and a reduction in the magnitude of the IPSP, a process that is inhibited by prior morphine exposure. In contrast, disruption of AKAP-mediated anchoring of PKA does not affect glutamatergic synapses onto DA neurons, suggesting that the PKA-AKAP-CaN complex is uniquely situated at GABAA receptor synapses where it regulate plasticity associated with GABAA receptor trafficking. Opiate-induced disruption of GABAergic LTP and LTD mediated through these recently elucidated signaling pathways in the VTA has the potential to persistently alter the output of individual DA neurons and of the VTA as a whole. These actions may contribute to the reinforcing and/or addictive properties of opiates. [Supported by the Whitehall Fndn and NARSAD (to FN) and USU].

No. 19 - Trypanosoma brucei as Novel Vaccine Platform for Oxycodone

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Vaccination against abused drugs holds promise as a means to prevent drug addiction, both as an aide to prevent recidivism in drug addicted persons in recovery, as well as to prevent progression from those in early stages of the drug addiction trajectory to prevent development of addiction. Prior attempts to develop such vaccines demonstrated that immune system production of specific antibodies to drugs of abuse can suppress the effects of these drugs, but indicate a need for better vaccine platforms with more sustained production of higher levels of antibodies, and with reduced individual variability. Trypanosoma brucei brucei, a nonvirulent (in humans) strain related to virulent T. brucei strains responsible for African sleeping sickness, has properties of immune stimulation suggesting it may serve as an improved vaccine platform compared to conventional approaches, e.g. non-specific hapten conjugation to proteins such as keyhole limpet hemocyanin, co-administered with adjuvant. Oxycodone was conjugated via the 6-ketone-position using amino-oxy-propanethiol linkage to a peptide which was then enzymatically coupled to the N-terminus of the single uniform coat protein of T.brucei, with up to 100 million uniformly presented oxycodone molecules present on each T.brucei organism. In our initial studies in mice, we detected oxycodone specific antibodies following 2 successive injections of 1.0x10⁶ T. Brucei with conjugated oxycodone. In parallel studies, we also detected antibodies specific for a different small molecule, nitrophenol, following inoculation with T. brucei coupled to nitrophenol. This seminal proof-of-concept study will serve as the basis of further studies of the immune response to T.brucei as a small molecule vaccine platform.

Supported by: NIH-NIDA-CEBRA grant R21-DA036365 (P.S., E.B.), NIH-NCATS-CTSA UL1-TR000043 Pilot Award (B.R., J.P.), Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (M.J.K.) The authors have no conflicts of interest.

No. 20 - Mass spectrometry analysis of agonist-induced MOP receptor phosphorylation in the mouse brain.

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Phosphorylation of the mu-opioid (MOP) receptor is thought to be one of the initial steps leading to the development of analgesic tolerance. Many in vitro data have shown that variation in the efficacy of several opioid drugs in terms of desensitization, arrestin recruitment and internalization is correlated to differential MOP receptor phosphorylation. High-efficacy agonists such as DAMGO induce higher levels of MOP receptor phosphorylation and internalization than low-efficacy agonists such as morphine. The relative importance of particular residues has been approached by site-directed mutagenesis and development of phosphosite-specific antibodies and, more recently, by mass spectrometry analyses which is the only method able to demonstrate the presence of multiphosphorylation on a single receptor. Phosphorylation of MOP receptor occurs primarily at S363 and within a cluster of serine and threonine residues (T370, and 375STANT379) within the cytoplasmic tail of the receptor. Depending on the drug, multiple phosphorylation sites have been identified and shown to be sequentially and differentially phosphorylated by diverse GRK. Most of these data have been obtained in cellular models and it is now essential to study the post-translational modifications of MOP receptors in vivo in order to understand the molecular basis for the diversity of acute and long-term opioid effects.

Here, we describe for the first time by using Nanoflow Liquid Chromatography-tandem Mass Spectrometry (NanoLC-MS/MS), the analysis of endogenous MOP receptor phosphorylation from the brain of mice administered with different opioids. The agonist-induced phosphorylated residues, including multi-phosphorylated clusters on a single receptor, were identified and their relative abundance quantified by a label-free method. The data contribute to demonstrate drug-selective and hierarchical multiphosphorylation of MOP receptor in vivo. A part of this work is reported in Glück et al. (Biol Psy, in press).

Financial support : Region Midi-Pyrénées and European funds (FEDER)

**No. 21 - The activity of HIV-1 Tat and morphine on Toll-like receptor 4 signaling
Implications for mechanism of actions
contributing to HIV-related
neuroinflammation**

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Opiate drug use by HIV-1 positive individuals is reported to exacerbate HIV-associated neuroinflammation. In addition to μ -opioid receptors, opiate drugs reportedly interact with the Toll-like receptor 4 complex (TLR4) in models of chronic pain and neuroinflammation. Likewise, TLR4 is a plausible target for HIV-1 virotoxins such as Tat; therefore, we aimed to clarify the role of morphine and Tat in TLR4 activation using acute HIV-morphine *in vitro* models. We initially employed a human, HEK cell line engineered to stably express CD14 and MD2 either with TLR4 [TLR4(+) cells] or without TLR4 [control, TLR4(-) cells], and containing a NF- κ B-inducible reporter that quantifies TLR4 activation. We found that an acute, 16-h exposure to HIV-1 Tat1-86 (100 nM) increased TLR4 reporter activity; however, at the time and conditions assessed, exposure to morphine (500 nM–5 μ M) did not activate the TLR4 reporter. Using an ELISA, we noted that a 24-h exposure to Tat, but not morphine, increased CCL2 release in TLR4(+), but not TLR4(-) cells. LPS-EK and LPS-RS were used as positive and negative controls to assure TLR4 specificity. We sought to confirm these results using acutely treated, primary, mixed-glia cultures isolated from wild-type and TLR4-null mice. Wild-type, but not TLR4-null mixed glia, showed increased release of CCL2 and CCL5 after 12 h of stimulation with TLR4 agonist LPS-EK, Tat, or combined Tat and morphine, but not morphine alone. Model validation using TLR2 (peptidoglycan) and TLR3 (Poly IC) agonists assessing the capacity of TLR4-null glia and macrophages to activate NF- κ B independently of TLR4 are planned. Overall, our results suggest that acute exposure to Tat, but not morphine, activates TLR4 and chemokine release. Thus, TLR4 expression plays a large role in the acute, Tat-induced chemokine response. Further experiments are required to explore more chronic HIV and opiate exposures, in which prolonged elevations in TLR4 levels may lead to fundamentally different interactions with morphine compared to acute models. Support: NIH T32 DA007027, K02 DA027374, and R01 DA034231. The authors declare no conflicts of interest.

**No. 22 - Adeno-associated virus mediated
gene transfer of arginine decarboxylase
reduces morphine analgesic tolerance.**

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Background: The enzyme arginine decarboxylase (ADC), decarboxylates arginine to produce agmatine. Agmatine has been shown to prevent the development of opioid analgesic tolerance when given by many routes of administration and in multiple models of tolerance. Since agmatine is produced endogenously, the opportunity arises to develop a method for enhancing expression of ADC and potentially the therapeutic effects of agmatine, locally and constitutively. We have recently characterized a method for delivery gene therapeutics to the central nervous system using simple intrathecal injection of adeno-associated virus (AAV) vectors by direct lumbar puncture in conscious mice. We report here the effects of gene transfer of ADC in a model of opioid induced tolerance.

Methods: ICR-CD1 male mice were pre-treated with either AAV5-ADC vector or saline eight weeks prior to testing. Thermal sensory responses were determined using the warm water tail flick immersion test (52.5°C). Mice were given seven subcutaneous (s.c.) injections of either morphine (3 mg/kg (4 times) and 5 mg/kg (3 times)) or saline over the course of 3 days. On day four, tail flick test was performed and cumulative dose-response curves to s.c. morphine (1, 3, 10, 20 mg/kg) were constructed in each of the four pre-treatment groups (Saline-Saline, Saline-Morphine, AAV5-ADC-saline, AAV5-ADC-morphine.) Relative potency (Tallarida 1987) was calculated for each dose-response curve and the development of morphine tolerance assessed in each of the two primary treatment groups.

Results: A significant rightward shift in the morphine dose-response curve was observed in control subjects, as expected. However, in subjects pre-treated with AAV5-ADC, morphine tolerance did not develop.

Conclusion: We conclude that the observed AAV5-ADC prevention in morphine tolerance is likely due to production of the decarboxylase product of ADC, agmatine, which has been shown repeatedly to prevent opioid tolerance.

No. 23 - The Delta-Opioid Receptor Affects Epidermal Homeostasis via ERK-Dependent Inhibition of Transcription Factor POU2F3

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The expression of several neurohormones, neurotransmitters, and neuropeptides, including pro-opiomelanocortin derivatives, such as β -endorphin and [Met5]-Enkephalin, as well as expression of the corresponding receptors has been shown in skin. In view of the constant environmental assaults that the skin must endure, the presence of this neuroendocrine system has been suggested to be important for cutaneous homeostasis and during wound healing. However, there is currently a lack of understanding of the molecular mechanisms by which neuropeptide signalling can modulate keratinocyte function.

Here, we show by Western blot that DOPr signalling specifically activates the ERK 1/2 MAPK pathway in human keratinocyte cell lines. Quantitative imaging suggested a reduced proliferation rate of DOPr activated keratinocytes in culture. Furthermore, DOPr activation markedly delays induction of keratin intermediate filament Keratin 10 (KRT 10) and KRT 1 during in vitro differentiation, observed both by quantitative real-time PCR and Western blot, and abolished the induction of KRT 10 in an organotypic skin model. This is accompanied by deregulation of involucrin, loricrin, and filaggrin, illustrated by a markedly reduced induction of their expression upon initiation of differentiation in vitro. Additionally, POU2F3 was identified as a transcription factor mediating the DOPr-induced regulation of keratinocyte differentiation related genes. It was revealed that DOPr-mediated ERK-dependent downregulation of this factor affects key aspects of keratinocyte function.

Complementing previous studies in DOPr-deficient mice, these data suggest that DOPr activation in human keratinocytes significantly influences epidermal morphogenesis and homeostasis, and might have a major impact during the wound healing process.

The authors have declared that no competing interests exist.

These studies were supported by funding from Swiss National Science Foundation (SNSF) grant 31003A-116811 to M. Bigliardi-Qi and by the Singapore Biomedical Research Council of the Singapore Agency for Science, Technology and Research (A*STAR).

No. 24 - Effect of Peripheral Opioid Receptor Blockade on Opioid Analgesic Demand: A Randomized Clinical Trial

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Peripherally mediated opioid analgesia is a promising approach to pain treatment that avoids the deleterious side effects of centrally acting opioids and nonsteroidal analgesics. To gauge the therapeutic potential of this strategy, we assessed the proportion of morphine analgesia mediated outside the central nervous system. We hypothesized that the selective blockade of peripheral opioid receptors by methylnaltrexone (MNX) will increase the patients' demand for morphine to achieve satisfactory postoperative pain relief. In a double-blind, placebo-controlled, sequential two-center trial, 50 patients undergoing knee replacement surgery were randomized to receive MNX (0.9 mg/kg s.c.) (hospital I: n = 14; hospital II: n = 11) or saline (hospital I: n = 13; hospital II: n = 12) at the end of surgery. Primary outcome was the cumulative amount of i.v. morphine administered over 8 hours postoperatively. Secondary outcomes were pain scores at rest and during movement, vital signs and adverse side effects. After MNX, demands for morphine (hospital I: 35.31 ± 12.99 mg vs. 25.51 ± 7.92 mg, $P=0.03$; hospital II: 35.42 ± 11.73 mg vs. 24.8 ± 7.84 mg, $P = 0.02$; pooled data: $P > 0.001$; means \pm SD) were strongly (by about 40 %) increased. Secondary outcomes were similar in all groups ($P < 0.05$). Thus, a significant proportion of analgesia produced by systemically administered morphine is mediated by peripheral opioid receptors. Drugs that selectively activate such receptors should have the potential to produce powerful clinical pain relief.

The study was funded by International Anesthesia Research Society, Helmholtz Gemeinschaft, and European Commission (FP7-602891-2).

No. 25 - SYNTHESIS AND EVALUATION OF DUAL-ACTIVITY OPIOID-NPFF LIGANDS FOR RECEPTOR AFFINITY, ANTINOCICEPTION AND TOLERANCE LIABILITIES.

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Neuropeptide FF (NPFF) receptor antagonists prevent morphine-mediated antinociceptive tolerance. Dual-acting compounds with opioid agonist and NPFF antagonist may produce antinociception without tolerance.

Methods: In an effort to produce selective, non-peptide NPFF probes, we identified compounds with dual affinity at NPFF and opioid receptors. The affinity and activity of these compounds for opioid and NPFF receptors was determined with radioligand competition binding assays as well as functional assays. Three compounds were selected for in vivo investigation in the mouse warm-water tail-withdrawal test, as well as an initial screen for acute antinociceptive tolerance. Antinociceptive activity and opioid selectivity of these compounds was determined after intracerebroventricular (icv) administration.

Results: A series of compounds were generated and examined in receptor binding and functional assays. Structure-activity relationships were identified in the series of compounds, conveying increased affinity in the nM range for opioid receptors and the uM range for NPFF receptors with opioid agonism and NPFF antagonism in functional assays. Compounds 1, 2 and 3 produced equipotent antinociception lasting at least 50 min, with ED₅₀ (and 95%CI) values of 6.9(4.7-9.5), 5.0(4.2-5.8) and 2.7(2.1-3.7) nmol, icv, respectively that was antagonized by pretreatment with MOR or KOR antagonists. Moreover, although repeat treatment with morphine shifted the ED₅₀ value 9.6-fold rightward, the 1.6-fold rightward shift caused by compound 1 did not produce significant acute antinociceptive tolerance.

Conclusions: Our investigations of structure activity relationships yielded ligands that retained the receptor affinity and activity of the parent compounds. Compounds selected from the in vitro screening demonstrated full antinociception, with one compound showing an absence of acute antinociceptive tolerance. These results suggest the development of dual-action opioid-NPFF ligands may offer therapeutic promise as analgesics with fewer liabilities of use.

Funding: Supported by funds from the University of Mississippi and the State of Florida

COI: none to declare

No. 26 - Platelet-Derived Growth Factor Receptor-beta Antagonism Restores Morphine Analgesic Efficacy Against Neuropathic Pain

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Background: Chronic, intractable pain is a problem of pandemic proportions. Pain caused by nerve injuries (neuropathic pain) is extremely difficult to treat. For centuries, opiates such as morphine have been the first-line treatment for severe chronic pain. However, opiates are often ineffective against neuropathic pain, leaving few options for suffering patients. In these studies, we determined whether PDGFR-beta inhibition could improve the effectiveness of morphine for neuropathic pain treatment.

Results and Findings: Spinal nerve ligation was performed in male Sprague-Dawley rats. The clinically used PDGFR antagonist imatinib did not relieve mechanical pain in a nerve injury model as determined by Von Frey assay. Surprisingly, combining imatinib with a previously ineffective dose of morphine led to complete pain relief. Scavenging released platelet-derived growth factor B (PDGF-B) also markedly augmented the analgesic effect of morphine. Tolerance to the analgesic effect of morphine did not develop when the PDGFR was inhibited or PDGF-B was scavenged.

Conclusions: These findings suggest the novel hypothesis that PDGF-B released by injured nerves renders animals resistant to morphine, implying that PDGFR-beta inhibition could potentially eliminate the tremendous suffering caused by neuropathic pain.

These studies were funded by CPRIT grant awarded to HG and PhRMA Foundation Fellowship to CD.

No. 27 - Intrathecal delivery of a bivalent ligand targeting a mu opioid Receptor mGluR5 Receptor heteromer reduces nerve-injury hyperalgesia

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Background: Reports on the presence of MOR and mGluR5 in the spinal cord, and the existence of MOR-mGluR5 heteromer in HEK293 cells coexpressed with MOR and mGluR5 has raised the likelihood that this putative heteromer may be present in the CNS. Such a heteromer could be involved in the inflammatory process, given the known functional interaction of mGluR5 with the NMDA receptor. Thus, a bivalent ligand (MMG22) that contains both mGluR5 antagonist and mu agonist pharmacophores should antagonize the mGluR5 protomer and activate the MOR protomer of the putative MOR-mGluR5 heteromer. This in fact proved to be the case, in that intrathecal MMG22 has been reported to produce exceptional antinociception without tolerance in mouse models of inflammatory pain (Akgün, E et al. 2013).

Methods: ICR-CD1 males mice were used. Sensory thresholds were determined using an electronic von Frey algometer. Mice were subjected to spared nerve injury (Decostered and Woolf, 2001). At multiple days post-injury, tactile hypersensitivity levels were measured to determine magnitude of hyperalgesia. MMG22, morphine, MPEP, or the combination of morphine + MPEP were administered intrathecally according to the method of Hylden and Wilcox (1980). Sensory thresholds were evaluated post-injection at 5, 12, 20, 60, 90, and 120 minutes post-injection. Data were analyzed by ANOVA and dose-response curves constructed by the method of Tallarida (1997).

Results: MMG22 (1, 100, 1000 pmol) was effective in reversing nerve-injury induced tactile hypersensitivity at 5 and 12 minutes post injection. Higher doses (1-10 nmol) were effective for a duration equivalent to that of effective doses of morphine, MPEP or the combination.

Conclusion: We conclude that MMG22 demonstrates anti-hyperalgesic efficacy in a mouse model of neuropathic pain. The potency of MMG22 appears to be greater than that of either morphine or MPEP given individually or in combination.

No. 28 - Novel ligands at the mu opioid receptor that display bias toward G protein coupling over β arrestin2 recruitment

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Introduction:

Advances in the understanding of functionally selective ligands, or drugs that differentially and preferentially activate or inhibit signaling pathways downstream of receptor binding, have led to drug development efforts specifically targeting certain pathways in the hope of enhancing the beneficial aspects of receptor activation while avoiding negative effects in vivo. While opioids, which mediate their effects through activation of the mu opioid receptor (MOR), are the gold standard for the treatment of moderate to severe pain, their therapeutic use is limited by their significant adverse side effects. Previously we have demonstrated in mice that the absence of the scaffolding and regulatory protein β arrestin2 (β arr2-KO mice) results in enhanced and prolonged antinociception to morphine without the development of antinociceptive tolerance, respiratory suppression and constipation. Based on these findings, we hypothesize that functionally selective compounds which activate MOR without inducing interactions with β arrestin2 would recapitulate the effects of morphine in the β arr2-KO mice and would serve as more effective analgesics with reduced side effect profiles.

Methods:

Herein, we utilize cell lines to evaluate novel ligands for their ability to activate MOR-mediated signaling cascades and induce β arrestin2 interactions with the receptor. Pharmacokinetic and antinociceptive profiles of lead compounds were then determined in C57Bl/6J mice.

Results:

Through this screening process, we have identified compounds that display bias towards the activation of MOR-mediated signaling cascades and away from β arrestin2 recruitment. These compounds are also brain penetrant and induce potent antinociception.

Conclusions:

These functionally selective MOR agonists may serve as useful tools in vivo for evaluating the contribution of β arrestin2 interactions with the receptor to MOR-mediated physiologies and may aid drug discovery efforts for the development of more effective opioid analgesics.

Funding from NIDA: DA033073

No. 29 - Cannabinoid CB1 Receptor Induction of Δ FosB Mediates Mu Opioid Receptor Cross-Sensitization in the Nucleus Accumbens

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Introduction. Repeated administration of abused drugs, such as opiates and Δ 9-tetrahydrocannabinol (THC), the major psychoactive constituent in marijuana, induces the AP1 transcription factor Δ FosB in the striatum. Repeated cannabinoid agonist treatment also cross-sensitizes rodents to the reinforcing and motor stimulant effects of mu opioid receptor (MOR) agonists. We reported that inducible transgenic expression of Δ FosB in D1/dynorphin positive medium spiny striatal neurons enhanced MOR signaling in the nucleus accumbens (NAc) and enhanced the rewarding effects of morphine without affecting MOR expression. Moreover, repeated THC administration induced Δ FosB in the NAc, but inducible transgenic expression of Δ FosB did not enhance cannabinoid CB1 receptor (CB1R) signaling in this region.

Methods. Mice were injected twice daily for one week with 10 mg/kg THC or a ramping dose paradigm of 10, 30 and 60 mg/kg THC, with the dose increasing every other day. Δ FosB induction was determined by immunoblot, and Δ FosB co-localization was determined by dual immunohistochemistry. G-protein activation was determined by agonist-stimulated [³⁵S]GTP γ S binding, either in membranes isolated from dissected brain regions or by [³⁵S]GTP γ S autoradiography.

Results. Repeated THC administration induced Δ FosB in the NAc and dorsal striatum. Δ FosB was co-localized with dynorphin, suggesting induction in D1/dynorphin positive medium spiny neurons. Similarly to transgenic over-expression of Δ FosB, repeated THC administration enhanced MOR-mediated G-protein activation in the NAc, whereas CB1R-mediated G-protein activity was not increased. This effect was limited to NAc because neither THC nor inducible transgenic over-expression of Δ FosB altered MOR-mediated G-protein activation in the dorsal striatum. Importantly, inducible transgenic expression of Δ cJun, a dominant negative inhibitor of AP1-mediated transcription, blocked THC-induced enhancement of MOR activity in NAc.

Conclusion. These results suggest that cross-sensitization of MOR-mediated G-protein activation by repeated THC is mediated by induction of Δ FosB.

Supported by DA014277, DA008227, DA030404 and DA030227 from NIDA.

No. 30 - Calmodulin-dependent Kinase II Protein is Increased in the Hippocampus of Oxycodone Self-administered Adult C57Bl/6 Mice.

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Prescription opioid (PO) abuse is reaching epidemic levels amongst all socio-economic groups in the United States. PO such as oxycodone are powerfully addictive, incur a high risk of fatal overdose, and lead to use of even more dangerous opioids. Progression to addiction involves learning to associate drug use with drug reward, thus the formation of drug-associated memories is an essential component of addictive disease. The hippocampus is acknowledged as the brain region critical for the formation/storage of memories. Memory consolidation in hippocampal neurons involves augmentation of glutamatergic synaptic strength through repeated activation of Calmodulin-dependent kinase II (CamKII). However, the mechanisms underlying drug-associated reward learning are still unclear for oxycodone addiction. To examine molecular correlates in the hippocampus with oxycodone-reward learning, we conducted an oxycodone self-administration experiment. Adult C57Bl/6 male mice were implanted with an intravenous catheter and allowed to self-administer oxycodone 2 hours/day for 14 days (0.25mg/kg/nose poke). Saline-yoked animals were used as control. Mice were then sacrificed and the entire hippocampus was dissected and protein lysate analyzed by Western Blot. We found a statistically significant increase in the amount of CamKII protein in the hippocampus of oxycodone-self administered mice compared to controls, though no difference in phosphorylated CamKII, or the memory-related factors NMDAR1 and Egr1, was observed. These data suggest that an increase of CamKII-dependent drug-associated memory formation may have occurred in the oxycodone-self administered animals and that CamKII may represent a potential therapeutic target for the disruption of drug-associated reward learning. This work was supported by the Kopf Family Postdoctoral Fellowship (DPS), NIH R01DA029147 (YZ) and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (MJK). No conflicts of interest to declare.

