INRC

Program

INTERNATIONAL NARCOTICS RESEARCH CONFERENCE

June 12-16, 2018
San Diego, California USA

Contents

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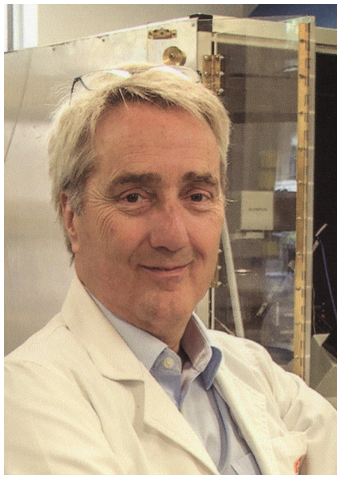
INRC 2018 AWARDEES

Founders' Lecture – Professor Macdonald Christie



Dr. Macdonald (Mac) Christie is Professor of Pharmacology, University of Sydney School of Medical Sciences. Mac is a neuropharmacologist, an internationally renowned electro-physiologist and expert in ion channel and synaptic physiology and pharmacology and the leading basic opioid pharmacologist in Australia. He leads a research team that studies cellular and molecular mechanisms in opioid receptor signaling in pain pathways, the biological basis of adaptations that produce chronic pain and drug dependence and is Chief Investigator on a NHMRC Program grant involving preclinical development of novel pain therapeutics. Professor Christie is internationally renowned for his studies on ion channel function and neural network plasticity in nervous system disorders such as chronic pain and drug dependence. His research is fundamentally important given the increasing use of opioids to manage moderate to severe chronic pain is complicated by adverse effects such as tolerance, opioid dependence and in some cases, addiction. Mac is a past Director (Basic Research) and Professor at the Pain Management Research Institute, Royal North Shore Hospital, and Director of Pain and Addiction Research at the Brain and Mind Research Institute, University of Sydney. He is a member of the Board of Directors of the Woolcock Institute of Medical Research and of the Sydney Cancer Institute and member of the NSW Tissue Resource Centre Management Committee.

Professor Christie's research has heralded numerous 'firsts'. He contributed to the first cloning of the D2 dopamine receptor (*Nature*) and achieved the first expression of a mammalian voltage-gated potassium channel (*Science*), and first functional demonstration of its heteropolymerisation (*Neuron*). His later research uncovered the mechanisms of plasticity of G-protein receptor coupling in neurons that are transforming thinking regarding opioid tolerance and ion channel plasticity in chronic pain states. His group discovered that μ and δ opioid receptors in nerve cells share common G-Protein receptor coupling mechanisms and provided the first evidence of direct coupling of μ-receptors to GIRK channels and of coupling of the opioid-related receptor ORL1, to GIRK channels in brain as well as in cannabinoid signaling. He discovered a novel signalling mechanism for G-Protein Coupled Receptors (GPCR) in synapses in brain (*Nature*). This provided a basis for seminal findings on plasticity of signaling in opioid withdrawal published in *Neuron* (2005) and recently in *Nature Neuroscience* (2011).

Mac received a BSc (Hons, First Class) from the School of Biological Sciences, Flinders University of South Australia in 1978 and Ph.D. From the Department of Pharmacology at The University of Sydney in 1983. Mac had postdoctoral fellowships at the University of Melbourne and MIT and was a Senior Research Associate at the Vollum Institute, Oregon Health Sciences University, where he produced his seminal work on opiate receptors with Dr. Alan North. He returned to the University of Sydney in 1990 and has been there ever since.

Young Investigator Award – Dr. Tuan Trang

Dr. Tuan Trang is Associate Professor of Physiology and Pharmacology at the University of Calgary. Tuan's research focuses on discovering the fundamental molecules and processes involved in chronic pain and enhancing the utility of opioid drugs in treating pain conditions. A strong focus of his research is the role of microglia, the immune cells in the central nervous system, and the complex interplay between microglia and neurons in chronic pain and opioid analgesia. Understanding the key molecules and processes that underlie chronic pain and that contribute to the unwanted side effects of opioid use is a major step towards improving current therapies and identifying novel targets for creating entirely new, more effective strategies for treating pain.

Dr. Trang graduated with first-class academic distinction from his undergraduate (1999) and graduate studies (2005) at Queen's University, one of the top Canadian academic institutions. He then completed a postdoctoral fellowship in the laboratory of Dr. Michael Salter, a world leading pain researcher at Toronto's SickKids Hospital. Tuan's productivity as a trainee was exemplary. From his postdoc alone he published 8 peer-reviewed articles, including first authored articles in the *Journal of Neuroscience*, *Nature Neuroscience*, and *Nature Medicine*. This adds to 8 papers published as a graduate student, 7 of which as first author in excellent journals such as *Pain* and *British Journal of Pharmacology*. He has published a total of 29 peer-reviewed articles and has a rising h-index of 18. Tuan's high level of achievement is evidence by prestigious scholarships and awards at each and every stage of his training.

Since arriving in Calgary, Tuan has been remarkably successful in obtaining competitive grants, including grants from NSERC and CIHR. Not only did he land a CIHR grant on his first attempt, but also his grant was the ***top ranked*** grant on the Pharmacology and Toxicology committee. Adding further to his success, Tuan has received numerous prestigious awards, including a CIHR New Investigator Award, and a Rita Allen Foundation Award in Pain, which is given to outstanding new investigators in Canada and the United States for distinguished achievement in pain research.

Tuan has built a vibrant and young lab with an upward trajectory. Most impressively, he recently published a senior authored paper in Nature Medicine – this is an outstanding achievement at any career stage, but it is especially remarkable for a young investigator only four and half years into a faculty position. He also has published a paper in the Journal of Neur*oscience*, and currently has manuscripts under review at the Journal of Clinical Investigation, and Pain. Tuan has therefore established a highly productive independent research program that is taking the opioid and pain research field in exciting new directions.