No. 31 - Oxycodone causes greater locomotor activation in female compared to male C57BL/6J mice

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Nonmedical use of the oxycodone is a major public health problem. Males and females may present different behavioral consequences related to its use. Unfortunately, many rodent studies of oxycodone's behavioral effects are conducted in mixed groups or males only and subsequently generalized to females. Here, we compare locomotor activity data in drug-naïve vs. oxycodone-exposed male and female mice.

Adult male and female C57BL/6J mice (10 weeks old) were randomly assigned to one of two groups (5 mice/sex/group), drug-naïve and oxycodone-treated, were injected (IP) 3x/day (every 6hr; 1st injection at 0800) for 14 days. Naïve mice received only saline at each injection. Oxycodone-treated mice received oxycodone (10 mg/kg) at each injection. Each day, immediately following the 1st injection, locomotor activity, defined as total distance traveled, was measured in a standard photobeam enclosure during a 2-hr session.

Three-way ANOVA, sex x drug x session, with repeated measures on the last factor, revealed a main effect of sex on locomotor activity in both oxycodone- and saline-treated mice [$F(1,16) = 29.06$, $p > 0.0001$] as well as a main effect of drug [$F(1,16) = 189.09$, $p > 0.00001$] with no significant interaction of sex and drug. Student-Neuman-Keuls post-hoc tests revealed a significant increase in locomotor activity in oxycodone-versus saline-injected animals in both sexes [$p > 0.0005$].

Taken together, these results suggest that sex-based differences produce unique oxycodone-induced locomotor-stimulating effects in male and female C57BL/6J mice.

This work was supported by NIH 1R01DA029147 (YZ), the Adelson Medical Research Foundation (BR & MJK), and the David Rockefeller Graduate Program (DTC). The authors declare no conflict of interest.

No. 32 - SYNTHESIS AND BIOLOGICAL EVALUATION OF A COMPACT, CONFORMATIONALLY CONSTRAINED BIFUNCTIONAL OPIOID AGONIST - NEUROKININ-1 ANTAGONIST

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The co-administration of an opioid agonist combined with NK1 receptor (NK1R) antagonists, as separate compounds, or as one hybrid compound, led to an enhanced antinociceptive potency, with suppressed tolerance effects upon prolonged administration.[1][2]

We reported the compact peptidomimetic Dmt-D-Arg-Ab-Gly-(3,5-(CF₃)₂)NMe-benzyl, (SBCHM01), possessing a mixed opioid agonist-NK1R antagonist profile (MOR Ki: 0.4 nM, DOR Ki: 10.4 nM, NK1R pA₂: 7.8), with potent antinociceptive properties in the mouse tail flick assay when given iv, indicating that it was able to cross the BBB.[3] The compound however induced tolerance. Control over the relative potency of a dual ligand with overlapping pharmacophores represents a major challenge. We have made modifications in SBCHM01 in order to influence opioid potency and NK1R antagonism, which identified Dmt-D-Arg-Ab-beAla-NMe-benzyl (KGCHM02), a ligand with improved opioid potency (MOR Ki: 0.08 nM, DOR Ki: 0.3 nM), and slightly reduced NK1R antagonism (pA₂: 6.44). In the mouse tail flick assay, after iv administration, KGCHM02 was less potent than SBCHM01, indicating that its BBB permeability is reduced. The hybrids were injected it and compared to their opioid parts lacking the NK1R part in a neuropathic pain model (CCI). The hybrids, but not the parent opioid parts decreased allodynia in the von Frey test, KGCHM02 being more potent than SBCHM01. Also in the cold plate test, the hybrids decreased hyperalgesia in contrast to the opioid parts, with a potency stronger than that of morphine.

[1] Misterek et al; Life Sci.54,393(1994)[2] Largent-Milnes et al.;Br. J. Pharmacol.161,986(2010)[3]Ballet J.Med. Chem. 54,2467(2011)

No. 33 - CO-LOCALIZATION OF MU AND DELTA OPIOID RECEPTORS IN THE NERVOUS SYSTEM USING DOUBLE FLUORESCENT KNOCK-IN MICE

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We have generated double knock-in mice expressing delta opioid receptors in fusion with the green fluorescent protein and mu receptors in fusion with a red fluorescent protein. We have used these animals to map the two receptors in the brain, spinal cord and dorsal root ganglia. Special attention was given to the profile of mu-delta co-expression at the cellular level. Our data indicate that neurons co-expressing the two receptors are particularly abundant within regions involved in nociception. This suggests potential physiological relevance and prompts to investigate in vivo the functional role of mu-delta heteromers to assess their potential as a new therapeutic target

No. 34 - Ligand-directed signaling at mu opioid receptors: differential mechanisms of MAPK activation by morphine and fentanyl

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INTRODUCTION: The ability of opioid ligands to selectively activate particular signaling pathways over others represents an intriguing opportunity in opioid pharmacology to develop more effective drugs. However, in order to exploit ligand-directed signaling at mu opioid receptors (MOR), a deeper investigation of the different signaling modalities elicited by opioid ligands is necessary. The aim of this research, therefore, has been to characterize the differential processes of JNK and ERK1/2 MAPK activation by two prototypical MOR agonists, morphine and fentanyl.

METHODS: Wild type, JNK1^{-/-}, JNK2^{-/-}, JNK3^{-/-}, or GRK3^{-/-} male C57/B16 mice were administered saline, morphine (10mg/kg, s.c), or fentanyl (0.3mg/kg, s.c.) 30-60 minutes prior to harvesting spinal cords. Spinal cord cell lysates were analyzed for phospho-JNK immunoreactivity. To further characterize mechanisms of JNK activation, additional experiments were carried out in MOR expressing HEK293 cells. Cells were pretreated with siRNA against arrestin-2 or arrestin-3, or inhibitors of Src family kinases or PKC, and then treated with morphine or fentanyl (10 μ M, 30 min). Cell lysates were analyzed for phospho-JNK and phospho-ERK1/2 immunoreactivity.

RESULTS: Both morphine and fentanyl selectively increase pJNK2-ir in vivo. Morphine activation of JNK was GRK3-independent, whereas fentanyl activation of JNK was GRK3-dependent. In vitro, morphine and fentanyl both activated ERK1/2 in a Src-dependent but arrestin-independent manner. Morphine activation of JNK was PKC- and Src-dependent, but arrestin-independent, whereas fentanyl-mediated activation of JNK was arrestin 2- and Src-dependent, but PKC-independent. These distinct modalities in JNK activation may represent the molecular basis of the differences observed between morphine vs. fentanyl-induced tolerance, which are JNK-dependent and JNK-independent, respectively.

Work supported by DA030074, DA07278, DA020570.

No. 35 - Nalfurafine is a KOR agonist that produces analgesia, pruritus, inhibition of luteinizing hormone release, and ERK1/2 MAPK activation, but has low potency for p38 MAPK activation

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Previous studies have shown the aversive properties of KOR activation to be GRK3/p38 MAPK-mediated, indicating that KOR agonists that selectively activate G-protein signaling without activating p38 may have therapeutic potential as non-dysphoric analgesics. Clinical trials investigating the efficacy of the KOR agonist nalfurafine in treating pruritus in dialysis patients did not report dysphoric side effects, indicating nalfurafine may have an attractive pharmacology for clinical use. This research aimed to characterize nalfurafine activation of ERK1/2 and p38 MAPK, as well as the in vivo effects of nalfurafine in analgesia, pruritus, and luteinizing hormone (LH) release.

HEK293 cells expressing human or rat KOR were treated with 10pm-10 μ M nalfurafine or 1 μ M U50,488 prior to analysis of phospho-ERK1/2 (5 min) or phospho-p38 (30 min) immunoreactivity to measure the efficacy and potency of nalfurafine for activation of these MAPKs. Analgesia was measured in male C57/BL6 mice using the warm water tail withdrawal assay before and 30 min after U50,488 (1-30mg/kg, i.p.) or nalfurafine (1-150 μ g/kg i.p.) To measure pruritus, male wild type, MOR^{-/-}, or KOR^{-/-} C57/Bl6 mice were administered saline or 50 μ g/kg nalfurafine 20 minutes prior to 5'GNTI (50 μ g/kg, s.c.) and scratches over 30 minutes were counted. To measure LH, blood was collected prior to or 20 minutes following administration of saline or 1-50 μ g/kg nalfurafine in female C57/Bl6 mice 13 days post-ovariectomy.

Nalfurafine was 250 fold more potent for ERK1/2 activation as compared to p38 activation in hKOR expressing HEK293 cells, and 20 fold more potent for ERK1/2 activation as compared to p38 activation in rKOR expressing HEK293 cells. U50,488 (15 and 30mg/kg) and nalfurafine (50 and 150 μ g/kg) produced a norBNI-sensitive analgesic response in the warm water tail withdrawal assay. 5'GNTI increased scratching in a KOR- and MOR-independent manner and nalfurafine pretreatment resulted in a KOR-dependent and MOR-independent reduction in scratching. U50,488 and nalfurafine both reduced LH levels in ovariectomized mice.

Work supported by DA030074, DA07278, DA020570.

No. 36 - Ligand-directed signaling at kappa opioid receptors: differential mechanisms of JNK MAPK activation by U50,488 and norBNI

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Exploiting ligand-directed signaling at kappa opioid receptors (KOR) represents an opportunity for the development of safer and more effective analgesic drugs, but further investigation of signaling modalities is needed. It has previously been shown that both U50,488 and norBNI can both stimulate KOR-dependent phosphorylation of JNK MAPK in vivo and in vitro; however norBNI activation of JNK results in receptor desensitization, whereas U50,488 activation of JNK does not. Therefore, the aim of this research is to characterize the process of JNK activation by these ligands to better understand the differential effects on desensitization. HEK293 or wild type mouse embryonic fibroblast (MEF) cells expressing KOR-GFP were treated with norBNI or U50,488 (10 μ M), and cell lysates were analyzed for phospho-JNK immunoreactivity. U50,488 resulted in biphasic JNK activation in HEK293 cells, with peaks at 15 and 60 min, whereas norBNI resulted in monophasic JNK activation peaking at 60 min. In contrast, U50,488 caused JNK activation in MEF cells peaking at 15min whereas norBNI was ineffective. To further characterize the signaling mechanisms, KOR expressing HEK293 cells were treated with norBNI or U50,488 following pretreatment with either Src family kinase inhibitors, Rho family GTPase inhibitors, or siRNA against arrestin-2 or arrestin-3. JNK activation by U50,488 had an early, arrestin-independent component (15min) and a slower arrestin-3 dependent component (60min). This is similar to the previously reported biphasic activation of ERK by U50,488. The monophasic norBNI activation of JNK was significantly reduced by both arrestin-2 and arrestin-3 siRNA. Inhibition of Rac1, RhoA, or Src prevented JNK activation by norBNI at 60min and U50,488 at 15 and 60 min. U50,488 and norBNI were both able to increase KOR/arrestin interactions, as assessed by BRET analysis using venus tagged arrestins and luciferase tagged KOR. These differences in signaling cascades mediating JNK activation by norBNI and U50,488 may account for the differences in JNK-mediation desensitization.

Work supported by DA030074, DA07278, DA020570.

**No. 37 - OPIOID DELTA
RECEPTOR-MEDIATED β -ARRESTIN
SIGNALING MODULATES CONVULSIVE
EFFECTS**

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Opioid δ receptor agonists are potential therapeutic agents for pain and various indications. However, convulsive effects through the δ receptor activation were reported in preclinical studies. Although successful separation between convulsions and the desired pharmacological effects has been reported for some δ agonists, its mechanism has not yet been explored.

We designed and synthesized the characteristic δ receptor agonists with a novel fundamental oxazatricyclodecane structure, and identified their unique pharmacological profile. With regard to four δ receptor agonists, which had a slightly different structure and showed similar G protein-mediated agonistic activities in cAMP assay using the δ receptor-expressing CHO cells, we analyzed their δ receptor mediated β -arrestin2 signaling activities in vitro and convulsive effects in mice. All compounds showed analgesic activities in acetic acid-induced writhing test and two compounds induced the convulsion. Interestingly, two δ agonists without the convulsion appeared to be a consequence reduced β -arrestin2 recruitment and followed internalization activities, suggested that the δ receptor-induced convulsive effects are mediated by β -arrestin2 signaling.

These results indicated that δ agonists preferred G protein-mediated signaling to β -arrestin2-mediated signaling could be useful therapeutic agents.

This study was supported by the Japan Science and Technology Agency (JST) grants.

**No. 38 - Endosomal Endothelin Converting
Enzyme-2: A regulator of opioid receptor
trafficking**

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Trafficking of opioid receptors following activation has been studied extensively, however very few studies have evaluated the fate of the internalized neuropeptide. It is generally assumed that the internalized peptide is hydrolyzed by endopeptidases; virtually nothing is known about specific enzyme(s) involved in this process. Previously we have shown endothelin-converting enzyme-2 (ECE-2), a metallopeptidase, to be involved in opioid peptide processing in vitro and opioid actions in vivo. In this study, we examined the involvement of ECE-2 in modulating μ and δ opioid receptor trafficking and its impact on receptor function. In cells stably expressing ECE-2 and μ or δ receptors, opioid peptide treatment leads to an increase in colocalization of μ or δ receptors with ECE-2 within the endosomal compartment. Following removal of the peptide, there is a decrease in colocalization with ECE-2, and this is prevented by ECE-2 inhibition. We also find that ECE-2 inhibition leads to substantial decrease in receptor recycling and signaling without significantly affecting receptor internalization. Interestingly we find that ECE-2 inhibition affects recycling of receptors internalized in response to only those agonists that are substrates of ECE-2. Similar results were obtained with dorsal root ganglion cells and primary cortical neurons that endogenously express the receptors and ECE-2. To assess the role of ECE-2 in vivo, we examined the effect of ECE-2 inhibition on intrathecal DAMGO or fentanyl-mediated antinociception. We find that the co-administration of the ECE-2 inhibitor prolongs DAMGO-mediated antinociception (as compared to controls) but not the fentanyl-mediated antinociception. Finally, we find that administration of the ECE-2 inhibitor significantly affects stress-induced analgesia in wild-type mice, but not in mice lacking δ receptors suggesting opioid receptors and ECE-2 in this process. Taken together these data reveal a novel role of ECE-2 in modulating the opioid system by postendocytic processing of opioid peptides.

Supported by NIH grants, DA019521 and NS026880 (to LAD); ENB was supported by the NIDA Postdoctoral Training Program DA007135.

No. 39 - Increased Emergency Department Visits Caused by Tramadol Abuse are Associated with Opioid-Related Over-Dose Death in the U.S.

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Introduction: Tramadol is an uncontrolled narcotic opioid in the U.S. and its increased abuse or misuse poses serious health risks and adverse outcomes. To better understand a geographic pattern associated to its adverse health effects, a comprehensive analysis of numbers of emergency department (ED) visits caused by Tramadol abuse and its correlation with opioid over-dose death were conducted.

Methods: The cohort data (2004 - 2010) was collected from three major public drug abuse surveillance systems including (1) drug abuse-related ED visits from Drug Abuse Warning Network (DAWN); numbers of individual deaths due to tramadol abuse from (2) The Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report and (3) The Annual Medical Examiners Report of Florida Department of Law Enforcement (FDLE).

Results: The results from DAWN ED visits demonstrated a substantial increase in the number of Tramadol abuse among the middle-aged group and that women comprise 58% of the ED visits. A correlation study of the numbers of ED visit and prescribed opioid over-dose death from CDC and FDLE showed that the increased ED visits involved in Tramadol are positively correlated with the increase in overdose death rates in the US ($r^2 = 0.741$; $r = 0.861$) nationally as well as in the state of Florida ($r^2 = 0.978$; $r = 0.989$). The analytic result is statistically significant ($p > 0.01$).

Conclusions: The study results showed that increased abuse of Tramadol positively correlated with increased deaths due to opioid over-dose and a further legal and regulatory implementation may be required to reduce the non-control access of Tramadol and subsequent public risk due to Tramadol abuse.

No. 40 - Tramadol is an effective analgesic in an acute and chronic rat pain model

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Charles River, Elanco

BACKGROUND:

The analgesic tramadol is a widely used drug for a variety of chronic pain conditions including osteoarthritis (OA) patients who have failed to respond to non-steroidal anti-inflammatory drugs (NSAIDs).

OBJECTIVE:

The aim of these studies was to assess whether tramadol would be efficacious in a rat model of OA pain which exhibits both NSAID sensitive and insensitive phases of joint pain.

METHODS:

Rat Formalin: Animals ($n=8$ per group) were administered either vehicle, tramadol (20-80mg/kg PO) or duloxetine before injection of formalin (30uL) into the left footpad. Nocifensive behaviours were captured by video camera and analysed later offline. Phase 1 (0-5mins) and Phase 2 (20-35mins) responses were quantified for all groups.

Rat Mono-iodoacetate (MIA): Joint pain was induced by injection of MIA (25uL) into the left knee of each animal ($n=8$ per group). Dynamic weight bearing (DWB) responses were quantified using the BioSeb™ system. Baseline responses were analysed either on Day 1 or Day 21 and animals were randomized to treatment groups. Tramadol was administered (60mg/kg PO) and DWB responses were measured at 0.5, 2, 4 and 48 hours. For each animal, data was quantified as weight borne on the left limb (injured) - right limb (uninjured).

RESULTS:

Treatment with tramadol resulted in a significant dose dependent reduction in both Phase 1 and Phase 2 responses in the rat formalin model. Based on these data, 60mg/kg (oral, BID) was evaluated in the rat MIA model. In the early NSAID sensitive phase, tramadol demonstrated significant efficacy (40-60%) at all timepoints following both single and BID dosing. In the late NSAID insensitive phase of the model, tramadol was similarly effective (35-55%).

CONCLUSIONS:

Tramadol is an effective analgesic demonstrating efficacy in both NSAID sensitive and insensitive phases of a chronic joint pain model. The rat MIA model employing a quantitative, non-subjective DWB endpoint, is a useful system to study OA joint pain.

No. 41 - Development of a Vaccine against Heroin

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One novel approach to the treatment of drug abuse is to develop vaccines that prevent the pharmacological effects of the drug. Although the vaccines do not reduce the physical dependence of the drug in addicts, vaccines will prevent the drug-induced euphoria and may also be important in preventing relapse and drug overdose. The mechanism of protection of vaccines is to induce antibodies that bind to the drug, sequester it in the blood and prevent it from the crossing the blood-brain barrier. However, the development of a vaccine to heroin has a number of challenges. Heroin is too small to be immunogenic when injected and therefore, surrogate heroin haptens must be coupled to a carrier to induce antibody responses to heroin. Both high titer and high affinity antibodies that have a long duration are required. Heroin vaccine development is particularly difficult because heroin is rapidly metabolized to 6-acetylmorphine and morphine after injection. Consequently, a vaccine for heroin must induce antibodies that bind not only to heroin, but also its metabolites. Two heroin haptens, DiAmHap and MorHap, were tested as antigens for a candidate heroin vaccine. MorHap is a morphine analog containing the functional group used for coupling at the 6 hydroxyl position. DiAmHap is a heroin analog in which amide groups are substituted for the acetyl groups at the 3 and 6 hydroxyl positions and the coupling group is at the bridge nitrogen at position 17. MorHap and DiAmHap were coupled to tetanus toxoid as the carrier and mixed with liposomes containing monophosphoryl lipid A as an adjuvant. Mice were immunized and boosted twice at 3 week intervals. Nine weeks after the primary immunization, sera were assayed for antibodies to the hapten and the animals were tested by a hot-plate nociception assay. Antibody titers were very high with endpoint titers of 3 and 2.5 million for MorHap and DiAmHap immunized mice, respectively. Both groups of immunized animals exhibited reduced antinociceptive effects of heroin after the injection of heroin. MorHap immunization induced antibodies that reacted with 6-acetylmorphine and morphine, whereas immunization with DiAmHap induced antibodies that reacted with heroin, 6-acetylmorphine and morphine. This study indicates that stable heroin haptens can be used in vaccine formulations and that resulting antibodies block the pharmacological effects of heroin.

No. 42 - Comprehensive analysis of factors influencing quantification of biased signalling at the mu-opioid receptor

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The dynamic structure of GPCRs allows different ligands to stabilise the receptor into unique active conformations, resulting in differential activation cell signalling pathways and, eventually, in distinct physiological outcomes. This phenomenon known as “signalling bias” or “functional selectivity” can be exploited to design new opioids that selectively activate signalling pathways that lead to analgesia whilst minimising side effects that are elicited by activation of other signalling pathways. However, bias is not an absolute quality, it is a dynamic and multidimensional phenomenon; it is always relative to a reference ligand, and it is dependent on both cellular protein complement as well as the spatiotemporal properties of the different signalling endpoints.

By quantifying signalling bias at the μ opioid receptor (MOR) across multiple different signalling pathways in two different cell lines, we show how the cellular background influences the direction of biased agonism. In addition, we have examined the impact of signalling kinetics on the detection of bias. This work has shown that the time points selected for measuring activation of different signalling effectors can significantly alter the bias observed across different pathways.

Altogether this demonstrates the changeable nature of ligand and bias, which presents a significant challenge when attempting to predict biased signalling in vivo. However these studies have also identified ligands that maintain unique signalling bias profiles in different cell lines which may be predictive of distinct physiological outcomes. A greater understanding about biased signalling in native tissue and in vivo, will enable links between distinct signalling bias profiles and specific physiological responses to be established, providing essential new information for the design opioids with effective analgesia and less side effects.

No. 43 - Supraspinal endomorphin analgesia requires exon 11 variants of the mu opioid receptor (MOR-1)

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Multiple mu opioid receptors (MOR-1) have been confirmed with the cloning of several mu splice variants. These variants are generated by distinct promoters associated with exon 1 and exon 11. Previously, our lab observed that the potency of morphine-6 β -glucuronide, heroin, and fentanyl analgesia was reduced in mice lacking truncated 6 transmembrane (6TM) exon 11 variants of MOR-1. In contrast, morphine analgesia was intact. We now present evidence that the mu selective peptides endomorphin-1 and endomorphin-2 also require exon 11 variants for their analgesic actions supraspinally. An endomorphin-2 analog, DAPP (dmt-DAla-Phe-Phe-NH₂), produces analgesia via exon 11 variants of MOR-1 as well. In vitro, approximately 50% of [125I]DAPP binding was lost in the brains of exon 11 knockout mice, whereas no specific binding was detected in mice lacking both MOR-1 exon 1 and exon 11 variants. Consistent with binding in mouse brain, we observed increased binding in CHO cells coexpressing MOR-1 and exon 11 6TM variant MOR-1G compared to CHO cells expressing MOR-1 alone. No binding was observed in cells expressing MOR1G alone. Together, our results indicate that 6TM exon 11 variants are involved in endomorphin analgesia and binding.

No. 44 - Pain inhibition by leukocytic opioid receptors

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Introduction: Traditionally, opioids produce analgesia by acting at opioid receptors on sensory neurons and blocking the release of excitatory neurotransmitters. Although opioid receptors are also expressed by leukocytes, their significance to pain transmission has not been addressed. Here we tested the hypothesis that activation of opioid receptors in leukocytes releases opioid peptide Met-enkephalin (ENK), which activates peripheral neuronal receptors, to ameliorate neuropathic pain.

Methods: As a model of neuropathy we used a chronic constriction injury (CCI) of the sciatic nerve in mice. Mechanical sensitivity was evaluated with von Frey filaments. Immune cells were isolated from damaged nerves, and ENK extracellular levels were measured by radioimmunoassay.