Schedule for INRC 2018

Tuesday June 12, 2018

2:00-5:00 PM -- Registration

5:30-7:00 PM -- Welcome Reception *Indigo Terrace*

Wednesday June 13, 2018

8:00 AM -- **INRC/CPDD Plenary Lecture** *Indigo BCFG*
Nora Volkow, NIDA

9:15 AM-12:00 PM -- **Symposium 1**, **INRC/CPDD** *Indigo BCFG*
Fentanyl
Chairs: Sandra Comer and Cathy Cahill

Speakers:
F. Ivy Carroll, *RTI International,* USA --
Synthesis of fentanyl: A little goes a long way

James Woods, *University of Michigan,* USA --
Behavioral pharmacology of fentanyl: Preclinical models

Alexander Walley, *Boston University School of Medicine,* USA --
Illicitly made fentanyl overdose: Prevention and management in real-world settings

Kim Janda, *The Scripps Research Institute,* USA --
Opioid use disorders: Vaccination as a therapeutic strategy

Sandra Comer, Columbia University, USA --
Fentanyl: How did we get here and what do we do about it?

INRC Hot Topics:

Rob Hill, University of Bristol, UK --
Fentanyl Depression of Respiration in Mice

Neil Varshneya, *Virginia Commonwealth University,* USA --
Antinociceptive and locomotor effects of Fentanyl-related substances in mice

12:00-1:30 PM -- Lunch *Aqua Salon EF*
and CPDD posters *Sapphire ABC/EFG*

1:30-4:00 PM -- **Symposium 2**, **INRC/CPDD** *Indigo BCFG*
Opioid Co-Morbidity

Chairs: Rita Valentino and Chris Evans

Speakers:
Tom Kosten, *Baylor College of Medicine,* USA --
Buprenorphine: Opioid dependence and depression

Brigitte Kieffer, McGill University, Canada --
Opioid receptors and diverse contributions to mood regulation

Charles Chavkin, University of Washington, USA--
Kappa/dynorphin systems and anxiety states

Louisa Degenhardt, *National Drug and Alcohol Research Centre*, Australia, --
Co-occurrence of mental health and opioid use disorders

Rita Valentino, National Institute on Drug Abuse, Discussant --
Comorbidity of opioid addiction and other psychiatric disorders

INRC Hot Topics:
Sarah Palumbo -- Clinical Characteristics of Opioid Use Disorder

Gregory Corder -- Dynamic encoding of aversive pain perception within hierarchical neural ensembles

Keith Olsen -- The non-selective opioid diprenorphine produces delta-opioid receptor-mediated rapid antidepressant-like effects in mice

4:15-4:30 PM -- **Data Blitz** *Indigo BCFG*

4:30-7 PM -- **INRC Posters** *Sapphire DHLP*
Please attend your poster on Wednesday if you have an odd numbered abstract and on Thursday if you have an even numbered abstract.

Thursday June 14, 2018

9:00-10:00 AM -- **INRC/CPDD Plenary Lecture** *Indigo BCFG*
George Koob, NIAAA

10:00 AM-12:30 PM -- **Symposium 3, INRC/CPDD** *Indigo BCFG*
Opioid Global Recommendations
Chairs: Gabriele Fischer and Fred Nyberg

Speakers:
Gabriele Fischer, *Medizinische Universitat Wien, Austria* --

Guidelines for the psychosocially assisted pharmacological treatment of opioid dependence within EU

Thomas Clausen, *The Norwegian Institute of Public Health, Norway* --
A Nordick Network promoting optimal service provision in addiction treatment in the Nordick region

Pauline Voon, *BC Centre on Substance Use, Canada* --
Opioid use disorder clinical guidelines: A Canadian model

Gavin Bart, *University of Minnesota, MN* --
Methadone treatment guidelines development for States in Southeast Asia

Fred Nyberg, Uppsala University, Sweden --
Opioid treatment programs – Aspects to deal with in the future perspective

INRC Hot Topics:
Caio Sarmento, *University of Kansas, USA* –
The trends of opioid prescription patterns among patients with Fibromyalgia

Insiya Attarwala, *University of Pittsburgh, USA* –
Computational modeling and analysis of Oxycodone suggest high-risk drug-drug interactions with commonly co-administered benzodiazepines

12:30 PM-2:00 PM *Aqua Salon EF*
and CPDD posters *Sapphire ABC/EFG*

2:00-4:30 PM -- **Symposium 4, INRC/CPDD** *Indigo BCFG*
New Targets for Drug Abuse Medications
Chairs: Tom Prisanzano and Nurulain Zaveri

Speakers:
Nicholas Cosford, *NCI-Designated Cancer Center, USA* --
Progress towards the development of novel therapeutics for drug dependence

Mei-Chuan (Holden) Ko, *Wake Forest University, USA* --
NOP receptor-targeted agonists as non-addictive analgesics and potential anti-opioid treatment in primates

Scott Runyon, *RTI International, USA* --
Dissecting the neuropeptide S system and its role in substance abuse

Huburt Yin, *University of Colorado, USA* --
Small molecule TLR modulators targeting neurological diseases

Corey Hopkins, *University of Nebraska, USA* --

The synthesis, optimization and biological characterization of a novel series of D_4 antagonists as a potential new treatment for drug abuse

INRC Hot Topics:
Aliza Ehrlich, *McGill University, Canada* –
Preserved functional selectivity of MOR ligands in HEK293 cells and MOR-Venus DRG neurons reveals the physiological reality of biased signaling.

Giordano de Guglielmo, *The Scripps Research Institute, USA* –
Nociceptin/orphanin FQ in the central nucleus of the amygdala selectively reduces oxycodone self-administration in rats with high addiction-like behaviors

4:45-5:00 PM -- **Data Blitz** *Indigo BCFG*

5:00-7:30 PM -- **INRC Posters** *Sapphire DHLP*
Please attend your poster on Wednesday if you have an odd numbered abstract and on Thursday if you have an even numbered abstract.

Friday June 15, 2018
8:30-9:30 AM -- **INRC Founders Lecture** *Aqua Salon DEF*
Macdonald Christie, University of Sidney

9:30 AM-12:15 PM -- **Symposium 5, INRC** *Aqua Salon DEF*
Kappa, Novel Uses
Chair: Lee-Yuan Liu-Chen

Speakers:
Jeff Liu, *Max Planck Institute of Biochemistry, Germany* --
In vivo Brain KOR Signaling Elucidated by Phosphoproteomics

Tao Che, *University of North Carolina School of Medicine, USA* --
Structure-guided ligand design of kappa opioid receptor

William Carlezon, *Harvard Medical School, USA* --
Sleep architecture regulated by selective alterations in the activity of dopamine D1 receptor-expressing cells within the nucleus accumbens

Frances Leslie, *University of California, Irvine, USA* --
Age- and sex-dependent interaction of kappa opioid receptors with nicotine and alcohol

Hot Topics:
Hannah Marie Harris, *University of Florida, USA* --
Evaluation of two peripherally-restricted kappa-opiod receptor agaonists for safer analgesia without CNS liabilities.

Qianhan Ma, *University of Bath, UK* --

Sex differences in kappa opioid receptor activated brain networks implicated in the response to stress and drugs of abuse

12:15 PM – Lunch --- Box lunches

Free Afternoon or
Research Society on Alcoholism, Satellite Symposium. *Aqua Salon DEF*

Saturday June 16, 2018

8:30-9:30 AM --**INRC Plenary Lecture** *Aqua Salon DEF*
Howard Fields, UCSF

9:30 AM-12:15 PM -- **Symposium 6, INRC** *Aqua Salon DEF*
Opioid Circuitry
Chair, Elyssa Margolis

Speakers:
Mary Heinricher, *Oregon Health and Sciences University, USA* --
Linking pain transmission to pain modulation

Tomohisa Mori, *Hoshi University, Japan* --
Opioid Circuits: Pain and Reward

Elyssa Margolis -- UC San Francisco, CA, USA
Mu opioid receptor activation inhibits hypothalamic inputs to the lateral habenula and relieves tonic pain

Dean Kirson -- *Oregon Health and Science University, USA*
Oxycodone self-administration dysregulates central amygdala kappa opioid receptor system

Hot Topics:
Will Birdsong, *Oregon Health and Science University, USA* --
Mu- and delta-opioid modulation of striatal glutamate release

Donita Robinson, *University of North Carolina, USA* --
Local mu-opioid receptor antagonism blunts evoked phasic dopamine release in rat nucleus accumbens

Kylie McPherson, *Oregon Health and Science University, USA* --
Diverse cell types within the vlPAG exhibit unique adaptations to membrane firing properties after inflammation

12:15-1:15 PM – Lunch *Aqua Salon foyer*

1:15-2:00 PM -- **Young Investigator Lecture** *Aqua Salon DEF*

Tuan Trang, University of Calgary
Targeting the immune system to alleviate opioid side effects

2:00-4:00 PM -- **Young Investigator Symposium** *Aqua Salon DEF*
Chair: Tuan Trang

Speakers:
Nicolas Massaly, *Washington University in St.Louis, USA* --
Pain-induced Negative Affect is Mediated via Recruitment of the Nucleus Accumbens Kappa Opioid System

Michael Morgan, *Washington State University, USA* --
Pain Enhances Spontaneous Opioid Withdrawal in the Rat

Tuomas Lilius, *University of Helsinki, Finland* --
Ketamine in the treatment of opioid tolerance: does the choice of opioid play a role?

Brad Taylor, *University of Pittsburgh, USA* --
Endogenous kappa opioid receptor inhibition of persistent pain

Hot Topic:
Catherine Thomas, *University of Calgary, Canada*
The effects of opioid sensitization on incentive sensitization and dopamine neurotransmission

4:00-5:00 PM -- **Business Meeting** *Aqua Salon DEF*

7:00 PM -- **Banquet: at Hotel** *Aqua Salon DEF*

Abstracts list

Please attend your poster on Wednesday if you have an odd numbered abstract and on Thursday if you have an even numbered abstract.

1 - The effect of mu-opioid receptor activation on synaptic transmission within the orbital frontal cortex
Brittany Ambrose

2 - Fentanyl Reexamined
Jessica Anand

3 - Labeling endogenous opioid receptors in living neurons via ligand-directed chemistry
Seksiri Arttamangkul

4 - Investigating the anti-addiction and chemotherapy induced neuropathic pain effects of MP1104, a novel mixed acting kappa, delta opioid receptor agonist in rodents
Diana Atigari

5 - Computational modeling and analysis of Oxycodone metabolism suggest high-risk drug-drug interactions with commonly co-administered benzodiazepines
Insiya Y. Attarwala

6 - Differential G-Protein Expression Alters Pharmacological Properties of Kappa Opioid Receptor
Miriam E. Barnett

7 - Pharmacological Properties of Buprenorphine and Samidorphan Alone and in Combination Being Developed as an Adjunctive Treatment for Major Depressive Disorder
Jean M. Bidlack

8 - mu- and delta-opioid modulation of striatal glutamate release
William Birdsong

9 - The neuropeptide system, BigLEN-GPR171, interacts with the opioid system to relieve pain and decrease tolerance
Erin Bobeck

10 - Morphine-induced condition place preference activates alpha-CaMKII but does not require its phosphorylation at Thr286.
Fernando Boix

11 - The active heroin metabolite 6-acetylmorphine has reinforcing properties and can sustain self-administration in the rat
Fernando Boix

12 - Cell type specific loss of striatal Gαolf signaling regulates morphine-induced behaviors in mice
Gloria Brunori

13 - Investigating agonist-dependent changes in the membrane organization of the μ-opioid receptor using fluorescence correlation spectroscopy
Meritxell Canals

14 - Risk factors attributing to severity of pressure ulcers in adult palliative care patients with cancer.
Minkyung Cho

15 - Neuroadaptations at nicotinic acetylcholine receptors and behavioral responses to nicotine in neuropathic rats
Andrea Cippitelli

16 - Dynamic encoding of aversive pain perception within hierarchical neural ensembles
Gregory Corder

17 - Nociceptin/orphanin FQ in the central nucleus of the amygdala selectively reduces oxycodone self-administration in rats with high addiction-like behaviors
Giordano de Guglielmo

18 - Role of mu-opioid receptors in indirect-pathway striatal neurons in cocaine reward
Lauren K. Dobbs

19 - Synthesis of FGF21, a Hormone Which Modulates the Dopamine Reward Pathway, Is Increased by GSK3 and HDAC Inhibitors
Louben Dorval

20 - G-protein biased kappa opioid receptor agonist attenuates cocaine self-administration in male mice
Alexandra Dunn

21 - Identifying kappa opioid receptor-protein interactions following activation by unbiased and G-protein biased ligands
Amelia Dunn

22 - Preserved functional selectivity of MOR ligands in HEK293 cells and MOR-Venus DRG neurons reveals the physiological reality of biased signaling
Aliza T. Ehrlich