Results: In vivo, exogenous μ -, δ - and κ -opioid receptor agonists injected at the CCI site infiltrated by opioid peptide-containing leukocytes produced analgesia, which was attenuated by ENK inactivation or leukocyte depletion. The decreased analgesia was restored by re-injection of wild type, but not of μ -, δ - or κ -receptor lacking leukocytes. In vitro, the exogenous opioids secreted ENK from leukocytes. This release was abolished by the respective opioid receptor antagonists, by blocking G α i or G $\beta\gamma$ subunits, by removing intracellular, but not extracellular calcium, and was attenuated by inhibitors of phospholipase C or inositol 1,4,5-trisphosphate receptors.

Conclusion: Our findings indicate that activation of G α i-coupled opioid receptors in immune cells leads to the intracellular calcium-regulated secretion of ENK, and consequently decreases neuropathy-induced mechanical sensitivity. The leukocytic opioid receptor-induced opioid secretion represents a previously unknown mechanistic basis for peripheral pain relief.

There is no conflict of interest. Supported by the Deutsche Forschungsgemeinschaft grant.

No. 45 - The role of beta-arrestin2 in the development of morphine tolerance in the enteric nervous system

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Clinical and anecdotal data suggest that tolerance to opioids does not develop to constipation upon long-term treatment. This is supported by studies demonstrating lack of tolerance development in the isolated mouse colon upon repeated morphine exposure. However, tolerance develops to upper gastrointestinal motility and in the isolated ileum. In the ileum, but not the colon, tolerance is associated with a decrease in β -arrestin2 (BARR2). We have previously shown that tolerance to morphine occurs in the colon from BARR2 knock-out (KO) mice. We have now examined a) localization of BARR2 in the myenteric plexus, b) development of tolerance in a single adult mouse enteric neuron from the BARR2 KO mice, and c) changes in the expression of the μ -OR in the ileum and colon following expression of morphine antinociceptive tolerance. Immunohistochemistry was performed on whole-mount longitudinal muscle myenteric plexus from mouse ileum. Enteric neurons were isolated, cultured and cell excitability examined by whole-cell patch clamp recordings. The mRNA expression for μ -OR was determined by qPCR. BARR2 was specifically localized in the cell bodies of neurons in the myenteric ganglia co-expressing ChAT, but not Substance P or nNOS. Isolated neurons from the ileum of wild-type mice exposed to morphine (10 μ M) overnight or from morphine-pelleted (5 days) mice, responded to naloxone (1 μ M) with an increased number of evoked action potentials and a decreased rheobase, indicative of enhanced excitability upon precipitated withdrawal. Neurons from the colon of wild-type mice remained unchanged. However, neurons from the colon of BARR2 KO mice when treated with morphine overnight, responded with enhanced excitability to naloxone treatment. In morphine-pelleted mice, the expression of OPRM-1 was significantly reduced compared to controls in the ileum but not the colon. The expression of OPRM-1 was reduced in the colon of BARR2 KO mice. The decrease in ileum of wild-type and colon of BARR2 KO mice was due to reduced mRNA stability. These studies suggest that the role of BARR2 in the enteric neurons is to maintain the expression of μ -OR. Supported by NIH DA024009.

No. 46 - Ligand ability to evoke $G_{\alpha i}$ activation and to promote internalization of DOR can predict response duration over a short period.

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Opioids are the most powerful analgesics but their prolonged use can cause the development of analgesic tolerance. The tolerance may be associated with the duration of response to cAMP. One approach to assess the production of cAMP in response to activation of adenylate cyclase is through the use of radiolabeled precursors. This method requires long periods of activation of the receptor to have a detectable level of cAMP production. During this period, we cannot distinguish the actual response regulated by the delta opioid receptor (DOR). For this study, we used a biosensor based on BRET technology that provides measurements of cAMP levels as a function of real time. The aim of our study was to determine whether there is a correlation between the internalization profile of DOR when stimulated by different ligands, with respect to the duration of signaling in the short-term (≤ 15 min) and long term (120 min). During the 15 min of stimulation, inhibition of cAMP production by DOR activation is characterized by an increase in the inhibition of cAMP, and after 6-7 minutes, a reduction in inhibition of production cAMP. This evolution of the duration of cAMP inhibition is biphasic and is explained in part by the efficiency of ligands to promote $G_{\alpha i}$ activation and by the profile of internalization for each of the different ligands used in this study. For example, deltorphin II which displayed high efficiency to promote $G_{\alpha i}$ activation and internalization shows a more pronounced decline in cAMP response. Unlike morphine which displayed low efficiency to promote $G_{\alpha i}$ activation, and a poor sequestration, displaying a minimal response decay of inhibition of cAMP. Blocking internalization of DOR by dynasore reduced response decay for internalizing agonists (e.g.: deltorphin II). However, ligand ability to promote internalization of DOR does not explain the kinetic profile of a persistent inhibition of cAMP over a longer period of 120 min. In conclusion this data indicates that a ligand's ability to evoke G protein activation or promote endocytosis were predictive of response duration over short but not sustained periods of cAMP inhibition.

No. 47 - Involvement of Microglial P2X7 Receptors in the Development of Morphine Analgesic Tolerance

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Introduction: Morphine is indispensable in the treatment of acute and chronic pain. However, its use is limited by the development of analgesic tolerance, such that higher and more frequent doses are required to achieve the same level of pain control. Growing evidence suggests that microglia are causally involved in the development of opioid tolerance. In the present study we examined the importance of ATP gated P2X7 receptors (P2X7R) expressed on spinal microglia in the development of morphine tolerance. **Methods:** Sprague Dawley rats were administered morphine (15mg/kg; IP) once daily over 7 days. Morphine anti-nociception was assessed using thermal tail flick and mechanical paw pressure tests. Cultured BV2 microglial cells were treated with 5 day morphine (1 μ M). Changes in P2X7R protein expression were assessed by Western blot, whereas modulation of P2X7R function was determined by whole cell patch clamp recordings and calcium imaging.

Results: We found that repeated morphine administration caused a progressive decline in morphine anti-nociception and a loss in morphine analgesic potency, the two key features of morphine analgesic tolerance. The development of morphine tolerance correlated with an increase in spinal dorsal horn microglial P2X7R protein expression. Pharmacologically blocking P2X7Rs with the selective antagonist A740003 attenuated the development of tolerance but did not reverse established tolerance. In BV2 microglial cells, repeated morphine treatment increased total P2X7R protein expression, an effect recapitulated by the mu-opioid receptor agonist DAMGO, and suppressed by the mu-receptor antagonist, CTAP. In addition to increased P2X7R expression, we found that repeated morphine markedly potentiated P2X7R-mediated calcium responses and inward current in BV2 microglia.

Conclusion: Taken together, our findings suggest that microglial P2X7Rs are causally involved in the development, but not expression, of morphine analgesic tolerance. We also discovered that microglial P2X7R expression and function are critically modulated by activity of mu-opioid receptors.

Acknowledgements: Supported by CIHR, NSERC and CFI
Authors report no conflict of interest

No. 48 - Toll-like receptor 4 mutant and null mice retain morphine-induced tolerance, hyperalgesia, and physical dependence

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Introduction: The innate immune system modulates opioid-induced effects within the central nervous system and one target that has received considerable attention is the toll-like receptor 4 (TLR4). Here, we examined the contribution of TLR4 in the development of morphine tolerance, hyperalgesia, and physical dependence in two inbred mouse strains: C3H/HeJ mice which have a dominant negative point mutation in the TLR4 gene rendering the receptor non-functional, and B10ScNJ mice which are TLR4 null mutants.

Methods: Opioid physical dependence was established using a 5-day escalating morphine dosing paradigm where mice received intraperitoneal injections of systemic morphine at 8 h intervals. On day 5 mice were challenged with a single injection of the opioid receptor antagonist, naloxone (2 mg/kg), to precipitate morphine withdrawal and signs of withdrawal were scored. Morphine analgesic tolerance and hyperalgesia were assessed in mice administered morphine (10mg/kg; i.p) once daily for 5 days or escalating morphine twice daily for 7 days, respectively. Anti-nociception was assessed using thermal tail flick and mechanical paw pressure tests. Gene expression was analyzed using quantitative real-time PCR and microglial activation and cfos immunoreactivity were assessed using immunohistochemical labelling of fixed spinal cord sections.

Results: We found that neither acute antinociceptive response to a single dose of morphine, nor the development of analgesic tolerance to repeated morphine treatment, was affected by TLR4 genotype. Likewise, opioid induced hyperalgesia and opioid physical dependence were not altered in TLR4 mutant or null mice. We also examined the behavioural consequence of two stereoisomers of naloxone: (-) naloxone, an opioid receptor antagonist, and (+) naloxone, a purported antagonist of TLR4. Both stereoisomers of naloxone suppressed opioid induced hyperalgesia in wild-type control, TLR4 mutant, and TLR4 null mice.

Conclusion: Collectively, our data suggest that TLR4 is not required for opioid-induced analgesic tolerance, hyperalgesia, or physical dependence.

Funding: CIHR (TT, CMC) and NSERC (TT, TAM)

No. 49 - Agonist-induced phosphorylation of Thr370 and Ser375 in the COOH-terminus of the mu-opioid receptor

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Agonist-induced activation of the mu-opioid receptor (MOPr) leads to phosphorylation of multiple residues in the COOH-terminus of the receptor (1,2,3). The role of these phosphorylation events in MOPr function is still largely unknown, however phosphorylation of Ser375 is thought to be mediated by GRKs (1,4) and leads to arrestin association and MOPr internalization (2). In the present study we used phospho-specific antibodies to study the agonist-induced phosphorylation of Thr370 and Ser375. HA-tagged MOPr was stably expressed in HEK293 cells and following agonist addition, the receptor was immuno-precipitated from cells (1,3) and subject to Western blotting.

Under receptor saturating concentrations, DAMGO, Endomorphin-2 and methadone all induced robust phosphorylation of Thr370 and Ser375 within 10 min. In contrast, morphine induced lower levels of phosphorylation at both these

residues. The role of PKC in phosphorylation of Thr370 and Ser375 was studied using the PKC inhibitor GF109203X (10 μ M, added 30 min before agonist). Pre-treatment with GF109203X abolished morphine-induced phosphorylation of Thr370 but did not affect morphine-induced phosphorylation of Ser375. Pre-treatment with GF109203X also reduced

DAMGO- and Endomorphin-2-induced phosphorylation of Thr370, but did not affect the ability of these agonists to induce phosphorylation of Ser375.

In summary, agonist-induced activation of MOPr leads to phosphorylation of Thr370 and Ser375 in the COOH-terminus of the receptor, with morphine inducing less phosphorylation than other agonists such as DAMGO and Endomorphin-2. Furthermore, whereas inhibition of PKC blocked morphine-induced phosphorylation of Thr370, it did not affect morphine-induced phosphorylation of Ser375. This indicates that PKC activity plays a key role (either directly or indirectly) in morphine-induced phosphorylation of Thr370.

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No. 50 - Synthesis and Biological Characterization of Tritium-Labelled HS665, a Highly Potent and Selective Agonist at the κ Opioid Receptor

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Introduction: Activation on the κ opioid (KOP) receptor leads to significant analgesia, while it is not involved in the unwanted side effects of the μ opioid (MOP) receptor. Besides the analgesic activity, KOP agonists have also shown other beneficial effects such as antipruritic, anti-arthritic, anti-inflammatory, and neuroprotective effects. On the other hand, inhibition of the KOP receptor may be useful for the treatment of depression, anxiety, stress and drug addiction. Several N-substituted diphenethylamine analogues have been synthesized and biologically characterized for opioid receptor activities. The N-cyclobutylmethyl substituted analogue (HS665) was identified as a novel highly selective KOP agonist with potent antinociceptive action mediated through KOP receptor mechanisms [1].

Methods: Bromination of HS665 with N-bromosuccinimide in dichloromethane afforded the dibrominated analogue, which was tritium labelled with tritium gas over PdO/BaSO₄ catalyst to yield [3H]HS665 with a specific activity of 30.65 Ci/mmol. Kinetic studies and opioid binding characteristics of [3H]HS665 were determined using radioligand binding assays.

Results: In Chinese hamster ovary (CHO) cells expressing the human KOP receptors (CHO-hKOP), binding of the new tritiated opioid ligand was demonstrated to be rapid, specific and saturable. [3H]HS665 specifically labelled a single class of opioid binding sites with an affinity in the subnanomolar range, together with an extremely low nonspecific binding (>10%). Furthermore, the ability of a number of type-selective opioid ligands to displace its binding from CHO-hKOP cells was assessed. The competition binding experiments proved the KOP receptor specificity of this novel radioligand.

Conclusion: Altogether, these properties clearly define the prospective of the tritium-labelled HS665 to become an important and useful research tool for investigating and understanding KOP receptor pharmacology.

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No. 51 - Anti-opioid NMDA receptor theory underlying the lack of morphine analgesia in fibromyalgia-like model mice

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We have developed an experimental fibromyalgia model in mice, which had been given intermittent cold stress (Mol Pain, 2008). As seen in fibromyalgia patients, these mice show long-lasting hyperalgesia and allodynia for many weeks. Female-dominant gender difference is also observed. From the point of therapeutic view, such abnormal pain behaviors could be effectively inhibited by gabapentinoids and many anti-depressants (Mol Pain 2011). Among all, no morphine analgesia is observed (Neurosci Lett, 2010). Based on the assumption that excess stress may drive endogenous opioid systems, we speculated that opioid tolerance might be caused by the actions of endogenous opioids. In addition, we have previously demonstrated that NMDA receptor subunit NR2A plays a crucial role in the development of morphine analgesic tolerance (J of Neurosci, 2003). In the present study we will discuss the possible roles of NMDA receptor system in such “endogenous opioid tolerance” by use of specific TG mice and therapeutic drugs.

No. 52 - Cyclin-dependent kinase 5 regulates Mu and Delta opioid receptor activities

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Introduction: We have shown that chronic morphine and CFA-induced inflammation increase the density of cell surface delta opioid receptors (DOPs) in rodent spinal cord and dorsal root ganglia neurons. Among other mechanisms, receptor phosphorylation represents an important step in the regulation of receptor trafficking and functions. Interestingly, the phosphorylation of DOP on Thr161 by Cyclin-dependent kinase 5 (Cdk5) was shown to be involved in receptor trafficking. The present study therefore aimed to determine the role of Cdk5 in regulating the functions of DOP in inflamed- and morphine-treated rats. Since mu (MOP) and DOP have been shown to be coregulated, we also sought to determine if Cdk5-mediated regulation of DOP also affects MOP.

Methods: The role of Cdk5 on the regulation of opioid receptors in inflamed- and morphine-treated rats was studied using roscovitine as a selective kinase inhibitor and C11-DOPi2, a cell-penetrant peptide mimicking endogenous DOP's second intracellular loop.

Results: We found that roscovitine dose-dependently (1-30 μg , i.t.) reduced the antinociceptive effect of deltorphine (Dlt II) in morphine-treated rats. Similarly roscovitine induced a robust decrease in Dlt II-induced antihyperalgesia in the CFA inflammation model. Administration of the C11-DOPi2 mimicking peptide also produced a robust decrease in Dlt II-mediated antinociceptive and antihyperalgesic effects. Interestingly, although MOP does not contain the putative phosphorylation motif for Cdk5, we observed that roscovitine significantly increased the DAMGO-induced antinociception and antihyperalgesia. Moreover, blockade of DOP phosphorylation with DOPi2 significantly increased MOP antinociceptive and antihyperalgesic effects.

Conclusion: Together, our results demonstrate that Cdk5 is a key player of DOP regulation in inflamed- and morphine-treated rats. Moreover, our results indicate that the regulation of DOP by Cdk5 is sufficient to modulate MOP functions by an indirect process. Further studies are required to understand the precise mechanisms of action of Cdk5 on MOP and DOP. Supported by CIHR, FRQS and NSERC. Authors declare no financial disclosure.

No. 53 - Molecular determinants of delta-opioid receptor recycling

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Opioids are the most potent analgesics for the treatment of severe pain. Delta opioid receptor (DOR) agonists induce their analgesic actions with fewer side effects than those of mu receptor, making them a target of interest for development of novel analgesics. However, they induce tolerance to analgesia. Recent hypotheses suggest that distinct profile of tolerance of different opioid analgesics could result from the stabilization of different ligand-specific conformations of the receptor, each having different trafficking profiles. Here, we compared different DOR ligands to assess whether they display different post-endocytic trafficking (recycling) properties. The experiments were done in HEK293 cells and in cortical neurons transfected with DORs. Our results indicate that in HEK293 cells, DORs internalized by DPDPE colocalize with the mannose-6-phosphate receptor (M6PR), a marker for late endosomes (LE) and trans-golgi network (TGN). Inhibition of Rab9 and TIP47 (two proteins involved in the transport between LE and TGN) affects DOR recycling. Furthermore, the kinase PKD (which is known to intervene in trafficking from TGN to the membrane), the Rab7 (which enables retromer-mediated transport from sorting endosome (SE) to TGN) and Rab11 (which mobilizes recycling cargo from perinuclear compartment to the membrane) were also involved in this process. We can conclude that in HEK293 cells, the DOR may recycle from the SE directly or via the LE and/or the TGN. Then we studied DOR recycling in neurons. Here too, DORs colocalized with M6PR and the recycling was dependant on Rab9 and TIP47. We can conclude to date that in neurons, translocation from LE to TGN constitutes a route for internalized DORs to reach the membrane. Our outlook is to study these mechanisms for different DOR agonists and to correlate these properties with the potential of drugs to induce tolerance. These results will enable the development of ligands with a longer analgesic activity. (CIHR, NSERC)

No. 54 - Analysis of Morphine Regulated Striatal Synaptic Networks using Proteomics and Network Analysis

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The clinical use of morphine in the treatment of chronic pain is limited by the development of tolerance, dependence and addiction. These undesired effects are thought to be due to alterations in synaptic transmission and neuroplasticity within the reward circuitry, particularly in the striatum. In this study, using a combination of subcellular fractionation, quantitative proteomics and systems biology approaches, we examined changes in striatal postsynaptic density fractions following chronic morphine administration. Differential isotopic labeling and mass spectrometry analysis of fractions from saline and morphine treated animals identified over 2,600 proteins of which 38 were differentially altered (25 upregulated and 13 downregulated) in response to morphine. Several of these differentially regulated proteins were validated by Western blot analysis. Next, using graph theory-inspired network analysis we identified a morphine-regulated network where upregulated and downregulated proteins were connected by 40 significant intermediates. Follow up studies with a few of the interesting intermediates in the network (such as caspase-3, receptor-interacting serine/threonine protein kinase 3 and E3-ubiquitin ligase neural precursor cell expressed developmentally down-regulated protein 4 (NEDD4)) revealed that they were localized to the postsynapse and upregulated in response to chronic morphine administration. Finally, since many of the proteins in the morphine-regulated network predicted alterations in proteasomal degradation, we hypothesized that the global ubiquitination state of the postsynaptic density proteins were substantially altered by chronic morphine treatment. Direct examination revealed a significant increase in the level of ubiquitination of postsynaptic density proteins in morphine treated animals as compared to saline treated animals. Taken together, these findings suggest an emerging role for posttranslational modification of proteins such as ubiquitination in opiate tolerance, dependence and addiction.

This work was supported by NIH grants DA008863, DA019521, and NS026880 to L.A.D.

No. 55 - Evaluating the optimal ratio of mixed MOR/KOR agonism to prevent liabilities of use and cocaine-conditioned place preference.

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Mixed activity mu opioid receptor (MOR) and kappa opioid receptor (KOR) agonists may prevent the acute rewarding effects of abused drugs. We used two macrocyclic tetrapeptide mixed opioid agonists with differing ratios of MOR and KOR agonism to evaluate the balanced of mixed MOR/KOR agonism that would minimize liabilities while preserving the therapeutic ability to suppress the rewarding effects of cocaine.

Methods: The ratio of MOR to KOR agonist activities possessed by the two peptides CJ-15,208 (Ross et al., 2012) and [D-Ala1,D-Trp4]CJ-15,208 (Aldrich et al., 2014) were determined in the 55oC warm-water tail-withdrawal test, using wild-type C57BL/6J, MOR knockout (MOR KO) and KOR knockout (KOR KO) mice. Sedation and respiratory depression were evaluated in the rotarod assay and CLAM system. Conditioned place preference (CPP) testing directly evaluated the rewarding effect of the peptides and their ability to prevent cocaine CPP.

Results: CJ-15,208 and [D-Ala1,D-Trp4]CJ-15,208 produced equipotent antinociception in wild-type mice, with ED50 (and 95%CI) values of 1.74(0.62-4.82) and 3.03(2.16-4.59) nmol, icv, respectively. CJ-15,208 showed an equal mix of MOR and KOR agonism, whereas [D-Ala1,D-Trp4]CJ-15,208 antinociception was predominantly KOR mediated. Neither compound produced sedation at doses up to 100 nmol icv. CJ-15,208 produced initial conditioned place aversion (CPA) at a 3 nmol icv dose and prevented cocaine-CPP, but at higher doses demonstrated MOR-mediated CPP. In contrast, [D-Ala1,D-Trp4]CJ-15,208 (3 nmol icv) was without effect in the conditioned place preference assay, but demonstrated CPA at higher doses that also prevented cocaine-CPP. Both of these effects were absent in KOR KO mice.

Conclusions: The addition of MOR agonism to KOR agonism alleviates some liabilities attributed to KOR-selective agonists, yet retains the ability to counteract the rewarding effects of cocaine. However, an even ratio of MOR/KOR agonist activity proved detrimental at higher doses, suggesting an upper limit to the desired amount of MOR agonism.

Funding: NIDA grant R01 DA032928 and funds from the State of Florida.

Conflicts of interest: none.

No. 56 - Mechanism for the allosteric effects of G proteins on orthosteric ligand binding to GPCRs

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G protein-coupled receptors (GPCRs) are allosteric machines that translate extracellular ligand binding into intracellular signaling via transition between distinct conformational subsets. Agonist-mediated stabilization of an active GPCR conformation, and thus promotion of G protein interaction, is a fundamental tenet of GPCR biology. In addition, G protein-mediated allosteric enhancement of agonist affinity has been observed in many GPCRs. However, the intricacies of this high-affinity state remain poorly understood, and the mechanism by which G proteins allosterically regulate orthosteric ligand binding is unclear. Based on structural data, we hypothesized that transition to an active receptor conformation leads to constriction of the orthosteric binding site around its ligand, thus slowing dissociation and enhancing affinity. We used equilibrium and kinetic radioligand binding assays to characterize the effects of G protein and G protein-mimetic nanobodies on the ligand-binding properties of multiple GPCRs, and also investigated the roles of individual residues within β 2-adrenergic receptor (β 2AR). When bound to nucleotide-free Gs heterotrimer, β 2AR displayed a Bmax decrease which was restored by GDP or GTP γ S. [3H]DHAP association was slowed when β 2AR was bound to nucleotide-free Gs heterotrimer, and GDP reversed this effect in a concentration-dependent manner. The Gs mimetic nanobody Nb80 slowed both [3H]DHAP association and dissociation. However, this effect was not antagonist-specific, as Nb80 also inhibited association of the agonist [3H]Formoterol. Together, these data suggest that transit into or out of the β 2AR orthosteric site is restricted due to a “closed” orthosteric site in the active β 2AR conformation (bound either to Gs or Nb80). Furthermore, it appears that high-affinity agonist binding is only supported when receptor is bound to nucleotide-free G protein. Interestingly, the M2 muscarinic acetylcholine receptor and mu opioid receptor displayed ligand-binding phenomena similar to those we observed for β 2AR, suggesting that this behavior may extend to GPCRs beyond β 2AR and the amine receptor family.

This work was supported by AHA 13PRE17110027.

No. 57 - Characterization of the Antinociceptive and Kappa Opioid Receptor Antagonist Activity of Analogs of the Macrocytic Tetrapeptide CJ-15,208

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The macrocyclic peptide CJ-15,208 (cyclo[Phe-D-Pro-Phe-Trp], Saito et al., *J. Antibiot.* 2002, 55, 847) exhibits mixed opioid agonist/kappa opioid receptor (KOR) antagonist activity in vivo (Ross et al., *Br. J. Pharmacol.* 2012, 165, 1097) and is a promising lead compound for developing novel therapeutics for the treatment of pain and drug abuse. We synthesized analogs of this lead peptide to explore the influence of the aromatic residues on the analogs' opioid activity profiles.

Methods. The analogs were synthesized by a combination of solid phase synthesis with cyclization in solution under optimized reaction conditions. The peptides were evaluated in radioligand binding assays for mu, kappa and delta opioid receptor affinities in vitro, and in the mouse 55 oC warm water tail withdrawal assay in vivo for antinociception and opioid antagonist activity.

Results. The analogs generally exhibited potent antinociception in vivo comparable to CJ-15,208 following both intracerebroventricular and oral administration in spite of reductions in opioid receptor affinities in vitro; multiple opioid receptors contributed to the antinociception of each analog. In contrast to the parent peptides, only one of the analogs exhibited KOR antagonism in vivo. Interestingly, when screened in an acute tolerance model, the antinociceptive tolerance observed varied markedly among the peptides, from no significant display of tolerance to a rightward shift in the dose-response curve equal to or greater than that exhibited by morphine.

Conclusions. Changes in the macrocyclic tetrapeptide structure were well tolerated with retention of antinociceptive activity, while the KOR antagonism was very sensitive to changes in the aromatic residues. The identification of compounds with potent antinociception that exhibit minimal tolerance in the initial screening is a promising development in the search for potential analgesics with improved liability profiles.

Research supported by NIDA grant R01 DA018832.

No. 58 - Broad spectrum analgesic efficacy of IBNtxA is mediated by exon 11-associated splice variants of the mu-opioid receptor gene

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Mu-opioids remain vastly important for the treatment of pain, and would represent ideal analgesics if their analgesic effects could be separated from their many side effects. A recently synthesized compound, iodobenzoylnaltrexamide (IBNtxA), acting at 6-transmembrane (6-TM) splice variants of the mu-opioid receptor gene, was shown to have potent analgesic actions against acute, thermal pain accompanied by a vastly improved side-effect profile compared to 7 TM-acting drugs such as morphine. Whether such analgesia can be seen in longer lasting and non-thermal algesiometric assays is not known. The current study demonstrates potent and efficacious IBNtxA inhibition of a wide variety of assays, including inflammatory and neuropathic hypersensitivity and spontaneous pain. We further demonstrate the dependence of such analgesia on 6-TM mu-opioid receptor variants using isobolographic analysis and the testing of Oprm1 (the mu-opioid receptor gene) exon 11 null mutant mice. Finally, the effect of nerve damage (spared nerve injury) and inflammatory injury (complete Freund's adjuvant) on expression of mu-opioid receptor variant genes in pain-relevant central nervous system loci was examined, revealing a complex pattern of changes. These findings are supportive of the potential value of 6-TM-acting drugs as novel analgesics.

The Louise and Alan Edwards Foundation (J.S.M.) and the National Institute on Drug Abuse of the NIH (DA02615, DA06241, DA07242, G.W.P.). JSW was supported by a Vanier Canada Graduate Scholarship from the Canadian Institutes for Health Research. None of the authors has a conflict of interest related to this research.

No. 59 - Functionally selective kappa opioid receptor agonist efficacy in pruritis

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Introduction: The only clinically utilized KOR agonist on the market is the partial agonist Nalfurafine which is used in the treatment of pruritis or itch. Conversely, KOR antagonists induce acute pruritis emphasizing that KOR may be a very good target for the treatment of pruritis. The mechanism through which KOR modulates this response remains unclear. In this study we behaviorally investigate the role of KOR and the GPCR regulatory protein, β arrestin2, in the itch response in mice.

Methods: Itch is evaluated by using an established mouse model of acute pruritis wherein a single subcutaneous (s.c.) injection of an itch-inducing irritant (either KOR antagonist or chloroquine phosphate) is used to induce robust scratching bouts that are quantified over a 60 minute observation period.

Results: Two KOR antagonists, 5'GNTI and norBNI, induce itch in C57BL/6J mice in a dose-dependent manner which is decreased (but not eliminated) in KOR KO mice supporting the involvement of the KOR system in pruritis. This response can be dose-dependently reduced with pretreatment of the conventional KOR agonist, U50,488H, as previously shown. A functionally selective KOR agonist that preferentially promotes G protein signaling over β arrestin2 recruitment is also efficacious in suppressing the itch response. Like U50,488H, the biased KOR agonist is also efficacious in preventing chloroquine phosphate-induced itch. To further test the β arrestin2 involvement in this response, itch was also evaluated β arr2 WT and KO mice.

Conclusions:

These observations suggest that agonist efficacy at KOR for treating pruritis may be due to its utilization of G protein signaling pathways. Further studies are needed to determine the mechanistic underpinnings of KOR function in the development and treatment of pruritis.

Support: NIH/NIDA: R01DA031927 (LMB and JA)

Disclosure:

No. 60 - DEVELOPMENT OF PERIPHERALLY AVAILABLE MIXED EFFICACY MOR/DOR LIGANDS THAT DISPLAY REDUCED DEVELOPMENT OF TOLERANCE

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It has been shown that the co-administration of a mu opioid receptor (MOR) agonist with a delta opioid receptor (DOR) antagonist displays MOR mediated analgesia, but also produces reduced negative side effects notably the development of tolerance and dependence, features that limit the clinical use of opioid analgesics. Administering drug cocktails has considerable disadvantages related to potential diverse pharmacokinetic properties. Therefore we have explored the development of multifunctional ligands that display both MOR agonism and DOR antagonism in a single molecule. We have generated a series of constrained mixed efficacy peptides using our previously published homology models of the opioid receptors to guide ligand design. These peptides displayed low nM affinity for MOR and DOR in vitro with selectivity over the kappa opioid receptor. This series of ligands also displayed MOR efficacy similar to morphine and reduced DOR activation, with some compounds showing a complete lack of stimulation at DOR. As low bioavailability is typical of peptides, we installed a C-terminal sugar moiety, in which the side chain hydroxyl of Ser is covalently O-linked to a glucose or lactose as a means of improving blood-brain barrier penetration. Both glycosylated and unglycosylated derivatives were assessed in vivo for antinociceptive properties. Those ligands that produced antinociception were further evaluated for the development of tolerance and dependence. Ligands in this series displayed a reduced development of acute tolerance, as desired. This demonstrates that although peptides are traditionally considered to be poor drug scaffolds, they can be viable tools for exploring therapeutic peptide analgesics as well as potentially developing molecules with reduced dependence liability and extended therapeutic use. This work was funded by NIH grants DA003910 (H.I.M.) MH083754 (J.R.T), DA007267 (V. P.-B.), DA007281 and GM007767 (J.P.A.)

No. 61 - Exploring pharmacological functions of mu opioid receptor carboxyl termini in gene targeted mouse models

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Mu opioid receptor (OPRM1) gene undergoes extensive alternative pre-mRNA splicing, creating an array of splice variants or isoforms that are conserved from rodent to human. These splice variants have been categorized into three major types based upon receptor structure, full-length carboxyl (C-) terminal variants with 7-transmembrane (TM) domains, truncated variants containing 6-TM domains and truncated variants with single TM. Structurally, all the C-terminal variants shared the entire receptor structure predicted from exons 1/2/3 with the original MOR-1, except that they have different intracellular C-terminal tails. The functional significance of these C-terminal variants has been suggested by differences in region-specific expression, mu agonist-induced G protein coupling, phosphorylation, internalization and post-endocytic sorting. However, little has been known for their in vivo function. In the present study, we generate three gene targeted mouse models: 1) mE3M in which all C-terminal tails are eliminated by introducing a stop codon in the end of exon 3; 2) mE4M and 3) mE7M, in which C-terminal tails encoded by exon 4 or exon 7 are disrupted by inserting a stop codon in the beginning of exon 4 or exon 7, respectively. All three mouse models displayed normal analgesic responses toward various mu opioids, but each mouse model exhibited unique profiles in terms of mu opioid-induced tolerance, physical dependence and rewarding. For example, mE7M homozygous mice showed resistance to morphine tolerance, contrasting to mE4M homozygous mice that were vulnerable to morphine tolerance. Eliminating C-terminal tails encoded by exon 7 had no effect on morphine-6 β -glucuronide (M6G) dependence. However, disrupting C-terminal tails encoded by exon 4 significantly reduced M6G dependence. Our data highlight the functional importance of the C-terminal tails in mediating the actions of various mu opioids, and suggest distinct roles of individual C-terminal tails in the complex mu opioid actions. (Supported by DA13997 & DA029244 (Y.-X.P) and DA02615 & DA07242 (G.W.P) from the National Institute on Drug Abuse; CA08748 (Core grant, MSKCC) from the National Cancer Institute)

No. 62 - Optimization of an active vaccine against heroin: immunotherapeutic approaches towards long-term blockade of heroin activity

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Heroin continues to be the most statistically likely drug to result in addiction among users, with few effective therapeutic options that result in long-term abstinence. While opioid antagonists can be administered in slow-release formulations, these treatments often preclude other common treatment options, have potential for side-effects, and require moderate medical monitoring. We have previously demonstrated a novel heroin vaccine hapten design that is capable of preventing heroin antinociception, conditioned place preference, reinstatement, and re-escalation of self-administration with high specificity without targeting opioid receptor activity. This vaccine attached heroin to keyhole limpet hemocyanin (KLH), via a thiol linker attached at the nitrogen bridgehead, allowing the metabolically labile portion of heroin to remain exposed. When delivered in alum adjuvant, the result was generally high titer levels of antibodies that had binding specificity for heroin ($4 \pm 1 \mu\text{M}$) and especially high affinity its primary metabolite, 6-acetylmorphine ($35 \pm 1 \text{nM}$).

Using serum antibody titer ELISA measurements and quantitative screening methods of heroin-induced hot plate and tail immersion responses (54°C) in mice, we have engaged in a multi-step optimization of the original vaccine. The process systematically examined all the components of the heroin vaccine formulation: route of administration, protein immunogen, chemical linkers, and adjuvants. We have carried the top performing variations forward through each successive test, with the final result being an optimized vaccine formulation that appears to have roughly 5-10 times the capacity in preventing heroin psychoactivity of the original vaccine, shifting heroin dose-response curves over 20-fold versus unvaccinated animals. The blockade of heroin activity is effective for at least several months, and may represent an effective adjunct therapeutic option for heroin addiction.

Research Supported by DA026625 and the Pearson Center for Alcoholism and Addiction Research. The authors declare no conflicts of interest for this research.

No. 63 - Identification of Novel Signaling Regulators of the Mu Opioid Receptor

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Chronic pain is a serious health concern with high costs and a significant impact on patient quality of life. A multi-decade drug discovery effort targeted at finding new pain therapeutics has met with limited success. Recent work has demonstrated that specific signaling cascades (i.e. β arrestin2) downstream of the mu opioid receptor (MOR) mediate specific behavioral effects, and that functionally selective drugs that specifically activate the desired cascades can have an enhanced therapeutic index. However, very few regulatory proteins which can be targeted in this manner have been described. In order to facilitate the identification of such targets, we have applied two methods of unbiased screening to find novel signaling regulators of the MOR. For one, we have created a MOR-CHO cell line, and used co-immunoprecipitation of drug activated receptor complexes followed by proteomic analysis in an attempt to find novel associated proteins. For the other, we will transfect a MOR-HEK cell line with a pooled shRNA library, covering 15,000 human genes. Populations of cells with increased or decreased signaling will be isolated by flow cytometry, and the candidate genes which regulate MOR induced signaling will be identified by next-gen sequencing. From these data, we will identify a list of potential signaling regulators of the MOR, and will further test the effect of candidate knockdown on MOR induced signaling. Using this approach, we have initial confirmation that a novel scaffold protein previously unassociated with the MOR regulates MOR induced ERK activation without altering receptor expression or activation. We have also begun to test other candidate regulators with a similar approach. Overall, we hope to use this data to further define the MOR signaling complex, and to identify new targets and strategies for drug discovery to treat chronic pain.

Conflicts: None

Funding: COBRE Pilot Project Grant (under P20GM103643)

No. 64 - Wireless devices for optogenetic and pharmacological dissection of neural circuits in behavior

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The rise of optogenetics provides a unique opportunity to advance both materials engineering and neuroscience. We report a series of novel devices for studying and perturbing intact neural systems through optogenetics, microfluidic pharmacology, and electrophysiology. Unlike optogenetic approaches that rely on rigid, glass fiber optics coupled to external light sources, these novel devices utilize flexible substrates to carry microscale, inorganic light emitting diodes (μ -ILEDs) and multimodal sensors into the brain. As with previous versions of these devices, wireless control is possible using radiofrequency (RF) power scavenging. Increasing the carrier frequency of this RF signal allows for miniaturization of these harvesters. We demonstrate that these devices can be accurately implanted into deep brain structures while mounted completely subcutaneously under the scalp. Because of their architecture, light from these devices can be delivered at different angles allowing complete dorsal-ventral targeting of deep brain structures. We demonstrate the utility of these independently-controlled μ -ILEDs in isolating anatomical subregions of dynorphinergic neurons. In addition to optogenetic control, we also present devices for wireless delivery of conventional and UV-caged opioid agonists and antagonists. These wireless microfluidic devices introduce a second wireless scheme based on battery-powered microprocessors for combined optogenetic and pharmacological manipulation of neural circuits. Here we demonstrate the ability to wirelessly deliver the μ -opioid receptor agonist, DAMGO, in a homecage environment. Finally, acute in vivo electrophysiological study of opioid circuits is possible with completely dissolvable recording and stimulating electrodes. These devices allow for chronic implantation of a transient electrode that is fully resorbed by brain tissue over time. In sum, we present a diverse toolset of devices capable of achieving previously untenable control of neuropeptide and monoamine neural circuits. Supported by NIDA R01DA037152 (MRB), NIH Common Fund NINDS R01NS081707 (MRB, RWG, JAR), and NIMH F31MH101956 (JGM).

No. 65 - Stress-induced increases in depression-like and cocaine-seeking behaviors are reversed by disruption of memories during reconsolidation

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Maladaptive behavioral responses characteristic of post-traumatic stress disorder (PTSD) are notably resistant to treatment after establishment. We hypothesized that disrupting memory reconsolidation during stress re-exposure may reverse previously established stress-induced increases in depression-like and cocaine-seeking behaviors. C57BL/6J mice were subjected to repeated social defeat stress (SDS), and examined for time spent immobile in a subsequent forced swim test (FST). Additional SDS-exposed mice were place conditioned with cocaine, and tested for conditioned place preference (CPP). To determine if memory disruption could reverse PTSD-like sequelae, mice were administered vehicle or drugs proven to disrupt memory formation during reconsolidation (propranolol (1 or 10 mg/kg, i.p.) or cycloheximide (2.2 or 4.4 mg/kg, i.p.)) both 1 h prior and 2 h after re-exposure to a single additional trial of SDS. Mice were then again tested for FST or CPP the next day. SDS-exposed mice displayed increases in socially defeated postures across SDS trials and subsequently demonstrated significant increases in time spent immobile in the FST or in the cocaine-paired chamber compared to controls. Vehicle-treatment followed by additional SDS-exposure did not alter these behaviors, but propranolol- or cycloheximide-treatment reversed each of the potentiated responses in a dose-dependent manner. Overall, these results demonstrate that while repeated exposure to a social defeat stressor subsequently increased depression-like and cocaine-seeking behaviors, pharmaceutical disruption of traumatic memories made labile by re-exposure to SDS during reconsolidation may have therapeutic value in the treatment of established PTSD-related behaviors.

Source of Funding: This research was supported by a Northeastern University Provost's RSDF award to JPM and funds from the State of Florida, Executive Office of the Governor's Department of Economic Opportunity.

Conflict of Interest: none declared

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No. 66 - Extrasynaptic GABAA receptors in the periaqueductal gray are involved in descending pain modulation and chronic inflammatory pain-induced plasticity.

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Plasticity changes induced by chronic inflammatory pain have been described in many areas of the descending modulatory pathway, but remain relatively unstudied in the periaqueductal gray (PAG). Opioid inhibition of GABAergic synaptic activity in the PAG activates a descending antinociceptive pathway. The extrasynaptic, tonic chloride current produced by delta-subunit containing GABAA receptors has been identified in the PAG, but its role in chronic inflammatory pain has not been explored. Extrasynaptic and synaptic GABAA currents were measured in male and female rats treated with Complete Freund's Adjuvant (CFA) or untreated (naïve). Electrophysiological recordings were made from slices containing the PAG six days after CFA injection. Basal GABA mIPSC frequency was increased in CFA (2.2 ± 0.5 Hz, n=12 cells) compared to saline pretreated rats (1.1 ± 0.2 Hz, n=8 cells). The amplitude of the GABA mIPSC was significantly increased in CFA treated rats (45 ± 4 pA, n=10), compared to naïve rats (32 ± 4 pA, n=11). Superfusion with the GABAA antagonist bicuculline (BIC) reduced the holding current by 18 ± 4 pA (n = 10) in naïve male rats. The GABAA antagonist picrotoxin (300 μM) changed the holding current by 7 ± 2 pA (n=6) and this effect was enhanced by CFA treatment in female rats (16 ± 6 pA, n=3). When measured using qRT-PCR, CFA-treated rats had significantly more GABAA-delta subunit mRNA (2.8 ± 0.4 (n=4) compared to naïve rats (1.1 ± 0.4 relative units, n=3). These studies indicate that the extrasynaptic GABAA current mediated by receptors containing the delta subunit may be an important site of plasticity during chronic inflammatory pain.

We used the delta subunit agonist THIP to examine how activation of the tonic current alters pain behavior. On its own, THIP (100 ng) microinjected into the vPAG had no effect on hot plate latency but significantly attenuated morphine (5 μg)-induced antinociception. In addition, DS2, a new allosteric agonist of the delta subunit, shifts the morphine dose-response curve of morphine to the right. These data support a role of the extrasynaptic GABAA receptor in pain modulation in vivo.

Support: AG023477 (KJT) and DA027625 (SLI)

No. 67 - STRATIFICATION OF OPIOID ANALGESICS ACCORDING TO BIAS FOOTPRINTS AT MU- (MOR) AND DELTA-OPIOID RECEPTORS (DORs).

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Opioid analgesics are elective in the treatment of severe pain but their use is restricted by severe side effects. Signaling bias has been proposed as a viable means for improving this situation but we still ignore the whether signaling preferences are indeed predictive of desired and unwanted actions of opioid analgesics. To start addressing these issues HEK293 cells were transfected with different BRET-based biosensors to compare clinically available opioids and selective DOR agonists in their efficiency to induce: activation of different G proteins ($G_{\alpha i/o/z}$), stimulation of analgesic effectors (Kir3.1/3.2 channels), inhibition of cAMP production and recruitment of β arr2 in presence or absence of GRK2, GRK5 and GRK6. Bias was calculated from operational transduction coefficients ($\log(\text{Tau}/\text{KA})$) using Met-enkephalin as the standard (Met-ENK). Responses elicited by rat DORs indicated that deltorphin II defined a group on its own being the only ligand with no bias between Kir3 and cyclase responses. For the rest of ligands Kir3 $\log(\text{Tau}/\text{KA})$ ratios were at least one order of magnitude greater than those observed for cyclase inhibition. Among them β arr2 responses allowed to define three additional groups: a) drugs that failed to elicit any β arr2 response (buprenorphine and TIPP); b) drugs whose $\log(\text{Tau}/\text{KA})$ ratios for different β arr2 readouts were not different from those corresponding to cyclase responses (morphine, oxycodone and DPDPE) and c) drugs whose efficiency to inhibit cAMP production was at least one order of magnitude lower than that displayed for β arr2 recruitment (loperamide, AR-M100390 and SNC-80). Although with different efficiencies, all ligands activated $G_{\alpha i/o/z}$ proteins, displaying no bias among these responses. Comparing results obtained with rat DORs to human DORs and to rat MORs indicated that bias profiles were reasonably maintained among DORs of different species but varied among receptor subtypes. If these signaling profiles are predictive of in vivo outcomes, they should allow to segregate ligands into groups with distinct potential for analgesia and/or side effects.

Funding: This work was supported by Pfizer (M.B and G.P).

No. 68 - Post-sampling changes in opioid peptide levels and the effect of tissue stabilization

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Aim of Investigation: Immediately after sacrifice of experimental animals or tissue excision during sampling, enzymes present in tissues may start to degrade proteins and peptides, and thus give rise to analytical results based on post-mortem conditions that are not representative to the in vivo conditions. The present project evaluates the use of conductive heat transfer for sample stabilization (enzymatic inactivation) in a routine radioimmunoassay protocol for measurement of opioid peptides in rat brain.

Methods: The Stabilizer T1 instrument (Denator AB, Gothenburg, Sweden) was used for stabilization. Different post-mortem treatments were tested on stabilized and non-stabilized tissue and the effect was evaluated on the levels of opioid peptides in rat hypothalamus (HT), striatum (Str), and cingulate cortex (Cg). Dynorphin A (Dyn A), Dynorphin B (Dyn B), Leu-enkephalin-Arg6 (Leu-Arg), and Met-enkephalin-Arg6Phe7 (MEAP) was analysed using radioimmunoassay (RIA).

Results: Levels of Dyn A, Dyn B, and MEAP in HT were higher in stabilized tissue than in non-stabilized. Leu-Arg, a degradation product from the dynorphins, showed an opposite pattern. Stabilization before freezing resulted in higher Dyn A, Dyn B, and MEAP compared to samples which were stabilized in a frozen state.

Conclusions: This work proves that samples enzymatically stabilized using the Stabilizer T1 instrument can be analyzed using standard RIA protocols and keep the sample integrity. All of the peptide levels were stable two hours post-stabilization in room temperature, proving the instrument's application in the clinic. However, to fully evaluate the results obtained in this project, complimentary mass spectroscopy analyzes of the different post-mortem treatment groups are necessary to determine from which peptide, or peptides, the measured immunoreactivity is derived from.