23 – Nociception-evoked impulsivity in rats and its reversal with morphine
Nidia Espinoza
24 - Endomorphin analog treatment expedites recovery from chronic inflammatory pain and protects against latent sensitization
Amy Feehan

25 - Intrinsic G-protein efficacy, not ß-arrestin 2 dependent signaling, confers reduced opioid-induced respiratory depression
Alexander Gillis

26 - Exploring rapid desensitization of mu-opioid receptors induced by G protein-biased agonists
Sam Groom

27 - Preclinical Characterization of Mitragyna Speciosa Alkaloids for the Treatment of Alcohol Use Disorder
Anna Gutridge

28 - Evaluation of two peripherally-restricted kappa-opioid receptor agonists for safer analgesia without CNS liabilities
Hannah Harris

29 - Specific PKC isoform mediates ongoing pain in a mouse model of migraine
YING HE

30 - Mechanisms of presynaptic suppression of GABA release by opioid receptors in the hippocampus
X. Jenny He

31 - Fentanyl Depression of Respiration in Mice
Rob Hill

32 - Co-administration of chemokine receptor antagonists with opioids: Potentiation of analgesic effects on incisional pain in rats
Saadet Inan

33 - Multidisciplinary Approach in the Optimization of a Highly Selective Sigma-1 Receptor Antagonist
Sebastiano Intagliata

34 - Evaluation of mixed efficacy opioid ligands in drug discrimination assays
Emily Jutkiewicz

35 - An enzymatic approach reverses nicotine dependence, decreases compulsive-like intake and prevents relapse in rats
Marsida Kallupi

36 - Pharmacological effects of modifying the N-substituent of hydromorphone
Sophia Kaska
37 - A selective bivalent antagonist for the mu-delta opioid receptor heterodimer potentiates oxymorphone anti-nociception while sharply reducing morphine withdrawal
Attila Keresztes

38 - DPP4 inhibitors, Ile-Pro-Ile (IPI) and vildagliptin produce diverse antihyperalgesic effects following intrathecal administration in inflammatory pain models in rats
Kornél Király

39 - Evaluation of the analgesic and side-effects of G-protein biased mu-opioid receptor Salvinorin A analogues kurkinorin and kurkinol
Bronwyn Kivell

40 - Enhanced opioid analgesia and loss of tolerance but exacerbated side effects in mice expressing G-protein biased, phosphorylation-defic
Andrea Kliewer

41 - The complex roles of μ-opioid receptor phosphorylation and ß-arrestins in mediating opioid analgesia with fewer side effects
Andrea Kliewer

42 - Contribution of β-arrestin and ERK signaling in the anxiolytic-like effects of the δ-opioid receptor (δOR) agonist SNC80
Mee Jung Ko

43 - Interruption of continuous morphine administration in mice negatively impacts brain and behavior
Emilia Lefevre

44 - Ketamine in the treatment of opioid tolerance: does the opioid have a role?
Tuomas O. Lilius

45 - Roles of Protein Kinase C in Kappa Opioid Receptor-Mediated Effects in vivo: Pharmacological and Phosphoproteomic Approaches
Jeffrey J. Liu

46 - Correlation of KOR phosphorylation in mouse brains with sedation, motor incoordination and aversion induced by agonists
Lee-Yuan Liu-Chen

47 - Identification of local GPCR protein interaction networks using proteomics and proximity labeling
Braden T. Lobingier

48 - Sex differences in kappa opioid receptor activated brain networks implicated in the response to stress and drugs of abuse
Qianhan Ma
49 - Dissecting Agonist-induced Nociceptin/Orphanin FQ Receptor Phosphorylation in vitro and in vivo
Anika Mann

50 - A Stable Heroin Analog Vaccine Formulation that Induces Long Duration Antibody Titers that Block the Antinociceptive Effects of Heroin and Hydromorphone
Gary Matyas

51 - Morphine and HIV-1 Tat-induced calcium flux in hippocampal neurons is dependent on L-type calcium channels and NMDAR signaling
Virginia D. McLane

52 - Diverse cell types within the vlPAG exhibit unique adaptations to membrane firing properties after inflammation.
Kylie McPherson

53 - Mobility of the mu opioid receptor as an effector independent assay for alterations in signaling state
Marissa Metz

54 - Multi-site phosphorylation is required for sustained interaction with GRKs and arrestins in mediating rapid mu-opioid receptor desensitization.
Elke Miess

55 - Chronic Pain Enhances Spontaneous Fentanyl Withdrawal in Rats
Michael M. Morgan

56 - Exposure of early life stress reduces the expression of μ-opioid receptor in the PAG of adult mice brain and morphine analgesia
Kazuo Nakamoto

57 - Inside the Net: morphine-induced conditioned place preference and effects on perineuronal nets in the ventral tegmental area
Toni A.E. Nigro

58 - Cannabinoid Type-1 Receptors can Mediate the Opioid-Sparing Effects of Delta-9-tetrahydrocannabinol in Nonhuman Primates
Mark R. Nilges

59 - Growth hormone and Insulin-like growth factor-1 promotes the recovery of neurons after methadone exposure
Erik Nylander

60 - The non-selective opioid diprenorphine produces delta-opioid receptor-mediated rapid antidepressant-like effects in mice
Keith M Olson
61 - Inhibition of alpha7 nicotinic receptors in heroin-primed reinstatement of conditioned place preference
Josephine C Palandri

62 - Clinical Characteristics of Opioid Use Disorder
Sarah A. Palumbo

63 - Development of vaccines to treat heroin and prescription opioid abuse suitable for clinical trials
Marco Pravetoni

64 - In vivo EEG signatures during chronic fentanyl exposure, spontaneous withdrawal, and protracted abstinence
Corey Puryear

65 - Differential Activation and Cellular Localization of Protein Kinases in the Periaqueductal Gray Following Morphine Treatment
Akila Ram

66 - Sex differences in plasticity and stress-related genes in the rat following oxycodone conditioned place preference
Matthew Randesi

67 - The effects of remifentanil dose on the acquisition and persistence of responding for drug-paired cues.
Stephen H. Robertson

68 - Local mu-opioid receptor antagonism blunts evoked phasic dopamine release in rat nucleus accumbens
Donita L. Robinson

69 - The Trends of Opioid Prescription Patterns Among Patients with Fibromyalgia
Caio Sarmento

70 - HA-epitope tag knockin mice demonstrate lack of μ-opioid receptor splice variants in mouse brain
Stefan Schulz

71 - The association of long-term opium use with overall and cause-specific mortality: Results from a long-term, prospective population-based study
Ramin Shakeri

72 - Predictive factors for quality of life and impact of physical activity in Korean breast cancer survivors
Hyun Hwa Shin

73 - A genetically encoded biosensor reveals location bias of opioid drug action
Miriam Stoeber
74 - The effects of opioid sensitization on incentive sensitization and dopamine neurotransmission
Catherine Thomas

75 - Modulation of opiate reward and intake, as well as antinociceptive effects by the OPRM1 A118G SNP.
Annika Thorsell

76 - Interaction between spinal high-mobility group box-1 and glial cells in global cerebral ischemia-induced mechanical allodynia
Shogo Tokuyama

77 - Identifying a role for gonadotropin-releasing hormone and kisspeptin neurons in the development of opioid-induced hypogonadism
Karen J. Tonsfeldt

78 - Further evidence for the utility of central μ-opioids for cancer patients.
Kazuhiro Torigoe

79 - Antinociceptive and Locomotor Effects of Fentanyl-Related Substances in Mice
Neil B. Varshneya

80 - AT-312 (1-(1-((cis)-4-isopropyl cyclohexyl) piperidine-4-yl)-1H-indol-2-yl) methanol) a novel therapeutic compound for opioid relapse in an adolescent rat model
Dolores Vazquez Sanroman

81 - Physical association between μ opioid and dopamine D1 receptors : Implications for modulation of locomotor sensitization in dopamine-independent manner
Yu-Jun Wang

82- AMPA Receptor Positive Allosteric Modulators Attenuate Opioid Tolerance and Dependence
Zaijie Jim Wang

83 - Characterization of JVA 4001, a novel mixed opioid agonist/ kappa opioid receptor antagonist that attenuates drug and stress-induced reinstatement of extinguished morphine-conditioned place preference
Lisa Wilson

84 - Comparative Characterization of Operant Oxycodone Self-Administration in Male BALB/c and C57Bl/6 Mice
Kyle Windisch

85 - Generation of conditional Oprm1 knockout rat models using Easi-CRISPR with long ssDNA donors
Jin Xu
86 - The roles of alternatively spliced mu opioid receptor intracellular C-termini encoded by exon 7 on fentanyl actions
Jin Xu

87 - The Relation between Serum Uric acid and Spirometric Values and the Risk of Respiratory Disease
Jiwon Yoon

88 - Increasing G-protein signalling bias for μ-opioid receptor agonists enhances protein kinase C dependent desensitization.
Arsalan Yousuf

89 - Profile of a Novel bifunctional NOP/MOP receptor agonist for Opioid Use Disorder and Non-addicting Analgesia: Efficacy and lack of opioid side effects in nonhuman primates
Nurulain Zaveri

90 - Structurally different Anabolic Androgenic Steroids induce neurotoxic effects in primary cortical cell cultures
Sofia Zelleroth

91 - Morphine induced alteration in gut microbiome contributes to analgesic tolerance by modulating the Gut-Immune-CNS Axis
Li Zhang

92 - Diarylurea-based allosteric modulators of the cannabinoid CB1 receptor: Structure-activity relationship studies on the pyrrolidinylpyridinyl group
Yanan Zhang

Symposium Abstracts

Joint CPDD/INRC Symposia

Wednesday June 13

Symposium 1 -- Fentanyl Abuse: From Medicinal Chemistry to Clinical Management

Chairs: Sandra Comer and Catherine Cahill

Opioid use disorder (OUD) and its associated morbidity and mortality currently are at epidemic levels in the U.S. Beginning in the early 1990's, the increased incidence of OUD was mostly attributed to abuse of prescribed opioids, but an increase in heroin use has been observed in recent years. Despite concentrated efforts to address the problem, the rate of opioid overdose deaths is surging nationwide. This surge is due in part to the introduction of illicitly made fentanyl and its analogs. Despite the fact that fentanyl has been used in clinical settings for decades, its abuse liability, toxicity, and responsiveness to treatment medications are not well characterized. In the current symposium, Dr. Ivy Carroll will describe the ease of synthesizing fentanyl and its analogs relative to that of obtaining natural opioids and their analogs, as well as the relative in vitro and in vivo properties of fentanyl. Dr. James Woods will describe the behavioral pharmacology of fentanyl in preclinical models, as well as the ability of opioid antagonists and partial agonists to

reduce its effects. Dr. Alex Walley will describe the observed course of fentanyl-related overdoses and the utility of naloxone to reverse them in real-world settings. Dr. Kim Janda will describe a fentanyl vaccine as a potential novel therapeutic approach to treating fentanyl abuse. Dr. Sandra Comer will serve as the Discussant. This symposium should provide a broad overview of the chemistry and pharmacology of fentanyl, as well as the clinical characteristics of fentanyl overdose and opportunities for treatment

Symposium 2 -- Comorbidity of Opioid Addiction and Other Psychiatric Disorders

Chairs: Rita Valentino and Christopher Evans

Psychiatric disorders, including post-traumatic stress disorder and depression, often accompany opioid use disorder (OUD). Chronic pain syndromes are also comorbid with anxiety and depressive disorders and individuals in pain are more likely to be using opioids, creating a direct pathway to OUD in comorbid patients. The symposia will merge both clinical and basic research perspectives on the role of different opioid systems, namely mu, kappa and delta, in both contributing to, and relieving mood-related disorders. Dr. Kosten will first review clinical data on depression and opiate dependence, highlighting results from treatment with the kappa antagonist/partial mu agonist buprenorphine. Dr. Kieffer will then review basic research on the role of different opioid receptors in mood, and present her more recent work on the contribution of mu-receptors in selective cell-types to aversive behaviors triggered during opiate withdrawal. Dr. Chavkin will discuss how stress-induced activation of the dynorphin-kappa opioid receptor system can produce dysphoric and anxiety-like states that increase the rewarding valence of addictive drugs, promote drug seeking behaviors, and trigger relapse of drug-seeking in abstinent animals. Finally, Dr. Louisa Degenhardt will discuss the clinical implications of co-morbidity in cohorts she has studied in Australia. Questions in the symposia will address the salience of opioid drugs to individuals with mental disorders and/or pain and the issue of self-medication combined with distress during withdrawal states. Insights into contributions of different opioid receptors to mediating or alleviating symptoms exhibited in various comorbid mental states with OUD could guide future treatment strategies.