Acknowledgments: Funding was provided from the Swedish Medical Research Council and ERAB

No. 69 - Probing Allosteric Modulation of the Mu Opioid Receptor

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The mu opioid receptor (MOPr), particularly the orthosteric site, is the major target for all clinically used opioid drugs. We have begun to investigate positive allosteric modulation of MOPr as a novel avenue for managing pain and have recently characterized the first positive allosteric modulator (PAM) of MOPr, BMS-986122 (BMS). The objective of the current study was to investigate the probe selectivity, and possible mechanism of action of BMS for a variety of orthosteric MOPr ligands by radioligand binding assays and by activation of G protein using the [35S]-GTP γ S assay. In membranes from C6 glioma cells expressing MOPr, BMS alone did not displace orthosteric ligands from MOPr, nor did it alter basal [35S]-GTP γ S binding. As a MOPr PAM, BMS increased the binding affinity of several clinically used opioid drugs, including methadone and loperamide, as well as endogenous peptides including methionine-enkephalin and β -endorphin. In addition, BMS enhanced the potency of these ligands to cause activation of G protein with allosteric cooperatively (α) values ranging from 6 to 18. In contrast, binding affinity and potency to stimulate [35S]-GTP γ S binding of the opioids morphine and congeners as well as fentanyl was unaffected by BMS. For these compounds, there was an enhancement of maximal G protein stimulation. Sodium ions are known to stabilize inactive states of G protein-coupled receptors, including MOPr, thereby reducing agonist affinity. To test the hypothesis that BMS promotes active-like states of MOPr, we compared the sensitivity of MOPr agonists to BMS with their sensitivity to Na⁺ ions and found an excellent correlation. Moreover, the potency of Na⁺ ions to reduce DAMGO binding was markedly attenuated in the presence of BMS. Overall, these findings show that BMS exhibits distinct probe dependence for MOPr agonists and that this may be related to an ability of BMS to interfere with the binding of Na⁺ ions to the receptor. Supported by T32 DA007267.

No. 70 - The involvement of brain GPR40 signaling in regulation of the descending pain inhibitory system

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Omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) have an antinociceptive effect against pain caused by inflammation and neuropathy; however, the underlying mechanisms remain poorly understood. Previously, we demonstrated that DHA-induced antinociception, effectuated by increased β -endorphin release, may be mediated by GPR40, a long-chain fatty acid receptor. Here, we examine the involvement of GPR40 signaling in the supraspinal area in the regulation of the descending pain inhibitory system comprising serotonergic and noradrenergic neurons. Immunohistochemistry revealed that GPR40 protein was colocalized with NeuN (a neuron marker) in the medulla oblongata, but not with GFAP (an astrocyte marker). In addition, GPR40 protein colocalized with tryptophan hydroxylase (TPH, a serotonergic neuron marker) and dopamine beta hydroxylase (DBH, a noradrenergic neuron marker). A single intracerebroventricular (i.c.v.) injection of GW9508, a GPR40 agonist, significantly increased the number of c-Fos-positive cells in the locus coeruleus and raphe magnus of the medulla oblongata compared with the 0.2%DMSO-treated group. In addition, the number of neurons double-labeled for c-Fos and TPH and DBH were increased in the mice treated with GW9508, compared with the 0.2%DMSO-treated group. The pre treatment of 6-hydroxy dopamine (6-OHDA), a neuron toxic, or p-chlorophenylalanine (PCPA) significantly inhibited GW9508-induced antinociception. Interestingly, the i.c.v. injection of GW9508 significantly increased noradrenaline and serotonin level in the spinal cord. Furthermore, the i.c.v. injection of GW1100, a GPR40 antagonist, significantly increased formalin-induced pain-related behavior in the late phase, but not in the early phase, compared with the 0.2%DMSO-treated group. In conclusion, our findings suggest that the GPR40 signaling pathway may play an important role in the regulation of the descending pain control system.

This study was supported by a Grant-in-Aid for Scientific Research (C) (24592364) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

No. 71 - Mapping delta opioid receptors in the rat central nervous system using an irreversible biotinylated ligand

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Over the last decades, growing evidence revealed that delta opioid receptor (DOP) agonists may be of interest for the treatment of chronic pain, emotional disorders, and neurodegenerative diseases. The expression pattern of DOP in the central nervous system (CNS), however, remains a matter of controversy. In fact, the selectivity of most commercially available DOP antibodies has been challenged. To address this issue, we used an antibody-free approach to map DOP in the brain and the spinal cord of rats. We combined an irreversible affinity label (AL) derivative of the DOP antagonist TIPP with a biotin derivative (DSB) to take advantage of the high affinity of biotin derivatives to bind avidin. Brain and spinal cord slices were incubated with 100 nM of TIPP-AL-DSB and then the labeled receptors detected using an avidin-biotin-peroxidase reaction followed by 3,3'-diaminobenzidine (DAB) staining. This pharmacological labeling of DOP revealed a wide distribution of the receptor throughout the CNS with high staining in the olfactory bulb, cortex, striatum, hypothalamus, thalamus, some pontine nuclei, and brainstem as well as in the spinal cord. At the cellular level DOP labeling appeared to be relatively homogenous within the neuronal cell bodies and dendrites sharing different morphological features in size and shape. Topographical analysis indicated a selective laminar organization of DOP mainly in cerebral cortex and spinal cord. While DOP staining in the somatosensory cortex was mostly observed in pyramidal cells of layer V, it was largely distributed throughout the gray matter of the spinal cord (mostly in laminae I-V of the dorsal horn and in some motoneurons of the ventral horn). Notably, the pattern of DOP expression observed with TIPP-AL-DSB appears to be very similar to previous anatomical studies (binding and in situ hybridization studies). In conclusion, TIPP-AL-DSB is a useful tool to study DOP and could potentially be used as an alternative to antibodies.

Financial support: Supported by CIHR grant MOP273137
No conflict of interest to declare

No. 72 - Opiate exposure state controls a molecular switch in opiate reward memory formation in the basolateral amygdala-prefrontal cortical pathway

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The potent rewarding effects of opiate class drugs facilitate the formation of strong associative memories linked to the drug experience that play a key role in triggering relapse. These opiate reward memories are encoded and stored in the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) along a temporal gradient. Interestingly, intra-BLA processing of opiate-related reward memories is mediated by dopamine D1R and D2R signaling as a function of opiate exposure state, where D1R receptors are required for acute opiate memory formation in the previously drug-naïve state, but D2R signaling is necessary for memory formation during opiate dependence and withdrawal. Linkages between D1R and ERK 1/2 and between D2R and CaMKII α suggest these signaling molecules may underlie the state-dependent opiate memory formation “switch”. Using an unbiased place conditioning procedure paired with targeted microinfusions, we show that associative memories are processed in the BLA via an ERK-dependent mechanism in the naïve state, but via a CaMKII α -dependent mechanism following the transition to dependence and withdrawal. Interestingly, intra-mPFC memory acquisition requires CaMKII α signaling in the drug-naïve state, but does not appear to require ERK1/2 in either opiate exposure state. Western blotting analyses demonstrated a further dissociation in these signaling molecules across the BLA-mPFC pathway. Protein analysis revealed a reduction in both ERK1/2 and CaMKII α expression in BLA tissue, but an increase in both CaMKII α and ERK1/2 in the mPFC. Together, these results demonstrate a functional interaction between the BLA and mPFC during the processing and storage of opiate-related associative memories, during the switch from the non-dependent, to dependent/withdrawn opiate exposure states.

Funding sources: CIHR grant to SRL

No. 73 - Functional selectivity at the kappa opioid receptor (KOPR): from in vitro to in vivo

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It has been previously shown that analgesia at the KOPR is G protein mediated and dysphoria is β -arrestin mediated. There have been several recent reports of ligand bias at the KOPR. However, there have been no comprehensive reports of the degree of bias for multiple available KOPR agonists at the human and mouse KOPRs. Here we examined the ability of over 20 KOPR agonists to activate G proteins and to internalize the receptor in vitro. We used clonal mouse neuroblastoma (N2a) cells stably transfected with the human or mouse KOPR (hKOPR and mKOPR, respectively) for our in vitro studies. We used agonist-induced [³⁵S]GTP γ S binding to membranes as a measure of G protein activation and KOPR internalization as a readout of β -arrestin recruitment employing on-cell western technique on live cells. The method of Ehlert and colleagues was used to quantify the degree of ligand bias. Several ligands were identified to be biased for either the G protein or β -arrestin pathway, with several species differences. We chose a G biased compound, MOM-Salvinorin B, an arrestin biased ligand, U50,488H, and a balanced ligand, nalfurafine (TRK820) identified in vitro at the mKOPR to perform in vivo behavioral studies in CD1 mice. Three different behavioral tests were used: (1) inhibition of compound 48/80-induced scratching, (2) the formalin test, and (3) conditioned place aversion. We hypothesized that there would be separation between the dose-response relationship for analgesia and dysphoria in the arrestin biased and G protein biased ligands. At all doses of the three compounds that produced analgesia and anti-scratching effects, significant aversion was seen with U50,488 and MOM-Salvinorin B, whereas nalfurafine only produced significant aversion at the highest dose of 20 μ g/kg. Nalfurafine was the only compound studied to have a separation between the ED₅₀ values for antinociception and anti-scratching vs. aversion. Thus, there are differences in ligand bias between in vitro and in vivo, the reasons for which will be discussed.

This work was supported by NIH grants T32 DA007237 (K.M.D.) and R01 DA017302 (L.Y.L-C). The authors declare no conflicts of interest.

No. 74 - GIRK channel activation in midbrain GABA neurons is not required for opioid-induced locomotor stimulation

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The ventral tegmental area (VTA) and interconnected brain regions, including the nucleus accumbens and medial prefrontal cortex comprise, in part, the mesocorticolimbic dopamine (DA) system. The mesocorticolimbic DA system mediates the reward-related behavioral effects of drugs of abuse, including opioids. Opioids are believed to indirectly stimulate dopamine (DA) release from direct inhibition of GABAergic neurons located in the VTA and/or rostromedial tegmental nucleus (RMTg). Innervation of VTA DA neurons by VTA GABA neurons is evident, however, the specific mechanism whereby these local GABA neurons disinhibit VTA DA neurons remains unclear. Reward-related effects of opioids, including locomotor stimulation, require activation of mu opioid receptors (MOR). MOR activation modulates downstream effectors that decrease neuronal excitability, including G protein-gated inwardly-rectifying K⁺ (GIRK/Kir3) channels. GIRK channels are localized to midbrain GABA neurons and have long been thought to underlie the direct inhibitory effect of opioids on VTA GABA neurons, and consequently, the disinhibition of VTA DA neurons and opioid-induced reward-related behavior. Here, we show that selective genetic and pharmacologic manipulations of GIRK channel activity in the VTA or RMTg do not disrupt the locomotor stimulatory effects of opioids. The prototypical GIRK channel is a heterotetramer formed by GIRK1 and GIRK2 subunits, however, VTA DA neurons do not express GirK1. Leveraging this knowledge, we microinfused a novel GIRK channel agonist, ML297, which selectively activates GIRK1-containing heteromers. This approach permitted selective inhibition of midbrain GABAergic, but not DAergic neurons. Neither intra-VTA or -RMTg infusion of ML297 was sufficient to promote locomotor stimulation. In contrast to our hypothesis, these data suggest that GIRK channel activation is not required for reward-related behavior induced by opioids.

Supported by DA034696, DA011806, DA007097

No. 75 - Influence of disulfiram on the development of tolerance on analgesic action of morphine

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INTRODUCTION

The use of opioids in the treatment of chronic pain is limited by a rapid development of opioid tolerance.

It is known that dopamine participates in the development of tolerance on analgesic activity of opioids. Since disulfiram and its metabolites inhibit the activity of dopamine-B-hydroxylase leading to increase of dopamine level, we hypothesize that disulfiram may delay opioid tolerance.

METHODS

The studies were performed on male Wistar rats. Two types of nociceptive stimuli, mechanical (Randall-Selitto test) and thermal (Tail-flick test and Plantar test) were used.

RESULTS

Disulfiram administered alone on 21 consecutive days slightly increased the threshold for mechanical (Randall-Selitto test) and thermal stimuli (tail-flick and plantar tests) in dose-dependent manner.

Morphine administered alone on 21 consecutive days gradually increased the threshold for mechanical and thermal stimuli. The antinociception reached its maximum on day 6 in Randall-Selitto test and tail-flick test or on day 4 in plantar test, then following repeated treatment slowly decreased and achieved baseline values on day 14 (Randall-Selitto test), on day 13 (plantar test) and on day 21 (tail-flick test).

Premedication with disulfiram (12.5 mg/kg p.o.) prevented the development of tolerance on analgesic activity of morphine (25 mg/kg p.o.) for consecutive 21 days. Similar but stronger effect was observed after premedication with disulfiram at doses 25 or 50 mg/kg p.o. before morphine.

CONCLUSIONS

Disulfiram delays the development of tolerance on analgesic action of morphine in acute pain model after mechanical and thermal stimuli.

Research subject implemented with CePT infrastructure financed by the EU – the European Regional Development Fund within the Operational Program “Innovative economy” for 2007-2013.

No. 76 - Effect of new form of magnesium salt on analgesic activity of tramadol

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INTRODUCTION

As previously reported, magnesium sulphate administered parenterally significantly increased an opioid analgesia in different kinds of pain. It seems that the combined administration of opioid and magnesium may offer in the future very valuable therapeutic possibilities, moreover the concomitant administration of those compounds orally would hypothetically allow for the use of this combination in outpatient conditions.

Since the typical form of magnesium salts are weakly and slowly absorbed from the gastrointestinal tract, we decided to investigate how magnesium salt in new and better absorbed form administered orally modify the opioid analgesia, in comparison with conventional form of magnesium salt.

METHODS

The studies were performed on male Wistar rats. Two types of nociceptive stimuli, mechanical (Randall-Selitto test) and thermal (Tail-flick test), were used.

RESULTS

Conventional and new forms of magnesium salts at a dose of 15 mg of magnesium ions/kg did not modify the nociceptive threshold after mechanical and thermal stimuli. Tramadol administered at a low dose of 125 mg/kg had only a little antinociceptive effect.

A pre-treatment with a new form of magnesium salt enhanced the analgesic activity of orally administered tramadol significantly faster and more effectively in comparison to the conventional form of magnesium salt.

CONCLUSIONS

A new form of magnesium salt enhances analgesic effect of tramadol. This observation may be relevant clinically.

Research subject implemented with CePT infrastructure financed by the EU – the European Regional Development Fund within the Operational Program “Innovative economy” for 2007-2013.

This work was supported by the National Centre for Research and Development of Poland.

No. 77 - Anti-inflammatory and Antinociceptive Action of 6 β -Tryptophan Substituted 14-O-methyloxymorphone in Mouse Models of Inflammatory Bowel Diseases

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Introduction: Inflammatory bowel diseases (IBD) constitute a large group of severe and chronic gastrointestinal (GI) disorders characterized by intestinal inflammation, mucosal damage, and severe pain that is still inadequately managed. μ Opioid (MOP) receptors are distributed throughout the central and peripheral nervous systems and various non-neuronal tissues. Several lines of evidence show that MOP receptors are involved in the pathophysiology of inflammatory GI disorders, directing toward the clinical relevance of targeting MOP receptors for the treatment of IBD. Herein, we describe the anti-inflammatory and antinociceptive actions of 6 β -tryptophan substituted 14-O-methyloxymorphone (HS1333), a novel peripherally acting MOP agonist, in mouse models of IBD.

Methods: Opioid receptor activities were determined using radioligand binding and [³⁵S]GTP γ S functional assays. A fluorescence cell-based assay was used to monitor the nuclear factor- κ B (NF- κ B) activity upon stimulation with tumor necrosis factor- α (TNF- α) and lipopolysaccharide (LPS). Anti-inflammatory effects were assessed in trinitrobenzene sulfonic acid (TNBS)- and dextran sodium sulphate (DSS)-induced colitis in mice. Antinociceptive action of HS1333 was evaluated in mouse models of abdominal pain after.

Results: HS1333 displays high MOP binding affinity and is a potent and full agonist toward the MOP receptor. It significantly inhibits, in a concentration-dependent manner, NF- κ B activation in TNF- and LPS-stimulated human monocytic THP-1 Blue cells. In mice, the MOP receptor agonist (0.1 and 1 mg/kg, s.c.) twice daily attenuates TNBS- and DSS-induced colitis. Dose-dependent and significant antinociceptive effects were produced by s.c. HS1333 in acetic acid-induced abdominal stretching and mustard oil-induced acute colitis in mice.

Conclusion: Novel MOP agonists acting in the periphery with combined immunosuppressive and antinociceptive properties may provide a new approach for the treatment of IBD. (Supported by the Austrian Science Fund (FWF): P21350, Tyrolean Research Fund (TWF): UNI-0404/949 and S&T Cooperation Fund Austria-Poland 2013-2014: PL13/2013.

No. 78 - Endomorphin analog analgesics lack reinforcement qualities and are promising candidate medications for the treatment of opioid addiction

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Endomorphins (EM) have the highest selectivity of any endogenous peptide for the pain modulating mu-opioid receptor (MOR). We have synthesized several metabolically stable, blood-brain barrier permeable EM analogs that have equal or greater analgesic potency than morphine, the current gold standard MOR agonist. Morphine and other exogenously derived opioid analgesics produce detrimental side effects such as respiratory depression, tolerance, glial cell activation, and have a high potential for abuse. We have shown that EM analogs do not induce respiratory depression, produced substantially less tolerance than morphine, and do not induce markers of microglia or astrocyte activation. In abuse liability assays, intravenous (i.v.) morphine produced a robust and dose-dependent conditioned place preference effect, but the analogs did not. In self-administration models, lever pressings for morphine progressively escalated as lever pressing requirements increased or the available mg/kg dose decreased, suggesting high abuse liability. By contrast, rats with access to i.v. EM analog infusions lever pressed no more than controls with access to i.v. vehicle, suggesting the analogs lack reinforcing qualities. In this study we trained rats to discriminate i.v. infusions of morphine from vehicle in a 2-lever food choice drug-discrimination (DD) task to assess the substitution potential of the analogs for morphine. Methadone fully substitutes for morphine, but suppresses motor/appetitive responses for food in the DD model. A compound which lacks self-administration but substitutes for morphine without motor/appetitive impairment has potential as a pharmacotherapy for opioid addiction. We found that analogs 2 and 4 fully substituted for morphine, but did not impair motor/appetitive responding. This suggests the analogs may provide a better therapeutic window than methadone since motor impairment is a major limitation of methadone maintenance therapy. These data indicate EM analogs have a better safety profile than morphine, lack reinforcement qualities, provide potent analgesia, and are novel candidates for opioid addiction pharmacotherapy.

Support: VA, DOD, ONR

No. 79 - Spatiotemporal Control of Mu-Opioid Signaling and Behavior

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Tools for isolating microcircuitry and kinetics of opioid action with discrete spatiotemporal control in freely moving animals are not yet available. We created optically sensitive mu opioid receptors (oMORs) for using optogenetics to drive mu opioid-like signaling in vivo at the level of microcircuitry with cell-type specificity. Here, we present the initial characterization of these receptors in vitro and in vivo. oMOR chimeric receptor constructs were designed to incorporate the extracellular loops of rat rhodopsin with the intracellular components of rat mu opioid receptors. First, we compared the pharmacology of oMOR vs. wild-type MOR in inhibiting cAMP, activating MAPKs, and internalizing in response to photo-stimulation. Sustained photo-stimulation of oMOR caused suppression of forskolin-induced cAMP, activated pERK MAPK and caused receptor internalization with kinetics that are consistent with endogenous MOR activation. For in vivo cell-type specificity, oMORs were cloned and packaged into a cre-dependent adenovirus (AAV5-EF1a-oMOR-WPRE) and injected into VGAT-IRES-Cre (GABA-selective) mice in brain structures relevant for mu opioid-driven behaviors such as conditioned place preference and analgesia. As expected, the virus was expressed in the VTA and RMTg of VGAT-IRES-Cre mice. Whole cell slice physiology recordings from these mice showed that photo-stimulation of oMOR caused a robust outward GIRK current, in a similar manner to DAMGO at wild-type MOR. Furthermore, 10mW 473nm laser light delivered through fiber optic ferrules caused significant real time place preference. Here we show that oMORs are light sensitive Gi-coupled GPCRs that signal and traffic similar manner as compared to MOR and are sufficient to promote reward-like behavior in mice. This tool will enable spatiotemporal dissection of neural pathways, behaviors associated with mu opioid receptor activation, and could generally be used to examine effects of inhibitory G-protein activation in neural circuits. Authors have no competing financial interests. Supported by R01DA037152 to MRB, and HHMI to MAB.

No. 80 - Repeated Morphine Promotes Bidirectional and Cell-Type Specific Adaptations in AMPAR plasticity in Medium Spiny Neurons of the Accumbens Core and Shell

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Introduction: In animal models of addiction, plasticity of glutamatergic synapses in the nucleus accumbens (NAc) exerts powerful control over drug-seeking behavior. However, little is known about whether, how or when experience with drugs may trigger synaptic plasticity in this key nucleus. Here we identify adaptations in glutamatergic signaling within the NAc following withdrawal from repeated morphine exposure.

Methods: Whole-cell voltage-clamp recordings were performed in medium spiny neurons (MSNs) in sagittal brain slices containing the NAc core or shell regions from adult C57BL/J6 and *Drd1a*-tdTomato mice. Mice received 5 daily injections of morphine (10 mg/kg, i.p.) or saline, followed by 10-14 d of withdrawal, at which point animals were left undisturbed (no challenge) or injected with saline or morphine 24 h before electrophysiological study (challenge).

Results: Initial studies in NAc shell MSNs of wild-type mice showed a significant increase in the ratio of AMPAR:NMDAR currents, a reduction in paired-pulse ratios, and an increase in miniature excitatory postsynaptic current (mEPSC) amplitude, but not frequency in morphine-treated mice following withdrawal (no challenge). In contrast, preliminary data from the core indicate that withdrawal from morphine does not significantly alter any of these parameters. Studies using *Drd1a*-tdTomato mice show that within the shell, AMPAR/NMDAR ratios, as well as mEPSC amplitude and frequency are increased in D1- but not in non-D1-MSNs (i.e., D2-MSNs), while a significant reduction in frequency was found in D2-MSNs of the core. Whether AMPAR:NMDAR ratios are altered in the core is currently being explored. In drug-challenged mice, data indicate that a single re-exposure to morphine during withdrawal becomes a potent stimulus for synaptic depression and reverses the initially observed potentiation within the shell. These effects, and the role of depotentiation in the expression of sensitization are currently being explored further.

Conclusion: Our results indicate that in vivo morphine exerts dynamic bidirectional control over excitatory synaptic strength in NAc that displays neuronal and anatomical selectivity.

No. 81 - Variants of stress-related genes and their role in heroin self-exposure, addiction, and response to treatment

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Here we report part of a larger study designed to identify genetic variants that are associated with susceptibility to develop heroin addiction and response to treatment thereof. The goal was to determine if specific variants (120) of stress-related genes (26) contribute to heroin addiction in a unique cohort of 597 Dutch Caucasian subjects, consisting of four groups: 1) subjects with no history of drug use (n=153); 2) subjects self-exposed to illicit opiates at least 5 times but not addicted (n=163); 3) former heroin-dependent subjects successfully methadone-maintained (n=143); and 4) treatment-resistant heroin-dependent subjects, offered heroin-assisted methadone treatment (n=138). Genotyping was performed using a custom GoldenGate array (Illumina). Initially, a one-way ANOVA was performed to test for differences in genotype frequency among the four groups of subjects for each single nucleotide polymorphism (SNP), separately. Four SNPs from three genes were identified as nominally significant ($p > 0.05$): arginine vasopressin receptor 1B (AVPR1B) SNP rs33933482; galanin (GAL) SNP rs3136541; and in the glucocorticoid receptor gene (NR3C1) SNPs rs10482672 and rs17339455. Analysis comparing the control group to those subjects self-exposed without addiction, found two SNPs in the neuropeptide Y gene (NPY), rs2234759 and rs6536721, as nominally significant. When the methadone maintained subjects were compared to the group that required augmentation with heroin, four SNPs in four genes were nominally significant: corticotropin releasing hormone receptor 1 (CRHR1) SNP rs4792887; glycine receptor, alpha 1 (GLRA1) SNP rs2964608; NPY SNP rs16148; and NR3C1 SNP rs10482672. Further analyses are in progress to determine if there are differences between the combined groups 1+2 and combined groups 3+4.

Supported by pilot funds from NIDA [MJK], CCBH ZonMw 31000465, and The Dr. Miriam & Sheldon G. Adelson Medical Research Foundation.

The authors report no conflicts of interest.

No. 82 - Delineating the dynamics of μ Opioid Receptor signalling and regulation

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Receptor signal compartmentalisation (the restriction of second messengers in space and time) provides a mechanism whereby receptors can direct the assembly of focused “platforms” that facilitate second messenger production, the organisation and scaffolding of effectors, and co-ordination of regulatory events. Decades of research have focused, without success, on the differential regulation and internalisation of μ OR by morphine compared to DAMGO, in an effort to design more effective analgesics. To date, there are no studies examining compartmentalised signalling of μ OR in live cells, no information on μ OR spatiotemporal signalling profiles, and no knowledge of whether this is regulated by receptor trafficking. Whether μ OR resides within biochemically-defined lipid-rich plasma membrane (PM) regions is controversial, and the lack of a unifying view is likely due to the invasive nature of methods used to isolate these domains. We now show that the regulation of μ OR trafficking and signal compartmentalisation are interdependent. We have used BRET to measure μ OR endocytic trafficking and have correlated this with signalling measured from bulk compartments in single cells using FRET biosensors. μ OR initially resides within a unique PM microdomain. Morphine stimulation of μ OR initiates a PM-localised $G_{\alpha i/o}$ - $G\beta\gamma$ -PKC activation. PKC phosphorylation of the receptor causes sequestration of μ OR and a sustained signalling profile: sustained membrane-PKC phosphorylation, and sustained cytosolic ERK phosphorylation. In contrast, DAMGO does not activate PKC allowing μ OR translocation within the PM and a transient signalling profile: transient $G_{\alpha i/o}$ -mediated cytosolic ERK phosphorylation, β Arr recruitment, receptor internalisation, and transient β Arr-mediated nuclear ERK phosphorylation. Our results suggest that μ OR-mediated signalling is highly compartmentalised and ligand-dependent and that distinct μ OR membrane localisations lead to diverse spatiotemporal signalling.

No. 83 - Postnatal changes in excitability and GABAergic transmission in rat rostral ventromedial medulla (RVM) neurons

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Neurons in the rostral ventromedial medulla (RVM) play critical and complex roles in pain modulation. Recent studies have shown that electrical stimulation of the RVM produces pain facilitation in young animals (postnatal day (PN) > 21) but biphasic pain facilitation and inhibition in adults, and that opioids applied in this region have different effects at different ages. The cellular mechanisms underlying these changes in the influence of the RVM on pain behaviors are not known. This is in part because whole-cell patch-clamp studies in RVM to date have been in young (PN > 16) animals and the fact that the organization and abundance of myelinated fibers in this region make it a challenging area for whole-cell patch-clamp recording in adults. Neurotransmission in several systems, including GABA and glutamate neurotransmission, undergo developmental changes that mature by PN 21-30. Thus, we focused on optimizing whole-cell patch-clamp recordings for RVM neurons in animals older than PN day 30 and compared the results to animals 10 - 21 days PN. Our results demonstrate that there are substantial differences in neuronal excitability, GABAergic neurotransmission and opioid action in RVM neurons between these time periods. Differences in these properties of RVM neurons may underlie the developmental changes in descending control of pain from the RVM to the spinal cord.

These studies were supported by the grants from NIH (DA027625, S.L.I.), and America Heart Association (13SDG14590005, M.H.L.).

No. 84 - Spinal P2X7R receptors are critically involved in the development of morphine physical dependence

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Introduction: Opioids, such as morphine, are among the most powerful and widely prescribed analgesics for managing pain. However, repeated opioid use can lead to physical dependence, a crippling condition that manifests as a withdrawal syndrome upon drug cessation. The spinal dorsal horn is a primary site of action for opioids, and cellular changes in this region are causally implicated in opioid physical dependence. In the present study, we examined the importance of spinal ATP-gated P2X7 purinoceptors (P2X7R) in the development of morphine physical dependence.

Methods: Opioid physical dependence was established using a 5-day escalating morphine dosing paradigm. Adult male Sprague Dawley rats were given twice daily intraperitoneal injections of morphine at 8 hour intervals (day 1: 10 mg/kg; day 2: 20 mg/kg; day 3: 30 mg/kg; day 4: 40 mg/kg). To examine the contribution of spinal P2X7Rs in the development of morphine physical dependence, intrathecal injections of the P2X7R antagonist brilliant blue G (BBG) or A740003 were co-administered with morphine. On day 5, rats received a morning injection of 45 mg/kg and 2 hours later challenged with a single injection of the opioid receptor antagonist, naloxone (2 mg/kg), to precipitate morphine withdrawal.

Results: We found that naloxone challenge precipitated a robust withdrawal syndrome in morphine treated animals. The severity of morphine withdrawal correlated with increased spinal expression of ionized calcium-binding adaptor molecule (iba-1), a cellular marker of microglial activation, and the upregulation of c-Fos, a marker of neuronal activation. Intrathecal administration of the P2X7R antagonist, BBG or A740003, significantly attenuated the behavioural signs of withdrawal. Blocking spinal P2X7R also prevented the naloxone-induced increase in iba-1 and c-Fos expression.

Conclusions: Blocking P2X7Rs during repeated morphine treatment decreases withdrawal symptoms and reduces activation of spinal microglia. Collectively, our findings reveal a novel and critical role of spinal P2X7Rs in the development of morphine dependence.

[Supported by grants from CIHR, NSERC, and CFI]

Authors declare no conflicts of interest.

**No. 85 - Dopaminergic system genes
polymorphisms and heroin addiction: further
support for a protective effect of casein kinase
1 ϵ (CSNK1E) variants**

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The dopaminergic reward pathways have been implicated in the etiology of drug addictions. We have previously reported a tentative association of CSNK1E (casein kinase 1 ϵ) SNPs and haplotype with heroin addiction in subjects of European ancestry.

The aims of this association study were to determine if genetic variants in genes encoding proteins of the dopaminergic pathway account for the heritable factors in susceptibility to heroin addiction, as well as to replicate our previous findings in a larger cohort. The study was performed on 828 unrelated former severe heroin addicts treated at methadone maintenance treatment programs in NYC, Las Vegas and Israel, as well as 233 healthy controls. The study was limited to subjects of predominantly European ancestry, verified by STRUCTURE analysis of 155 AIMs. A total of 118 SNPs in 13 genes were genotyped using the Illumina GoldenGate platform. After filtering for quality, HWE, MAF and complete LD, 82 SNPs were analyzed for association by logistic regression using the PLINK program.

Nominally significant associations were observed at 12 SNPs located in the genes CSNK1E, DRD2/ANKK1, DRD3, and DBH ($P > 0.05$). The association of the intronic CSNK1E SNP rs1534891 (a protective effect of the minor allele) remained significant after correction for multiple testing. This study supports our previous report of association of this SNP and a related haplotype with protection from heroin addiction, in subjects of European descent. CSNK1E encodes a serine/threonine-selective enzyme isoform that interacts with several signaling pathways including circadian rhythms and DARPP-32 regulation of the dopaminergic pathway. Csnk1e has been implicated in negative regulation of sensitivity to stimulants and opioids, in rodents. CSNK1E may be a target in the treatment of drug addiction.

Supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation and 2UL1 RR024143-06(JC). The authors have no conflict of interest.

**No. 86 - Angiotensin II type 1
receptor-mediated increase in spinal p38
MAPK phosphorylation leads to the
induction of nociceptive behavior in mice**

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It has been demonstrated that angiotensin II (Ang II) participates in either the inhibition or the facilitation of nociceptive transmission depending on the brain area. However, the role of spinal Ang II in nociceptive transmission remains unclear. Therefore, in order to elucidate the role of Ang II in nociceptive transmission in the spinal cord, we examined the effect of intrathecal (i.t.) administration of Ang II into mice. I.t. administration of Ang II produced a behavioral response in mice mainly consisting of biting and/or licking of the hindpaw and the tail along with slight hindlimb scratching directed toward the flank. The behavior induced by Ang II was dose-dependently inhibited by intraperitoneal injection of morphine, suggesting that the behavioral response is related to nociception. The nociceptive behavior was also inhibited dose-dependently by i.t. co-administration of losartan, an Ang II type 1 (AT1) receptor antagonist, and SB203580, a p38 MAPK inhibitor. However, the Ang II type 2 (AT2) receptor antagonist PD123319, the upstream inhibitor of ERK1/2 phosphorylation U0126, and the JNK inhibitor SP600125 had no effect on Ang II-induced nociceptive behavior. Western blot analysis showed that the i.t. injection of Ang II induced phosphorylation of p38 MAPK in the lumbar dorsal spinal cord, which was inhibited by losartan, without affecting ERK1/2 and JNK. Furthermore, we found that AT1 receptor expression was relatively high expressed in the lumbar superficial dorsal horn. Our data show that i.t. administration of Ang II induces nociceptive behavior accompanied by the activation of p38 MAPK signaling mediated through AT1 receptors. This observation indicates that Ang II may act as a neurotransmitter and/or neuromodulator in the spinal transmission of nociceptive information.

No. 87 - Analogs of salvinorin A bearing sulfur-containing substituents at position C2 are high-affinity kappa-opioid receptor partial agonists with a potentially novel binding mode

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The mechanism by which the highly affine and non-basic agonist salvinorin A and its analogs interact with and modulate the function of the kappa-opioid receptor (KOP) is not only intriguing from a receptor–ligand interaction standpoint but also highly relevant to the potential treatment of several disease states, including but not limited to those associated with visceral pain (e.g. irritable bowel disease; IBS). Here we report the affinity, potency and putative interaction modes of 19 salvinorin A analogs with either an S-alkyl or S-aryl thiocarbonate or dual ester/S-aryl- or ester/S-alkyl-thioether substituent at the C2-position at the human KOP receptor. Affinity data for the compounds at the wild-type KOP receptor were determined using a competitive binding assay with the arylacetamide agonist [3H]U69,593 as the radioligand. Nine of the compounds were shown to have substantial binding affinity (K_i 4–177 nM). Notably, positional isomers were found to have greatly varying K_i values. Functional data for these compounds at the wild-type KOP receptor was determined using a cellular cAMP response assay and showed that each of the tested compounds have partial agonist activity (EC_{50} = 35–1850 nM) relative to salvinorin A. Experimentally-guided automated docking routines were used to derive putative binding modes for the partial agonists using the crystal structure of the human KOP receptor bound to the antagonist JD₁Tic (PDB ID = 4DJH). Interestingly, a “reverse” binding mode not previously reported was identified in which the 2-substituent interacts with transmembrane (TM) helix 2, TM3 and extracellular loop 2 (EL2), while the diterpenoid salvinorin scaffold interacts with TM3, TM6 and TM7. These results suggest that the uncharged nature of the ligands combined with the spacious and largely symmetric orthosteric binding pocket allow salvinorin A analogs to effectively bind and stabilize active conformations of the KOP receptor through alternative binding modes.

Support: NIH-NIMH MH-R01DA017204 (BLR).

No. 88 - Surface plasmon resonance as a label-free approach to monitor the mu opioid receptor-mediated signaling.

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Nowadays, most analgesics used for the treatment of moderate to severe pain conditions target the mu opioid receptor (MOP). Interestingly, all MOP agonists do not activate the numerous receptor-mediated signaling cascades to the same level. In this study, we used Surface Plasmon Resonance (SPR) spectroscopy as a label-free approach to investigate the contribution of G protein coupling, ERK1/2 activation and receptor internalization to the SPR signals generated by various ligands in living HEK cells expressing the human Flag-MOP.

In the present study, we found that SPR signals triggered by DAMGO and morphine were similar. However, we observed that they were differently affected by various pharmacological inhibitors. Indeed, U0126, dynasore and cholera toxin (CTX) all affected the amplitude of the signal induced by DAMGO and morphine. CTX also affected the maximal slope of morphine SPR response. However, it had no effect on the agonist-induced ERK phosphorylation. The SPR signals induced by DAMGO and morphine were completely abolished by pertussis toxin (PTX) pretreatment, indicating an important role for $G_{\alpha i}$. We also evaluated four MOP antagonists (CTAP, CTOP, naloxone and naltrexone). We found that CTOP, naloxone and naltrexone, which intriguingly is the lone ligand of our study to induce a rapid decrease in reflectance, all generated PTX-sensitive SPR signals. Our BRET analysis further revealed that MOP antagonists can trigger conformational changes within the $G_{\alpha i}\beta\gamma$ heteromer. Together, our results suggest that CTOP, naloxone and naltrexone are partial agonists while CTAP is likely an inverse agonist.

We demonstrated that SPR spectroscopy is a sensitive approach allowing for the monitoring of aggregate cellular responses occurring as a result of receptor activation. With this cell-based, label-free approach we show that it is possible to detect responses for ligands for which no responses are normally detected in formal biochemical assays.

Conflict of interest: authors have no conflict of interest to declare.

Funding: Supported by NSERC-CIHR Collaborative Health Research Project grant #RPCS-385882-2010 (M.G and L. G) and by NSERC grant #311997 (G. P)

No. 89 - Opioid signaling in early-life adversity: a translational study

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Introduction. Childhood maltreatment (CM) represents an extreme form of emotional pain that affects children of all ages, race, economic, and cultural backgrounds. There is a strong relationship between CM and mental health outcomes. Accordingly, sexual and physical abuse, as well as profound emotional neglect, are among the strongest predictors of psychiatric pathology, such as depression and suicide.

Hypothesis. We hypothesize that CM disrupts opioid signaling in brain structures processing emotional pain, thereby increasing the risk of depression and suicide throughout life. We also propose that epigenetic adaptations in the opioid system genes may account for such long-term effects.

Methods. Our project combines the study of human post-mortem tissues and a robust and validated rat model. We focus our analyses on three brain regions critically regulating emotional pain: the anterior insula, the anterior cingulate cortex and the dorso-medial thalamus.

In human, we measure the expression (quantitative PCR) and G-protein coupling ($[^{35}\text{S}]\text{-GTP}\gamma\text{S}$) of opioid receptors, as a function of past histories of severe CM, depression and suicide. In rat, we quantify the expression of the opioid system as a function of naturally occurring variations in maternal care. Our group of investigators has provided important evidence that low levels of maternal care in this model are relevant to the study of neurobiological adaptations to early-life adversity, which may be conserved across species.

Results. Our results indicate that a history of severe CM associates with a significant decrease in kappa opioid receptor mRNA levels in the human anterior insula. Surprisingly, CM did not modify the maximal coupling efficiency of the kappa opioid receptor (E_{max}) in this brain region.

Conclusion. This is the first study reporting how CM may impact the opioid system in the human brain. As a next step, we are currently investigating potential epigenetic mechanisms accounting for maladaptive opioid signaling associated with CM, focusing on DNA methylation.

Funding sources:

Canadian Institute for Health Research
Fondation Fyssen (PEL) and Fondation Bettencourt-Shueller (PEL)

No. 90 - Differential effector coupling does not underlie resistance or susceptibility to desensitization of presynaptic mu opioid and GABAB receptors

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Mu opioid receptor (MOR)-mediated inhibition of GABA release onto proopiomelanocortin (POMC) neurons in the hypothalamus resists acute desensitization. In contrast, acute desensitization of GABAB receptor (GABABR)-mediated inhibition of GABA release is observed at roughly one quarter of POMC neurons. It is currently unclear whether this differential susceptibility to desensitization is due to differing physical properties of presynaptic MORs and GABABRs, or due to differential effector coupling. In the present study, whole-cell voltage clamp recordings were made in POMC neurons. The ability of MOR and GABABR agonists to suppress inhibitory postsynaptic potentials (IPSCs) was measured in the presence of pharmacological blockers that target known effectors of MORs and GABABRs. Neither DAMGO- nor baclofen-induced inhibition of IPSCs was occluded when voltage-dependent K⁺ channels were blocked or when a Ca²⁺-free external recording solution was used. DAMGO and baclofen also induced robust inhibition of IPSCs induced by ionomycin-mediated Ca²⁺ influx into terminals presynaptic to POMC neurons. Together, these data suggest that MOR- and GABABR-mediated inhibition of GABA release onto POMC neurons does not require the activation of voltage-dependent K⁺ channels or the inhibition of calcium influx. However, when recordings were made in an external solution containing Sr²⁺ instead of Ca²⁺, Sr²⁺-evoked IPSCs were robustly inhibited by both DAMGO and baclofen. This result suggests that MOR- and GABABR-mediated inhibition of GABA release can occur through the inhibition of voltage-dependent Ca²⁺ channels (VDCC). Together with the observation that presynaptic inhibition of GABA release by MORs and GABABRs is not occluded by unregulated calcium influx due to ionomycin, it appears that inhibition of release from both types of receptors can be mediated by either the inhibition of Ca²⁺ channels or by direct inhibition of vesicular release. Altogether, the results demonstrate that presynaptic MORs and GABABRs are coupled similarly, and that differential receptor-effector coupling is unlikely to explain differential desensitization of presynaptic receptors. NIH grant R01DA032562

No. 91 - Bimodal role for nucleus accumbens dynorphinergic neurons in aversion and reward

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The dynorphin/kappa opioid system is implicated in stress and vulnerability to drug abuse. It is thought that stress causes dynorphin release activating kappa-opioid receptors (KOR) within dopaminergic and serotonergic nuclei and their ventral striatal targets. Consequently, much attention has focused on these systems in the modulation of KOR-mediated responses. Despite our current knowledge of central dynorphinergic cell body populations, a clear description of the axonal projections of these neurons is unknown. To address this we crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse expressing Cre recombinase under the same promoter as dynorphin (Dyn-Cre) so only dynorphinergic cells express tdTomato, allowing complete visualization of dynorphinergic circuitry throughout the brain. We show robust dynorphin expression in cell bodies throughout the brainstem and forebrain. We were also able to use these mice in conjunction with viral retrograde approaches to isolate and identify NAc dynorphinergic projections throughout the brain. Dynorphinergic neurons within the accumbens are particularly implicated in stress and drug abuse. Therefore we investigated whether specific modulation of dynorphinergic neuronal firing in the NAc is sufficient to induce aversive behaviors. We virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and optogenetically activated neuronal populations in both the dorsal and ventral NAc shell. Activation of dorsal NAc shell induces a place preference and is positively reinforcing in an FR1 operant task paradigm while activation of ventral NAc shell drives conditioned and real-time aversive behavior. This photoactivation of dynorphinergic neurons in the ventral NAc also increased dynorphin release, as measured using microdialysis and mass spectroscopy. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates negative affective behaviors will provide valuable insight into potential treatments for drug abuse and depression. Work supported by NIDA R01DA033396 (M.R.B, R.A), NIH Common Fund, NINDS R01NS081707 (M.R.B), NIMH F31MH101956 (J.G.M). Authors declare no conflict of interest.

No. 92 - Novel small molecule triazoles modeled from naltrindole potently inhibit the proliferation of human multiple myeloma cells.

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Multiple myeloma (MM) is an incurable malignancy derived from plasma cells that proliferates in the bone marrow. In 2014, the American Cancer Society estimated that 24,050 people will be diagnosed with MM, and 11,090 will die of the disease. Current therapies have improved the 5-year survival estimate to 40%, however, there is an obvious need for new, effective treatment options. We reported that naltrindole (Nti) inhibited human MM cell proliferation in vitro and in a murine xenograft model in vivo, by interaction with a non-opioid receptor target (Mundra et al., 2012). Molecular modeling of the Nti pharmacophore led to the synthesis of a series of trisubstituted triazoles, and in the present study, we have discovered a lead compound, MM-900, that is 50-fold more potent than Nti in inhibiting the proliferation of MM cells. For cell proliferation assays, human U266 cells are plated in 12-well dishes at a density of 100,000 cells/ml in RPMI 1640 medium containing 10% FBS. U266 cells were incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO₂ in the presence and absence of Nti or MM-triazoles at concentrations ranging from 5-50 μ M. Following incubation, cells were gently dispersed and aliquots were analyzed in duplicate in a Vi-Cell instrument to quantify the number of viable cells/ml and the overall percent viability. In these assays, Nti inhibits MM cell proliferation with an EC₅₀ of 20 μ M. Approximately 20 MM-triazoles have been screened, and the majority displayed EC₅₀'s in the 10-80 μ M range, however, 3 compounds inhibited MM cell growth at significantly lower concentrations. Thus, MM-900, MM-902 and MM-902S reduced U266 cell proliferation with EC₅₀'s of 400, 490 and 520 nM, respectively, reflecting an increase in potency relative to Nti of 40-50-fold. We have shown that Nti significantly inhibits human MM tumor growth in SCID mice with daily dosing of 15 mg/kg, ip, therefore, the most active MM-triazoles that are obtained will also be tested in vivo in the mouse/human xenograft model. It is anticipated that this approach will yield novel therapeutic compounds to be used alone or in combination with current drugs used to treat MM.

No. 93 - Tolerance to morphine respiratory depression: reversal by ethanol

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Ethanol has been reported to reverse both morphine cellular tolerance in rat locus coeruleus neurones (1) and morphine antinociceptive tolerance in vivo in mice (2). The present investigation examined the effect of a low dose of ethanol on tolerance to morphine respiratory depression in mice.

Respiration (minute volume) was measured by whole body plethysmography in male mice (CD-1) breathing 95% air/5% CO₂. To measure changes in respiration, the data for each mouse was normalised to pre-drug baseline and the area under the curve (AUC) calculated in arbitrary units for a 30 min post-drug period. All data are given as mean \pm standard error.

Morphine (10 mg/kg ip; n = 8) rapidly depressed respiration. In comparison to saline injected animals the AUC for morphine was -11.0 ± 1.3 . Ethanol (0.3 g/kg ip; n = 6) caused no significant depression of respiration compared to saline (n=8) with AUC = -1.4 ± 0.6 Vs -0.7 ± 0.6 respectively.

Tolerance to morphine was induced by subcutaneous implantation of a 75mg morphine pellet. After 6 days of pellet implantation respiration had returned to pre pellet implantation levels (minute volume pre pellet 154 ± 5 ml/min, n = 12; 6 days after pellet implantation 149 ± 4 ml/min, n = 12).

In morphine pelleted mice, challenge with morphine (10 mg/kg ip; n = 6) on day 6 caused significantly less depression of respiration than in placebo pellet implanted mice (AUC = -3.3 ± 0.6 for morphine treated Vs -10.4 ± 0.6 for placebo, $P > 0.001$). When morphine pelleted mice (n = 6) were injected simultaneously with morphine (10 mg/kg) and ethanol (0.3 g/kg) there was a significant depression of respiration compared to morphine alone (AUC = -8.3 ± 0.8 Vs -3.3 ± 0.6 , $P > 0.001$).

These data provide evidence that ethanol reverses tolerance to morphine respiratory depression as previously observed with both cellular and antinociception tolerance (1, 2).