Thursday June 14

Symposium 3 -- International Opioid Guidelines
Chairs: Fred Nyberg and Gabriele Fischer

Over the past decade, the prescribing of opioids has increased dramatically in Western countries and in particular in North America. In parallel, increases in opioid addiction, overdose, and associated deaths have been seen. A similar situation occurs in Europe. According to the EMCDDA assessment of the drug-induced deaths, next to Estonia, Sweden is the country with the most drug-induced deaths per million population in Europe. From 2006 to 2014 a 40% increase in drug-related deaths has been recorded in Sweden, mainly due to opioids. This increase has been seen in both sexes. Moreover, it should be noted that a great part of the increase is seen in opioid deaths combined with benzodiazepines. Although opioid addiction is effectively treated using a multidisciplinary approach including agonist opioid treatment and psychosocial intervention there are some difficulties. Misuse and diversion of pain medicines, like fentanyl and buprenorphine comprise a significant problem in Sweden, but also in other Nordic countries with some of the highest frequencies in Europe. These inconveniences are associated with poor treatment compliance and increases in risk of blood-borne infections, crime, and mortality. To address this problem, changes in medicines used in treatment in Finland and Iceland have already been implemented and considerations are under way in Norway and Sweden. At this symposium, international well-known speakers will highlight current perspectives on Guidelines for opioid treatment as well as opioid overdoses and related deaths in Nordic countries but also in other countries within EU, North America and in Southeast Asia.

Symposium 4 -- New Targets for Drug Abuse Therapy
Chairs: Thomas Prisinzano and Nurulain Zaveri

This symposium will highlight new and emerging therapeutic approaches for the treatment of drug abuse. Among the topics covered will be receptor modulators, neuropeptide S receptor antagonists, toll-like receptor modulators, and dopamine receptor antagonists.

INRC Symposia

Friday June 15

Symposium 5,
Kappa, Novel Uses
Chair, Lee-Yuan Liu-Chen

In vivo Brain KOR Signaling Elucidated by Phosphoproteomics

Jeffrey J. Liu[1], Yi-Ting Chiu[3], Luca Zangrandi[2], Chongguang Chen[3], Sean J. Humphrey[1], Kirti Sharma[1], Mariana Spetea[4], Christoph Schwarzer[2], Lee-Yuan Liu-Chen[3], Matthias Mann [1,5]*

[1]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried 82152, Germany [2]Department of Pharmacology, Medical University of Innsbruck, 6020 Innsbruck, Austria [3]Center for Substance Abuse Research and Department of Pharmacology, Temple University Lewis Katz School of Medicine, Philadelphia, PA 19140, USA [4]Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, 6020 Innsbruck, Austria [5]Novo Nordisk Foundation Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Copenhagen, 2200, Denmark

A systems view of G protein-coupled receptor (GPCR) signaling in its native environment is the key in development of GPCR therapeutics with fewer side effects. Using the kappa-opioid receptor (KOR) as a model, we employed high-throughput phosphoproteomics to investigate signaling induced by structurally diverse agonists in five mouse brain regions. We employed the classic KOR agonist U50,488H as the reference and comparatively studied downstream phosphorylation changes induced by 6'GNTI and newly reported compounds in one study, and Nalfurafine in another. From these two different phosphoproteomic studies, we observed strong regional specificity of KOR signaling, due to differences in protein-protein interaction networks, neuronal contacts and the different tissues in neuronal circuitries. Agonists with distinct signaling profiles elicited differential dynamic phosphorylation of synaptic proteins, linking GPCR signaling to the modulation of brain functions. The large-scale de-phosphorylation of synaptic proteins in striatum after 5 min agonist stimulation was partially blocked by Protein Phosphotase 2A (PP2A) inhibitors, underscoring the involvement of PP2A in KOR mediated synaptic functions. Pathway analysis in both phosphoproteomic studies revealed enrichment of mTOR signaling by agonists associated with aversion. Consequently, mTOR inhibition during KOR activation abolished aversion, while preserving beneficial antinociceptive, anti-scratch and anticonvulsant effects. Our results establish high-throughput phosphoproteomics as a general strategy to investigate GPCR in vivo signaling, enabling prediction and modulation of behavioral outcomes.

Structure-guided ligand design of kappa opioid receptor

Tao Che[1], Daniel Wacker[1], Susruta Majumdar[2], Magdalena Korczynska[3], Els Pardon[4], Jan Steyaert[4], Ivy F. Carroll[5], Brian K. Shoichet[3], Bryan L. Roth[1]

[1]*Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA;* [2]*Molecular Pharmacology Program and Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, NY, USA;* [3]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, USA; [4]*VIB Center for Structural Biology, VIB, Brussels, Belgium;* [5]*Research Triangle Institute, Research Triangle Park, NC, USA*

The kappa-opioid receptor (KOR) mediates the actions of psychotomimetics and proposed non-addictive analgesics. A complete understanding of KOR activation is necessary to elucidate the pharmacological properties of this important drug target. While there exist several orthosteric KOR ligands with diverse chemotypes, the allosteric mechanism of KOR still remains poorly understood due to the lack of such modulators. Here we present two distinct crystal structures of the KOR in complex with either a positive or negative allosteric nanobody. Comparing these multi-state structures reveals: 1) a mechanism for opioid receptor activation and ligand selectivity; 2) key residues that explain the pharmacology, function, and biased signaling of the KOR; 3) new avenues for opiate-based therapy and design of allosteric modulators.

Sleep architecture regulated by selective alterations in the activity of dopamine D1 receptor-expressing cells within the nucleus accumbens

Kenneth McCullough, Galen Missig, **William A. Carlezon Jr.**

Department of Psychiatry, Basic Neuroscience Division, McLean Hospital, Harvard Medical School, Belmont MA, USA.