1.Llorente et al, Mol Pharmacol 84:252, 2013

2.Hull LC et al, J Pharmacol Exp Ther 345:512,2013

No. 94 - Differential Modulation of the Signaling of μ -Opioid Receptor Agonists by the Positive Allosteric Modulator, BMS-986121

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The search for novel opioids with strong analgesic properties and reduced side effect profiles has been ongoing for decades. Efforts have focused on compounds acting at the orthosteric, "opiate" site on the μ opioid receptor (MOR). Recently, BMS-986121 was described as a positive allosteric modulator (PAM) acting on the MOR. Understanding how such a PAM molecule modulates signaling of μ agonists via G protein and β -arrestin2 (β -Arr2) pathways may aid the discovery of novel, analgesic μ PAMs with reduced side effect profiles. We examined the effects of BMS-986121 on MOR activation of G protein and β -Arr2 signaling by a panel of opioid agonists. BMS-986121 effects on agonist-stimulated MOR internalization and MOR/ β -Arr2 co-localization were also examined. Methods: Agonist-stimulated GTP γ S binding was used to measure G protein signaling. Signaling events in the β -Arr2 pathway were studied with: a β -Arr2 recruitment assay (DiscoverRx); fluorescent SnapTag-MOR and β -Arr2-ZsGreen proteins with high content imaging analysis on a GE INCell 6000 device were used to measure MOR internalization and co-localization with β -Arr2. Results: DAMGO was a full agonist for GTP γ S binding and β -Arr2 recruitment with no biased signaling observed; it also exhibited full efficacy for MOR internalization, with internalized MOR and β -Arr2 highly co-localized. BMS-986121 increased DAMGO potency for all parameters with no effect on efficacy, leaving the unbiased signaling profile intact. Morphine was a G protein-biased, partial agonist, with modest internalization of MOR and minimal subsequent co-localization with β -Arr2. BMS-986121 increased the potency and efficacy of morphine for both G protein and β -Arr2 signaling. The PAM enhanced morphine potency and efficacy for MOR internalization as well as co-localization with β -Arr2. Buprenorphine was a weak, partial agonist for G protein signaling with negligible recruitment of β -Arr2 giving a G protein-biased profile. Buprenorphine produced no MOR internalization or MOR/ β -Arr2 co-localization. BMS-986121 enhanced buprenorphine efficacy for various parameters, with little impact on potency.

No. 95 - Abuse liability of oxycodone and morphine in buprenorphine-maintained participants with or without chronic pain

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Abuse of prescribed opioids is currently a serious public health concern in the U.S. The problem is complicated by the fact that it is difficult to differentiate between opioid abusers who do not need these medications for pain and patients who may be misusing opioids (e.g., taking larger doses than prescribed) because they are experiencing inadequate pain relief. The purpose of our study is to examine the subjective and reinforcing effects of morphine (MOR) and oxycodone (OXY) in participants meeting DSM-V criteria for opioid use disorders who either do or do not have chronic pain. All participants live in the hospital and are maintained on sublingual buprenorphine (BUP; 4 mg BID) throughout the 4-week study. Following a 1-week BUP stabilization period, participants receive MOR (360 mg), OXY (120 mg), or placebo (PBO) orally during separate laboratory sessions. The drugs are administered under single-blind conditions and labeled Drug A, B, or C. Participants then have the opportunity to choose to self-administer Drug A, B, or C during 5 separate choice sessions (1 choice opportunity per day). Preliminary analyses of the data were conducted for 7 participants with chronic pain (6M,1F; average pain score (0-10): 4.3 ± 1.2) and 9 without pain (8M,1F; average pain score: 1.3 ± 0.6). Ratings of "High" and "Good effects" significantly increased after administration of MOR in those with pain and after OXY in those without pain compared to PBO, but drug "Liking" only increased in those without pain and only after OXY. Furthermore, the percentage of drug choices did not differ in patients with pain ($37 \pm 11\%$, $37 \pm 15\%$, $26 \pm 13\%$ for PBO, MOR, and OXY, respectively), but choice of OXY ($58 \pm 12\%$) was greater than PBO ($24 \pm 9\%$) and MOR ($16 \pm 11\%$) in those without pain. The reasons cited for drug choice also differed between the groups. The majority of participants without pain chose OXY and MOR in order to "Get high," whereas those with pain chose MOR most often to "Enhance the study medication (BUP)" and OXY to "Get high." These data suggest that opioid-seeking behaviors and the reasons for them differ between opioid users with and without chronic pain. Supported by DA16759 (SDC).

No. 96 - Reversal by Tamoxifen of tolerance to morphine-induced respiratory depression.

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Opioid analgesics are the leading class of prescription drugs that cause unintentional overdose deaths through respiratory depression. In single neurones and in studies of antinociception, tolerance to morphine is reversed by both inhibitors of protein kinase C (PKC) and ethanol (Hull et al., 2013; Llorente et al., 2013). The investigation of PKC activity in respiration experiments in freely moving animals is limited by the lack of PKC inhibitors that cross the blood-brain barrier. Here we investigate the effects of Tamoxifen, a drug known to inhibit PKC (O'Brian et al., 1985), on tolerance to morphine-induced respiratory depression.

To induce tolerance mice were implanted with a 75 mg morphine pellet (sc) for 6 days. On day 6, respiration was recorded using individual plethysmograph chambers. To assess the level of tolerance mice were given a challenge dose of morphine (10 mg/kg ip). Changes in minute volume were recorded for 30 minutes post challenge and analysed as a percentage of pre-challenge baseline.

Morphine treated animals demonstrated significant tolerance to the respiratory depressant effects of morphine, when compared to naïve controls. On morphine challenge, respiration was $92.8 \pm 3.5\%$ and $60.7 \pm 4.3\%$ of pre-drug levels respectively, $p > 0.001$. Tamoxifen (0.6 mg/kg ip), injected 30 minutes before morphine, significantly reversed tolerance to respiratory depression in morphine treated animals (on morphine challenge respiration was $59.3 \pm 5.3\%$ of pre-drug levels $p > 0.001$). Tamoxifen alone had no effect on respiration in morphine pelleted or naïve animals.

We have demonstrated that tolerance to respiratory depression by morphine can be reversed by the PKC inhibitor, Tamoxifen. Previously we have reported that ethanol reverses tolerance to both the antinociceptive and respiratory depressant effects of morphine. This could suggest that ethanol exerts its effects by inhibition of PKC.

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Research funded by the BBSRC.

**No. 97 - SYNTHESIS OF
3,4,5-TRIMETHOXYCINNAMIC ACID
DERIVATIVES AND THEIR
ANTI-NARCOTIC EFFECT**

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The purpose of this study is to characterize the putative anti-narcotic effects of 3,4,5-trimethoxycinnamic acid (TMCA) constituent of *Polygala tenuifolia*. A simple synthesis and biological evaluation of 3,4,5-trimethoxyphenyl acrylamides 1a-f as novel antinarcotic agents is described. TMCA derivatives were administered 30 min prior to the injection of morphine in C57BL/6 mouse for 7 days. It was found that compounds 1d-f exhibited good inhibitory effects on the morphine withdrawal syndrome in mice. Interestingly, TMCA derivatives showed specific binding affinity on serotonin receptors, especially on the 5-HT_{1A} receptor. The cells were treated with 3,4,5-trimethoxyphenyl acrylamides 1a-f (10 μ M) for 30 min, pERK expression was elevated by tested compounds in cortical neuronal cells. The elevation of pERK expression by 3,4,5-trimethoxyphenyl acrylamides treatment were suppressed by WAY 100635 (1 μ M), 5-HT_{1A} receptor-specific antagonist. Compounds 1a and 1c demonstrated significant action to pERK expression and good response to WAY 100635 in cultured cortical neurons among those analogues. These results suggest simple synthesis of 3,4,5-trimethoxyphenyl acrylamides and key fragments are useful for the development of antinarcotic agents.

**No. 98 - Opioid modulation of inhibitory
synapses in striatal patch compartments**

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Opioid peptides and their receptors are prominent in the dorsal striatum, a brain region critical for the generation of purposeful movements and goal-directed behavior. Mu-opioid receptors (MORs) are highly enriched in striatal patch compartments, distinct limbic microcircuits embedded within the predominately sensorimotor dorsal striatum. Previous work has revealed patch specific suppression of inhibition by the MOR agonist DAMGO, however it is not known which pre- and post-synaptic neurons within patches are targeted by DAMGO. In addition, the action of opioid peptides, which can activate multiple opioid receptors, is not understood. Using a combination of transgenic mice, optogenetics, and photoactivatable peptides, we identified the inhibitory synapses suppressed by opioid peptides, investigated the underlying receptors, and defined the kinetics of modulation.

We validated a transgenic mouse line that allows simultaneous observation of patches and post-synaptic cell identity. We confirmed that enkephalin (enk) – the only endogenous ligand for MORs in striatum – suppresses local inhibition onto both direct and indirect pathway striatal projection neurons selectively in patches. To determine the synaptic targets of enk we conditionally expressed ChR2 in specific cell types and found that particular subsets of inhibitory synapses were suppressed by enk. Investigation of the underlying receptors revealed that MOR is not the only relevant opioid receptor. Finally, we probed the time course of opioid peptide modulation with caged enk, allowing instantaneous delivery of saturating peptide concentrations. Surprisingly, maximal synaptic suppression required the presence of peptide for several minutes. These results delineate how opioid release in striatum could shift the balance of limbic-associated striatal output by suppressing a subset of inhibitory synapses in patches.

This work was supported by the Charles A. King Trust (MRB), National Institute on Drug Abuse (K99DA034648 to MRB), Canadian Institute of Health Research (SQN), the National Institute of Mental Health (R01MH085418 & R01MH100568 to BLS), and Howard Hughes Medical Institute (BLS).

No. 99 - Involvement of ERM proteins in the Development of Morphine Analgesic Tolerance through P-glycoprotein at the Blood-Brain Barrier

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Introduction: Many of opioid analgesics such as morphine are recognized as substrate for P-glycoprotein (P-gp), a drug efflux transporter. Therefore, P-gp has considerable impact on pharmacokinetics and pharmacological effects of opioid analgesics. Cumulative evidence proposes that chronic treatment with morphine increases P-gp expression in the brain capillary endothelial cells (BCECs), an integral component of blood brain barrier (BBB), leading to the development of morphine analgesic tolerance. However, the detailed mechanism remains unexplained.

Ezrin/radixin/moesin (ERM) are scaffold proteins regulating the plasma membrane localization of some drug transporters such as P-gp in peripheral tissues, although a few reports focus its role in the central nervous system as well. In this study, we investigated the involvement of ERM in the development of morphine analgesic tolerance through altered P-gp expression in BCECs.

Methods: Male ddY mice aged 4 weeks were given morphine (50 mg/kg/day) subcutaneously for 5 days and its analgesic effect against thermal stimuli was evaluated with tail-flick test every day during experimental period. Each protein expression in the membrane fraction of BCECs was determined using western blotting. The protein-protein interactions between P-gp and each ERM proteins, or their immunohistological localization were analyzed by means of immunoprecipitation or immunofluorescence assay, respectively.

Results & Conclusion: Repeated treatment with morphine decreased its analgesic effect and increased P-gp protein expression in BCECs. Under the same condition, the levels of total ERM proteins were similar between saline and morphine group. The protein expression of moesin was significantly increased whereas that of ezrin was significantly decreased and no change was observed in that of radixin. Furthermore, immunoprecipitation or immunofluorescence studies revealed the interaction between moesin and P-gp along with co-localization. In conclusion, an increase in moesin accounts, at least in part, for the increased expression of P-gp in BCECs, leading to the development of morphine analgesic tolerance.

No. 100 - Interleukin 4 mediated by HSV vector attenuates morphine tolerance and physical withdrawal in rats

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Introduction:

Morphine is one of the most effective and potent analgesics currently available. Their utility in the management of pain is well recognized, however, extending the application of opioids to the management of chronic pain has met with considerable resistance. This resistance is due in part to concerns related to the development of tolerance, incorrect drug usage, and addiction. Recent evidence suggests chronic morphine may induce the release of proinflammatory cytokines which are involved in morphine tolerance.

Methods:

In the morphine tolerance model, chronic morphine was administered intraperitoneally for 7 days. Thermal latency was measured using hotplate test. HSV vector expressing IL-4 or control vector was injected into the hindpaws. Neurochemical factors in the spinal cord and/or DRG were examined using western blots and/or immunohistochemistry. In the physical withdrawal model, rats were treated with escalated morphine for 5 days followed by naloxone precipitation. HSV vector expressing IL-4 or control vector was injected into the periaqueductal gray (PAG) 1 week before morphine. Physical withdrawal symptoms were observed.

Results:

Subcutaneous inoculation of these vectors into hindpaws delayed the development of chronic morphine tolerance in rats. IL-4 overexpression mediated by the HSV vectors reduced TNF α and the phosphorylation of p38 in morphine tolerance in the DRG and the spinal dorsal horn in chronic morphine

Furthermore, we found that HSV-mediated IL-4 overexpression in the PAG reduced the morphine withdrawal.

Conclusion:

These results support the concept that proinflammatory factors may play an important role in the pathogenesis of morphine tolerance induced by chronic morphine. These studies indicate that the nonreplicative HSV-derived vectors expressing immunomodulatory molecules might prove a novel approach (gene therapy) to morphine tolerance/physical withdrawal.

The study was supported by the NIH DA20078 (S.H.), DA026734 (S.H.), and DA034749 (S.H.). The authors declare that there are no competing interests in the work.

No. 101 - Substance Dependence Treatment Data Set; According to the Comparative Study of Substance Dependence Treatment Information System

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Addiction is a phenomenon that causes structural changes in different systems of society. Studies show for planning of addiction prevention and treatment, it is necessary to create an information management system. In addiction treatment, without access to identification and causal information, distribution of personal trusteeships and treatment equipment won't be possible. In order to plan for addicts' treatment, information has to be collected by interviewing and examining patient and regarding substance using history and social activity. This information helps clinicians to specify treatment plan for detoxification and continuing supportive treatment. The aim of this study was to establish substance dependence treatment data sets according to a comparative study on America, Australia, and England Minimum Data Set. In this study, which was an applied one and from comparative-exploratory study, data sets of substance dependence treatment information systems in America, Australia, and England were compared. Sample countries were chosen based on related available information on the internet and also development of these countries in health information management. Information source included library sources, electronic sources and counseling with specialists inside and outside of country. Findings analysis was done in theory-comparative method and by using comparative table. In America admission data set and discharge data set, in Australia established-level items and episode-level items, and in England adults and young people, were titles of main groups in data set of substance dependence treatment. Presence of a standard data set in substance dependence treatment field is base of establishing a treatment information system and so base of this disease prevention and treatment. Thus, according to lack of this data set in Iran, we hope that data sets studied in this research can be a good guidance to establish a Minimum Data Set (MDS) for substance dependence treatment.

Key words: Data Set; Minimum Data Set; Information System; Substance Dependence; Substance Dependence Treatment

Conflict of interest: Nil

No. 102 - The Advantages and Barriers to Implement Substance Dependence Treatment Information System (SDTIS)

Sima Ajami, Zahra Mellat-Karkevandi

Isfahan University of Medical Sciences

Addiction is a phenomenon that causes structural changes in different systems of society. Studies show for planning of addiction prevention and treatment, it is necessary to create an information management system. In addiction treatment, without access to identification and causal information, distribution of personal trusteeships and treatment equipment won't be possible. The aim of information system is informing others, thus information system gives proper information to users based on their information need. The information system is a set of organized components which is responsible for collecting, storing and distributing of information. Substance dependence information systems refer to systems which collect, analyse and report data related to substance dependence information. The aim of this study was to identify advantages and barriers to implement Substance Dependence Treatment Information System (SDTIS). We employed the following keywords and their combinations in different areas of articles. In this study, 22 of collected articles and reports were selected based on their relevancy. The most important ones are recording sufficient, complete and accurate information and easy and timely access to them; supporting clinical decision making; supporting audits of clinical practice; helping prevention, control and treatment planning; monitoring and enhancing the quality of care; evaluating risk factors; being effectively used for research purposes; helping creation a network among treatment centers and administrative centers and allowing longitudinal tracking of client health-related outcomes by having linkages with other healthcare information systems. Also, it may be necessary to modify the assessment to transfer information when patients move between levels of care. In general, it is a useful and credible tool in measuring improvements in healthcare and one of vital prerequisites for creating and using an Electronic Health Record (EHR) in every country. Developing formal protocols to examine data quality and consistency shortly after admission can help us to solve the last problem. **Key words:** Registry, Management Information System, Substance Dependence

No. 103 - Dissecting Nociceptin Receptor Modulation of Reward

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Nociceptin/Orphanin FQ Opioid Receptor (NOPR) and its endogenous ligand, nociceptin (NOFQ) have been shown to affect the rewarding properties of drugs of abuse. Both NOPR and N/OFQ are highly expressed in the Ventral Tegmental Area (VTA), widely regarded as a critical anatomical region for both drug and natural reward. The circuitry of the NOPR/NOFQ receptor system in the context of reward, however, remains unknown. The aim of this study was to dissect the role of NOPR and NOFQ in reward modulation. First, we pharmacologically targeted NOPR receptors with potent and selective NOPR agonist, SCH 221510. Consistent with prior studies on NOPR agonism, we demonstrated that SCH221510 significantly attenuates drug-seeking behavior in a cocaine conditioned place preference (CPP) model. Then, to determine whether the effect of SCH221510 was specific to NOPR expressed in dopaminergic VTA (DA-VTA) neurons, we targeted a cre-dependent NOPR virus into the VTA of NOPR knockout crossed with Tyrosine hydroxylase-Cre mice. Our preliminary data suggests activation of NOPR specifically within the dopaminergic neurons of the VTA is sufficient to attenuate drug seeking behavior in a CPP model. To determine the source of endogenous nociceptin to the VTA that modulates reward behavior, we generated a novel Cre mouse line, Noci-IRES-Cre, in which the promoter for the NOFQ gene drives Cre recombinase expression. We then created a reporter mouse by crossing a Noci-Cre mouse with a Cre-dependent tdTomato (Ai9) reporter mouse. In this reporter mouse, tdTomato is expressed in NOFQ positive neurons and allows us to examine both cell bodies and projection sites. We show NOFQ is expressed in the expected cell bodies of brain loci involved in reward and affective behavior, including the Rostromedial Tegmental nucleus (RMTg) and the VTA, which inhibit DA-VTA neurons, and also the Central Amygdala (CeA). We optogenetically targeted these nociceptin containing cells and evaluated their effects on reward and aversion behavior. These data provide the groundwork for NOPR containing neural circuits that regulate reward and aversive-like behavior.

No. 104 - Analysis of the second intracellular loop of DOP: A role in the membrane targeting of DOP

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Université de Sherbrooke

We have previously shown that activation of the mu opioid receptor and inflammation increase the plasma membrane density of delta opioid receptors (DOPs) in neurons. Interestingly, this effect is accompanied by an increased analgesic efficacy of DOP agonists. In a recent study, we found that inhibiting cyclin-dependent kinase 5 (cdk5) impairs the regulation of DOP triggered by morphine and inflammation. The regulation of DOP by cdk5 is likely mediated by the phosphorylation of T161 localized within the second intracellular loop (ICL2) of DOP. The exact mechanisms involved in the cdk5-mediated DOP regulation are however unknown.

We have identified a putative COPB1 binding site (KxK) near to the cdk5 consensus phosphorylation motif in the ICL2 of DOP. Since COPB1 is involved in the retrograde transport of proteins from the Golgi to the endoplasmic reticulum (ER), we hypothesized that the cdk5-mediated phosphorylation of T161 attenuates the ability of COPB1 to bind the 164KAK putative ER retention motif, thus promoting the export of DOP to the cell surface. To study the role of this putative retention motif in the regulation of DOP, we generated a mutation in the ICL2. Using ELISA, we found that the K166A mutant has a higher level of cell surface expression compared to the wildtype Flag-DOP. The K166A mutation also induced a mobility shift of Flag-DOP on SDS-PAGE compatible with a variation in the glycosylation levels. Finally, our preliminary results suggest that COPB1 can be co-immunoprecipitated with wildtype Flag-DOP. A reduction in the association of COPB1 with the K166A mutant remains to be determined. Our results suggest that the KxK motif localized in the ICL2 of DOP is part of the cellular mechanisms involved in the retention of DOP. Mutation of this motif possibly decreases the ability of COPB1 to associate and retain DOP in the ER. Because this motif is adjacent to the putative cdk5 phosphorylation site, we now propose that cdk5 regulates DOP by promoting the phosphorylation of T161, which interferes with the association of COPB1 with DOP.

Financial support: Supported by CIHR grant MOP273137
No conflict of interest to declare

No. 105 - Platelet-derived growth factor circuitry underlying opioid tolerance

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For centuries, opioids have been the gold standard for treating severe pain. However, with repeated dosing, the analgesic effect of opioids decreases. The mechanisms underlying opioid tolerance remain poorly understood. We recently made the groundbreaking discoveries that inhibition of the platelet-derived growth factor receptor beta (PDGFR- β) signaling prevented morphine tolerance from occurring and completely reversed established tolerance. Based on these findings, we hypothesize that 1) PDGFR- β signaling is a general mediator of opioid tolerance; 2) PDGFR- β expressing cells in the pain processing pathways form neural circuits underlying tolerance; and 3) These circuits are modified by opioid exposure. Using immunohistofluorescence and confocal microscopic imaging, we have defined the specific types of cells expressing the PDGFR- β and the platelet-derived growth factor-BB (PDGF-BB) in the dorsal root ganglia (DRG) and the substantia gelatinosa (SG) under basal conditions. These proteins were characterized in various cell subtypes of the SG and the DRG. Experiments are underway investigating the co-localization of PDGFR- β and the MOR under basal conditions. We are also determining whether the distribution and co-localization of these markers is altered by chronic administration of various opioids. These studies will provide unique insights into the neural substrates underlying opioid tolerance.

The authors declare no conflict of interest.
This research is funded by a CPRIT grant to H.B. Gutstein.

No. 106 - Quantitative Analysis of Functional Selectivity at the Nociceptin Opioid Receptor

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The Nociceptin/Orphanin FQ Opioid Receptor (NOPR) is the most recently discovered and least characterized G-protein coupled receptor in the opioid receptor family. The actions of NOPR and its endogenous ligand, nociceptin, have been thought to play a role in neuromodulation in the contexts of pain, opioid tolerance and addiction, as well as the modulation of stress and anxiety behaviors. It is thought that the NOP receptor, like other opioid receptors and GPCRs, may bind functionally selective ligands that can bias signal transduction, resulting in diverse behavioral effects. The potential for functional selectivity at the NOPR has yet to be explored. To quantitatively study the signal transduction profiles of NOPR ligands in-depth, we used real-time live-cell cAMP and Bioluminescence Resonance Energy Transfer (BRET) assays to study the ligand-induced $G\alpha$ -protein activation and arrestin 2/3 recruitment at NOPR. Additionally, we employed high-resolution confocal microscopy to identify differences ligand-induced receptor internalization. We screened multiple NOPR-selective ligands, as well as novel, custom-designed small molecules aimed at elucidating structural significance in signal transduction. We have found that the NOPR, like other opioid receptors exhibits different signaling profiles, depending on the ligand type. Furthermore, we show that minute changes in ligand structure can illicit opposing signaling profiles ranging from agonism to inverse agonism. Additionally, we propose plausible ligand-receptor docking configurations that may help to elucidate the relationship between conformation and signal transduction at the NOP receptor. Finally, we show that ligands are capable of eliciting different degrees of receptor internalization. Together, these data provide new and quantitative insight into the structural relationship between ligands and signal transduction at the NOP receptor, which will ultimately facilitate additional studies examining NOPR ligand bias in behavior. Supported by: NIH R00 DA025182 and T32 DA007261.