Stress plays a critical role in the neurobiology of mood and anxiety disorders. We recently showed that chronic social defeat stress in mice causes persistent alterations in sleep architecture. Older work shows that stress activates the transcription factor CREB within the nucleus accumbens (NAc), and that non-selective elevations in NAc CREB function produce depressive-like effects whereas disruptions in CREB function produce antidepressant- and anxiolytic-like effects. Elevated NAc CREB function is associated with increases in expression of dynorphin (an endogenous agonist at kappa-opioid receptors) and neuronal excitability. However, the ways in which dopamine D1- and D2-expressing medium spiny neurons (MSNs) within the NAc contribute to the persistent effects of stress is unclear. Here we examined how selective manipulation of D1 MSNs, which co-express dynorphin, affects sleep architecture using a wireless EEG system that enables continuous data collection in freely-moving mice. We used viral vectors to express excitatory or inhibitory DREADDs in the NAc of mice expressing cre-recombinase in D1 MSNs (GENSAT FK-150). Chronic activation of D1-MSNs (via hM3Dq) produced decreases in paradoxical sleep (PS) without perturbing slow wave sleep (SWS) or wakefulness (W) times. Conversely, chronic inhibition of D1-MSNs (via hM4Di) produced increases in PS without affecting SWS or W. These effects persisted following a 5-day DREADD ligand washout, suggesting that even transient activation or inhibition of this neuronal population produces long-lasting effects on sleep. Our findings indicate that alterations in the function of NAc MSNs resembling those caused by stress are sufficient to produce complex effects on sleep and wakefulness.

Support: MH063266 (to WAC)

Age- and sex-dependent interaction of kappa opioid receptors with nicotine and alcohol

Frances M. Leslie*, Armando Lárraga, & Sarah J. Cross

Department of Pharmacology, University of California, Irvine CA, USA

Concurrent use of nicotine and alcohol represents a major public health concern, and use of both substances typically begins during adolescence. We have studied the role of the kappa opioid receptor (KOR) in initial acquisition of intravenous drug self-administration in adolescent and adult rats, and have found both drug- and sex-differences. Blockade of KOR by pretreatment with norbinaltorphimine (norBNI) enhances initial self-administration of ethanol (EtOH) in adolescent males but not adults, and in adult females but not adolescents. In contrast, self-administration of nicotine + EtOH is enhanced by norBNI pretreatment in adult males but not adolescents. KOR blockade does not significantly affect combined drug intake in females, or nicotine self-administration in either sex. These data suggest that KOR activation may differentially block drug reinforcement in an age- and sex-dependent manner. To test the hypothesis that KOR activity is both age- and sex-dependent, we used an autoradiographic method to assess U69,593-stimulated GTPγS binding in specific brain regions. KOR activity in drug naïve animals was found to differ across age and sex, with adolescent males having greater KOR activity in the ventral tegmental area and median raphe compared to adult males, whereas adult females showed higher KOR activity in the basolateral amygdala than adolescent females. Overall, these findings suggest that KORs undergo sex- and region-dependent functional maturation during adolescence that may underlie observed differences in the reinforcing effects of nicotine and alcohol.

Saturday June 16

Symposium 6,
Opioid Circuity
Chair, Elyssa Margolis

Linking pain transmission to pain modulation

Mary Heinricher

Oregon Health and Sciences University, Portland OR, USA

Descending modulatory pathways are a primary site of action of centrally acting analgesic drugs, including opioids and cannabinoids. The principal output node mediating descending control is the rostral ventromedial medulla (RVM). A significant focus of research in the last several decades has been on defining the *outputs* from the RVM, showing that bidirectional control from this region is mediated by two physiologically defined cell classes, “ON-cells” and “OFF-cells,” that respectively facilitate and inhibit dorsal horn nociceptive transmission under different conditions. However, *ascending* transmission pathways are intimately intertwined with this descending control system, and RVM pain-modulating neurons also *receive* noxious input: ON-cells are activated by noxious stimulation, giving a “burst” of activity, whilst OFF-cell firing is suppressed, producing a “pause” in any ongoing activity. We showed recently that the lateral parabrachial complex is a major relay of acute noxious information to the RVM. PB is a nociceptive and autonomic processing and relay center that, at least in rodent, is the target of the bulk of supraspinal projections from the superficial dorsal horn. We will discuss recent evidence that PB projects directly to RVM to modulate the activity of identified pain-modulating neurons in the region, including how that circuit switches from a contralateral to ipsilateral dominance in persistent pain states. We will also consider recent evidence for plasticity in cannabinoid regulation of this circuit.

Opioid Circuits: Pain and Reward

Mori T, Watanabe M, Hamada Y, Narita M, Kuzumaki N, Navratilova E, Porreca F, Narita M

Department of Pharmacology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan, Department of Pharmacology, Arizona Health Sciences Center, University of Arizona, Tucson, Arizona

Opioid research has been mostly focused on drug dependence and pain control. Pain is a subjective experience and could be modified by affective and motivational factors such as attention, distraction, mood and/or pleasure. Pain itself can negatively alter pleasure or motivation, indicating that there is cross-talk between pain and motivation. The mesolimbic dopaminergic system integrates positive and negative motivational signals and determines the appropriate behavioral response. Therefore, we hypothesized that activation of mesolimbic dopaminergic neurons by systemic administration of morphine could be altered by pain. Interestingly, we found that morphine-induced reward could be suppressed under pain conditions accompanied by inhibition of the mesolimbic dopaminergic neurons, suggesting that pain alters activation of dopaminergic system and suppresses the acquisition of rewarding effect of morphine. On the other hand, optogenetic or chemogenetic activation of mesolimbic dopamine neurons using, respectively, light sensitive opsins or designer receptors exclusively activated by designer drugs (DREADDs) reduced hyperalgesia in animal models of chronic pain. To further investigate the involvement of mesolimbic dopaminergic neurons in morphine-induced analgesia, we generated transgenic mice expressing enhanced natronomonas pharaonis halorhodopsin (eNpHR-EYFP) under the control of the c-fos promoter using a tamoxifen inducible Cre-loxP system (c-Fos-eNpHR mice), allowing us to label morphine-activated cells with eNpHR-EYFP. Optical suppression of morphine-activated (c-fos positive) dopamine neurons in the ventral tegmental area (VTA), which expressed eNpHR-EYFP, significantly inhibited morphine-induced analgesia. We next generated transgenic mice expressing Gq-coupled human muscarinic M3 DREADD (hM3Dq), which can be specifically targeted by clozapine N-oxide (CNO) to induce neuronal activation, or channelrhodopsin-2 (ChR2), under the control of the c-fos promoter using a tamoxifen inducible Cre-loxP system. Reactivation of morphine-activated neurons in the VTA through the DREADD system had no effect on pain thresholds under normal conditions, whereas optical reactivation of morphine-activated neurons in the VTA alleviated thermal hyperalgesia in animal models of neuropathic and cancer pain. These findings provide further evidence that morphine-activated VTA-dopamine neurons may, at least partially, contribute to morphine-induced analgesia and modulate pain transmission.