No. 107 - Utilizing the Ugi multi-component reaction to synthesize opioids

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In multicomponent reactions (MCRs), two or more starting materials react simultaneously to yield a single product. High structural diversity can be achieved in just one synthetic step, reaction yields are generally high and the purification of products is simple. Thus, MCRs are suitable for the quick and convenient synthesis of drug-like libraries.

Our approach utilizes the isocyanide-based four-component Ugi reaction to obtain synthetic opioid scaffolds in a one-pot, single step fashion. Reaction between N-phenethylpiperidone (ketone), aniline (amine), propionic acid (carboxylic acid) and isocyanides yielded a small library of bis-amide analogs of carfentanyl. Our lead compound showed high affinity for mu (MOR) and delta (DOR) opioid receptors with poor affinity for kappa (KOR) receptors in radioligand binding assays. It had moderate analgesic potency in vivo but showed a reasonable side-effect profile over morphine.

This MCR approach has the promise to deliver more potent and efficacious small-molecule opioids with an advantageous side-effect profile and potential clinical applicability.

This work was supported by research grant from the National Institute on Drug Abuse (DA034106) to SM and (DA06241) to GWP.

No. 108 - THE CONTRIBUTION OF Gi/o PROTEIN TO OPIOID ANTINOCICEPTION IN AN OXALIPLATIN-INDUCED NEUROPATHY RAT MODEL

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Purpose: Oxaliplatin is a chemotherapeutic agent that induces chronic refractory neuropathy. To determine whether opioids are effective for this type of neuropathy, we investigated the efficacies of morphine, oxycodone, and fentanyl, and the mechanisms underlying opioid antinociception, in oxaliplatin-induced neuropathy in rats. **Method:** We assessed the antinociceptive effects of opioids in rats with oxaliplatin-induced neuropathy using the von Frey test. To investigate the μ -opioid receptor function of each opioid in the oxaliplatin model, a [35S]-GTP γ S binding assay was performed.

Result: Rats exhibited significant mechanical allodynia following 2 weeks of chronic oxaliplatin administration. Within the range of doses that did not induce sedation, morphine (3 mg/kg, subcutaneously [s.c.]) and oxycodone (0.3–0.56 mg/kg, s.c.) completely reversed oxaliplatin-induced mechanical allodynia, whereas fentanyl (0.0056–0.03 mg/kg, s.c.) showed partial antinociception. In the oxaliplatin model, the antinociceptive effects of the optimal doses of morphine and oxycodone were completely inhibited by pertussis toxin (PTX; 0.5 μ g/rat, i.c.v.), a Gi/o protein inhibitor, while the partial effect of fentanyl was not affected by PTX. In the [35S]-GTP γ S binding assay, activation of μ -opioid receptor by fentanyl, but not by morphine or oxycodone, in the mediodorsal thalamus of oxaliplatin-treated rats was significantly reduced.

Conclusion: Morphine and oxycodone showed robust antinociceptive effects via PTX-sensitive Gi/o protein activation in oxaliplatin-induced neuropathy. The partial antinociception of fentanyl in the oxaliplatin model might result in part from the loss of that protein activation. The degree of Gi/o protein activation might be related to the potency of antinociceptive effect of opioids on oxaliplatin-induced neuropathy.

Conflict of interest

None of the authors have any conflicts of interest to disclose relating to this submission.

No. 109 - Association of the OPRK1 intronic SNP rs6473797 with prolactin response to dynorphin administration in normal male volunteers

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Pituitary prolactin (PRL) secretion is under inhibitory hypothalamic tuberoinfundibular dopaminergic control in humans. Extra pituitary brain prolactin has anxiolytic and anti-stress properties through activation of PRL receptors. The kappa opioid receptor (OPRK1) and its ligands dynorphin peptides (Dyn) modulate the activity of the mesolimbic, nigrostriatal and hypothalamic dopaminergic neurons. This study evaluated the effect of an OPRK1 variant, which we have previously shown to be associated with heroin addiction, on prolactin response to dynorphin administration in healthy males. **Method.** Twenty four healthy male volunteers participated in the study conducted in the Rockefeller University Hospital. On each testing day, subjects received Dyn1-13 i.v. at doses of 0, 120 and 500 $\mu\text{g}/\text{kg}$. Serum prolactin levels were determined at sequential time points from 0 to 120 min by immunoradiometric assay. The change in prolactin response of each dynorphin dose from the saline (delta area under the curve, AUC) was calculated for each subject. OPRK1 intronic SNP rs6473797 was genotyped using Illumina GoldenGate array. A two-way ANOVA, Genotype by Condition (Low Dose, High Dose), with repeated measures on the second factor, was used to examine delta AUCs. All subjects gave consent for genetic studies. **Results.** There was no significant main effect of Genotype, $F(2,21)=2.41$, $P=0.11$. There was a significant main effect of Condition, $F(1,21)=36.50$, $p>0.00001$, and a significant Genotype X Condition interaction, $F(2,21)=3.94$, $p>0.05$. While there was no difference among genotypes at the low dose, individuals with the GG genotype had significantly lower response to the higher dynorphin dose than individuals with AA and AG ($p>0.05$). **Conclusion.** The results suggest that the OPRK1 intron 2 variant alters prolactin levels in response to Dyn1-13 administration. This alteration may be due to lower expression of the receptor and therefore less inhibition of dopaminergic tone in the hypothalamus. **Support:** NIH NIDA-P60-05130 (MJK), NIH-NCRR CTSA, UL1-TR000043, Dr. Miriam and G. Sheldon Adelson Medical Research Foundation. The authors declare no conflict of interest.

No. 110 - Subcutaneous injection of DALDA inhibits ongoing neuropathic pain and persistent inflammatory pain in rats

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Introduction & Methodology: Patients with tissue or nerve injury commonly suffer from ongoing and/or paroxysmal spontaneous pain. Although ongoing pain is the most common and bothersome clinical complaint, most preclinical animal studies rely on reflex response to evoked sensory hypersensitivities which does not always correlate with analgesic efficacy in humans. Here, we used the conditioned place preference (CPP) to examine if subcutaneous injection of dermorphin [D-Arg2,Lys4](1-4) amide (DALDA), a mu-opioid receptor (MOR) agonist, can reduce ongoing pain associated with nerve injury and tissue inflammation

Results: Compared to the preconditioning, rats with L5 spinal nerve ligation (SNL) surgery spent significantly more time in the DALDA (5, 10 mg/kg s.c., n=8-12/dose) paired chamber during post-conditioning. Importantly, neither dose of DALDA (5 and 10 mg/kg, s.c.) induced CPP in naive rats, suggesting that DALDA-induced CPP in SNL rats is likely due to the reward from pain relief, but not due to drug penetration into central nervous system (CNS) which will directly activate CNS reward circuitry, an action known to CNS-penetrating mu-opioids (e.g., morphine). Intraperitoneal (i.p.) pretreatment with methylnaltrexone (5 mg/kg), a peripherally restricted MOR-preferring antagonist, at 10 min before DALDA injection blocked DALDA-induced CPP in SNL rats, further suggesting a peripheral mechanism for DALDA-induced relief of ongoing pain. Gabapentin (60 mg/kg, i.p.), which we used as a positive control, also induced CPP in SNL rats, but not in naive rats. In a rat model of persistent inflammatory pain induced by intraplantar injection of formalin (50 μL , 1%), DALDA pretreatment (10 mg/kg s.c.) significantly inhibited spontaneous pain behavior at 15-60 min (phase II) after formalin injection, as compared to saline pretreatment.

Conclusions: Current findings suggest that subcutaneous injection of DALDA may alleviate ongoing pain associated with neuropathic and inflammatory insults in rats, and the drug action may be preferentially by targeting MORs in the peripheral nervous system. Further studies are required to examine the underlying neurophysiological mechanisms.

Grant/Other Support: NIH Grant NS70814, NS26363.

No. 111 - Antagonistic effect of angiotensin (1-7) on angiotensin II-induced nociceptive behavior in mice

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We have recently demonstrated that intrathecal (i.t.) administration of angiotensin II (Ang II) induces nociceptive behavior in mice accompanied by a phosphorylation of p38 MAPK mediated through Ang II type 1 (AT1) receptors (Nemoto et al., *Mol. Pain* 2013;9:38). The N-terminal fragment of Ang II, Ang (1-7), plays a pivotal role in counterbalancing many of the well-established actions induced by Ang II. However, the role of Ang (1-7) in spinal nociceptive transmission remains unclear. Therefore, we examined whether i.t. administration of Ang (1-7) can inhibit the Ang II-induced nociceptive behavior in mice. The nociceptive behavior induced by Ang II was dose-dependently inhibited by the co-administration of Ang (1-7) (0.03-0.3 pmol), whereas Ang (1-7) (0.3-30 pmol) alone did not elicit nociceptive behavior. The inhibitory effect of Ang (1-7) was reversed by the co-administration of A779, a Mas receptor antagonist, at a dose of 0.1 pmol, suggesting that the inhibitory effect of Ang (1-7) was mediated through Mas receptors. Western blot analysis showed that the increase in spinal p38 MAPK phosphorylation following the i.t. administration of Ang II was also inhibited by Ang (1-7), and the Ang (1-7) induced-inhibition was prevented by A779. Furthermore, we found that spinal Mas receptors are co-localized with AT1 receptors in the superficial dorsal horn. Our data show that the i.t. administration of Ang (1-7) attenuates an Ang II-induced nociceptive behavior and is accompanied by the inhibition of p38 MAPK phosphorylation mediated through Mas receptors. This observation indicates that Ang (1-7) may modulate nociceptive transmission in the spinal cord.

No. 112 - Involvement of kappa opioid receptors in chronic escalation alcohol drinking in mice

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Alcohol consumption affects multiple neurobiological systems including the dynorphin/kappa opioid receptor (KOP-r) system. Specifically, activation of the KOP-r system has been implicated in the negative reinforcement aspects of alcohol addiction. Though KOP-r antagonists are found to reduce alcohol consumption in alcohol-dependent rats, it is unclear whether KOP-r activation is involved in high alcohol drinking in mice. Therefore, the present study was undertaken to determine if pharmacological blockade of KOP-r could alter alcohol drinking (consumption & preference at 7.5, 15 and 30% concentrations) in male adult C57BL/6J mice, using two protocols: 1) 5-day drinking-in-the-dark (DID) with 1 recording/day (4-h); and 2) 3-week chronic escalation drinking (CED) (2-bottle choice & each other day) with 3 recordings/day (4, 8 & 24-h). Selective KOP-r antagonist JDtic (3, 10 or 20 mg/kg, ip) administration was given 1 day before the test after mice established drinking behavior. Saccharin DID drinking (0.2%) with JDtic were tested as control. The alcohol (2g/kg) induced conditioned place preference (CPP) was tested with JDtic at 20mg/kg. Mice exposed to the DID for 5 days at each of 3 alcohol concentrations established stable drinking behavior, with 4-6 g/kg/4-h alcohol consumption. JDtic had no effect on alcohol or saccharin DID drinking. With the CED protocol, mice exposed to 15% alcohol vs. water gradually developed high alcohol consumption from 12g/kg/day at the start to 20g/kg/day after 3 weeks, with increased preference ratio [PR] from 0.4-0.6 to 0.7-0.8. In contrast to the DID, after 3-week CED drinking, JDtic at 10 & 20mg/kg (but not 3mg/kg) decreased alcohol drinking at all 3 time points. The total daily consumption was reduced to 15g/kg after 20mg/kg JDtic, with no effects on the PR. The alcohol CPP expression was unaltered by JDtic at 20mg/kg. The results show that pharmacological blockade of KOP-r reduced the CED, but not the DID binge, drinking or CPP rewarding expression, providing new evidence for the involvement of the KOP-r system in alcohol addiction. Support: Dr. Miriam & Sheldon G. Adelson Med Res Foundation, DA09045. No conflict of interest.

No. 113 - Tetrahydroisoquinoline-based orexin 1 receptor antagonists: structure-activity relationships at the 1-benzyl position

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Blockade of the OX1 receptor has been suggested to be a promising strategy for the treatment of drug addiction. Unfortunately, currently available orexin antagonists are either mixed OX1/OX2 antagonists designed to treat sleep disorders or OX1 selective antagonists with undesirable pharmacological properties. To address the pressing need for potent and selective OX1 antagonist, we have developed orexin antagonists based on a tetrahydroisoquinoline scaffold, the core structure of both the dual OX1/OX2 receptor antagonist ACT-078573 and the OX2 selective antagonist TCS-OX2-29. Recently, we reported the synthesis and in vitro and in vivo evaluation of a series of substituted tetrahydroisoquinolines, one of which showed excellent in vitro OX1 potency and selectivity and attenuated cocaine-induced conditioned place preference in rats (*J. Med Chem.*, 2013, p6901). We herein report our efforts in further exploring the structure-activity relationships focusing on the 1-benzyl position of the tetrahydroisoquinoline. Structural features important for OX1 potency and selectivity have been identified including the requirement for aromatic functionality at the 1-position. Combined structural modifications from several positions have been conducted which resulted in compounds having low nanomolar potency at the OX1 receptor and excellent selectivity over the OX2 receptor. These findings will help expedite the development of potent and selective OX1 antagonists as potential medications for the treatment of OX1-mediated disorders such as drug addiction. This work was supported by research grants from the National Institute on Drug Abuse (DA032837 and DA026582).

No. 114 - Deciphering agonist- and species-dependent KOPR phosphorylation using LC-MS/MS and phosphospecific KOPR antibodies

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We found that etorphine caused lower phosphorylation and internalization of the KOPR than U50,488H. Here we examined the sites of phosphorylation by the two agonists. The human and mouse KOPRs were tagged at N-terminus with FLAG and at C-terminus with 6xHis (FhK6H and FmK6H, respectively) and stably expressed in Neuro2A mouse neuroblastoma cells. Cells were treated with vehicle or 10 μ M U50,488H for 30 min. FhK6H and FmK6H were affinity-purified with Ni-NTA agarose followed by anti-FLAG M2 antibody-agarose, resolved with SDS-PAGE and stained. The FhK6H or FmK6H protein, a diffused band of 52kDa, was excised and in-gel micro-digestion was performed with Glu-C. LC-MS/MS analysis revealed that U50,488H promoted hKOPR phosphorylation at S356, T357, S358 and T363 with T363 having the most hits. U50,488H enhanced mKOPR phosphorylation at S356, T357, T363 and S369 with S369 having the most hits. Peptides containing 1, 2 and 3 phosphates were detected. Rabbit antiserum was generated against KLH conjugates of four phosphopeptides containing pS356/pT357, pS358, pT363, and pS369. Antiserum was tested for phosphopeptide specificity by dot-blot and those with high specificity were purified. Cells were treated with saline, U50,488H (10 μ M) or etorphine (1 μ M) for 30 min, solubilized and receptor was partially purified with a Ni-NTA agarose. For FhK6H and FmK6H, purified phosphopeptide antibodies all detected diffuse bands of 52kDa. Staining intensity of hKOPR with anti-pS358 and anti-pS363 was in the order of U50 < etorphine < control, whereas that with anti-pS356/pT357 was U50 < etorphine < control. The U50,488H effect was blocked by naloxone. Staining intensity of mKOPR with anti-pS356/pT357 was U50 < etorphine < control; with anti-T363 was U50 << etorphine < control; with anti-S369 was U50 < etorphine << control. These results indicate that KOPR phosphorylation is agonist- and species-dependent. We will further investigate the dose-response relationship and time courses of the two agonists in phosphorylating different residues to disclose possible differences in site and hierarchy of phosphorylation in both KOPRs.

This work was supported by NIH grant DA17302. The authors have no conflicts of interest.

No. 115 - A heroin addiction severity-associated intronic single nucleotide polymorphism modulates alternative pre-mRNA splicing of mu opioid receptor gene, OPRM1, as a splicing modifier via hnRNPH interactions

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Single nucleotide polymorphisms (SNP) in the OPRM1 gene have been implicated to associate with vulnerability of dependence on opioids such as heroin. The functional significance of several SNPs has been demonstrated in influencing opioid binding, G protein coupling, and receptor expression, as well as promoter activity. Yet, little has been known for the role of SNPs on OPRM1 alternative splicing. The present studies identify association of an intronic SNP (rs9479757) with the heroin addiction severity among Chinese male heroin abusers, and describe its roles as an OPRM1 alternative splicing modifier. Although no significant difference between heroin abuser (n=332) and normal control (n=190) groups, this SNP were significantly associated with the heroin addiction severity (Mild. Moderate and Severe groups) defined by criteria including route, duration and daily dose of heroin intake among 332 heroin abusers. Haplotype-based analysis together with additional 22 SNPs further supported strong association of rs9479757 with the heroin addiction severity, suggesting that this SNP is a candidate marker for predicting degree of heroin addiction in Chinese male heroin abusers. In vitro EMSA and UV-crosslinking studies indicated that hnRNPH is the major binding partner for the G-containing SNP site, whereas the A to G transition significantly lowers affinity toward hnRNPH. Minigene studies indicate that the A to G transition facilitates exon 2 skipping, leading to altered expression of the OPRM1 splice variants, which was consistent with the data from human postmortem brains, suggesting that the G-containing SNP site functions as an intronic splicing enhancer. The roles of this SNP in modulating OPRM1 alternative splicing through hnRNPH was further demonstrated by using siRNA, antisense morpholino oligos, and RNA affinity purification-coupled LC-MS/MS approaches in human neuroblastoma Be(2) cells. Our studies suggest a functional link between a SNP-containing splicing modifier and the heroin addiction severity through the underlying mechanisms involving hnRNPH.

No. 116 - EFFECT OF THE NOCICEPTIN/ORPHANIN FQ PEPTIDE RECEPTOR ANTAGONIST JTC-801 ON CYTOKINE EXPRESSION FOLLOWING EXPOSURE TO THE SINGLE PROLONGED STRESS MODEL OF PTSD

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Post-traumatic stress disorder (PTSD) is frequently associated with increased inflammatory cell activity and higher levels of circulating cytokines in plasma. Cytokine profiles in animal models of PTSD have not been reported to date. We previously reported that single-prolonged stress (SPS), an established animal model for PTSD, induces long-lasting mechanical and thermal allodynia. Further, JTC-801, an N/OFFQ peptide (NOP) receptor antagonist, reversed SPS-induced pain- and anxiety-like behavior, as well as elevated N/OFFQ levels and NOP receptor expression in brain. To test our hypothesis that neuroinflammation is a pivotal component of the mechanism underlying pain and/or PTSD, we examined the effect of JTC-801 on cytokine protein and gene expression in brain after SPS. Male Sprague-Dawley rats were subjected to sham or SPS, followed by once daily injection of JTC-801 (6 mg/kg, i.p.) or vehicle from day 7 until 21 days after initiation of SPS. Cytokine mRNA levels were measured using real-time quantitative PCR. JTC-801 alleviated SPS-induced mechanical allodynia and reversed thermal threshold to baseline levels. JTC-801 reversed increased IL-2 and IL-6 mRNA in amygdala after SPS. In hippocampus, there was a significant interaction effect between SPS and JTC-801 on IL-6 mRNA levels. SPS increased IL-1 α and IL-2 mRNA in periaqueductal grey (PAG), and TNF- α mRNA in hippocampus, but this was not reversed by JTC-801. When cytokine proteins were examined using Rat Cytokine 10-Plex Panel, there was a significant interaction effect between SPS and JTC-801 on IL-6 level in spinal cord. Higher IL-4, IL-10, IL-12, GM-CSF and IFN- γ levels were noted in hippocampus after SPS, which were not reversed by JTC-801. These results suggest that inflammatory cytokine expression in brain was differentially affected by SPS, and only partially mediated through the NOP receptor; expression of neuroinflammatory mediators may contribute to the maintenance of PTSD symptoms. Animal protocol was approved by the OUHSC IACUC committee and U.S. Army Research and Materiel Command ACURO office. This study was supported by the Department of the Army DMRDP W81XWH-11-2-0077. No conflict exists.

No. 117 - Rescue of IBNtxA analgesia, but not morphine, in a double E1/E11 MOR-1 knockout mouse model using lentiviral-mediated gene delivery of the 6 transmembrane (6TM) domain variant mMOR-1G

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Extensive alternative pre-mRNA splicing of the mu opioid receptor (OPRM1) gene creates multiple variants or isoforms that are conserved from rodent to human. Expression of these variants is controlled by two distinct promoters associated with exon 1 (E1) and exon 11 (E11). Almost all the full-length (7TM) splice variants and the single TM variants are under the control of the E1 promoter whereas the truncated 6TM variants are transcribed through the E11 promoter. The E11 knockout (KO) mouse has provided important functional information on the 6TM variants. Loss of the E11 variants eliminates IBNtxA (3-iodobenzoyl-6 β -naltrexamide) analgesia while morphine analgesia was intact. IBNtxA is a new potent opiate analgesic lacking the traditional side effects associated with classical opiates, including respiratory depression, constipation, physical dependence and reinforcing behavior. In contrast, morphine analgesia is lost in an E1 knockout mouse while IBNtxA analgesia remained intact. Recently, we developed a new KO mouse model in which both E1-associated and E11-associated variants are disrupted (E1/11 double KO), leading the loss of all MOR-1 splice variants. No mu opioids show analgesic activity in this E1/11 double KO mouse, including morphine, M6G and IBNtxA. In the current study we used a lentiviral-mediated gene delivery of the 6TM variant mMOR-1G to determine if we could rescue (i.e. restore) IBNtxA analgesia in the E1/11 double KO mouse. Intrathecal (i.t.) or intracerebroventricular (i.c.v.) administration of lentiviral particles expressing mMOR-1G in E1/11 double KO mice restored IBNtxA analgesia, but not morphine analgesia. These studies confirm a role for E11 variants in IBNtxA analgesia and illustrates the utility of Lentiviral-mediated gene delivery in studying in vivo the function of the OPRM1 splice variants and as a potential therapeutic strategy to pain management. (Supported by DA13997 & DA029244 (Y.-X.P) and DA02615 & DA07242 (G.W.P) from the National Institute on Drug Abuse; and a core grant CA08748 from the National Cancer Institute to MSKCC.

No. 118 - RGS4 and RGS2 proteins, new modulators of the κ -opioid receptor signaling

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Previous studies have shown that RGS4 associates with the C-termini of μ - and δ -opioid receptors in living cells and plays a key role in Gi/Go protein coupling selectivity and signaling of these receptors (Georgoussi et al., 2006; Leontiadis et al., 2009). To deduce whether similar partners are involved in κ -opioid receptor (κ -OR) signaling and define the ability of members of B/R4-RGS family to interact with this receptor we performed pulldown experiments using GST fusion peptides encompassing specific regions of the κ -OR. These experiments indicated that RGS2 and RGS4 interact within a specific amino acid stretch of the carboxyl terminus of κ -OR. Co-immunoprecipitation studies indicated that RGS2 and RGS4 associate with κ -OR constitutively and upon receptor activation and confer selectivity for coupling with a specific subset of G proteins. Functional assays have shown that both members of B/R4-RGS family attenuate κ -OR mediated inhibition of adenylyl cyclase and ERK1,2 phosphorylation displaying a differential amplitude in their functional effect. Collectively, our results demonstrate that RGS2 and RGS4 are new interacting partners and negative modulators of κ -OR signaling. This work was supported by the EU grant “Normolife” (LSHC-037733) and the European COST Action CM1207 (GLISTEN).

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