Mu opioid receptor activation inhibits hypothalamic inputs to the lateral habenula and relieves tonic pain

Maggie W. Waung[1], Joseph R. Driscoll[1], **Elyssa B. Margolis**[1];

[1]Department of Neurology, University of California, San Francisco, CA, USA 94143

The lateral habenula (LHb) is a small, phylogenetically conserved, diencephalic nucleus implicated in depression, drug abuse, and the processing of aversive or disappointing information. Composed of physiologically homogeneous glutamatergic neurons, the LHb receives inputs from and projects to many components of the limbic and basal ganglia circuits. LHb neurons are activated by acute and persistent pain, raising the possibility that our previously reported inhibition of LHb neurons and their glutamatergic inputs by mu opioid receptor (MOR) activation (Margolis and Fields, 2016) decreases the aversive LHb output signal. We have found that in rats with neuropathic pain, microinjecting the MOR agonist DAMGO directly into the LHb reversed the mechanical sensitivity induced by the injury. Rats also develop a place preference for the context paired with intra-LHb DAMGO, but only when experiencing chronic pain, not following sham injury, suggesting the preference is driven by pain relief, rather than positive reinforcement. By optogenetically isolating different inputs to the LHb, we found that MOR inhibition of glutamate release is strongest in LHb terminals arising from neurons in the lateral preoptic area (LPOA) of the hypothalamus. Barker et al. (2017) recently reported that optogenetic stimulation of this glutamatergic projection from LPOA to the LHb is aversive in naïve animals, consistent with the possibility that MOR inhibition of this circuit can cause relief by decreasing activity in this aversive input. Together, these observations implicate the LHb and its hypothalamic inputs as novel targets for inhibiting the aversive component of tonic pain.

Oxycodone self-administration dysregulates central amygdala kappa opioid receptor system

Dean Kirson

The Scripps Research Institute, La Jolla, CA, USA

Non-medical opioid abuse is a significant global problem, with an estimated 2 million people in the US having a prescription opioid related abuse disorder and prescription opioid related overdose deaths drastically increasing over the last two decades. Despite the growing impact of prescription opioids on public health, few studies have investigated synaptic changes resulting from abuse of oxycodone, one of the most commonly prescribed medications. The negative affective state associated with chronic exposure to drugs of abuse is thought to be mediated in part by dynorphin/kappa opioid receptor (KOR) signaling. Increased KOR activity is aversive and KOR antagonists are currently being investigated as potential treatments for stress-induced drug-seeking behaviors and withdrawal-related anhedonia. The CeA is involved in the processing of internal and external emotional stimuli related to anxiety and stress and thus is a critical mediator of escalated drug intake. The CeA is a primarily GABAergic nucleus that also contains numerous stress-related neuropeptides, and dynorphin/KOR inhibition of GABAergic transmission becomes dysregulated in the CeA following escalated intake of other drugs of abuse, suggesting that alteration of CeA KOR-mediated signaling may be involved in the escalation of drug intake. We investigated the effects of the KOR agonist (-)-U-50488 hydrochloride (U-50488) and KOR antagonist nor-binaltorphamine dihydrochloride (norBNI) on locally evoked and action potential independent spontaneous (mIPSC) GABAergic signaling in the central amygdala of rats that self-administered oxycodone on short and long access schedules. We find that 12 hour oxycodone access on a 5 day a week schedule selectively reverses the effects of norBNI from increasing mIPSC frequency (increasing GABA release) to decreasing it (decreasing GABA release). This effect has been seen with other drugs of abuse and is usually accompanied by a reversal in the effects of U-50488, which we do not find here. Rats that had 12 hour access on a 7 day a week schedule, decreasing abstinent time, or were pretreated with norBNI, to antagonize KOR signaling, had decreased dysregulation similar to rats that received shorter 1 hour oxycodone access periods. Thus, we find that chronic oxycodone intake dysregulates CeA KOR system uniquely from other drugs of abuse and dysphoric signaling from intermittent long withdrawal periods further exacerbates this dysregulation.

Young Investigator Award

Targeting the immune system to alleviate opioid side effects

Young Investigator Lecture -- Tuan Trang,

University of Calgary, Calgary, Alberta, Canada

Microglia are immune cells in the central nervous system that are key targets of opioid action. Repeated opioid treatment drives microglial activation, which critically underlies a variety of side effects associated with opioid use. How opioids modulate microglial function is poorly understood. Here, we identified an essential transcription factor expressed in microglia that is modulated by opioid treatment. Pharmacological and genetic manipulation of this transcription factor alters the microglial response to opioid treatment and impacts the development of opioid tolerance, dependence, and hyperalgesia. Collectively, our results identify a transcription factor that critically modulates microglial function and response to opioid treatment.

Symposium 7

Young Investgator Symposium

Pain-induced Negative Affect is Mediated via Recruitment of the Nucleus Accumbens Kappa Opioid System.

N. Massaly

Washington University in St.Louis, St.Louis, MO, USA

Prolonged negative affect significantly impacts quality of life for patients suffering from pain. These maladaptive emotional states can lead to severe depression, suicide, involuntary opioid overdose, and related neuropsychiatric comorbidities. The nucleus accumbens (NAc) shell, which integrates both the aversive and rewarding valence of stimuli, exhibits allostatic changes in the presence of pain. In discrete regions of this structure, activation of the kappa opioid receptor (KOR), either by dynorphin, its endogenous agonist, or pharmacological ligands, acutely decreases the reinforcing properties of rewards and induces dysphoria and aversive behaviors. Using a wide range of complementary techniques including pharmacology, optogenetics, chemogenetics, physiology, biochemistry and in vivo positron emission tomography (PET) imaging, our current findings demonstrate that *in vivo* recruitment of NAc shell dynorphin neurons, acting through KOR, is both necessary and sufficient to drive pain-induced negative affect.

Furthermore, we reveal that the presence of inflammatory pain impacts patterns of consumption of fentanyl using an intra-venous self-administration paradigm. Those particular patterns, where rats in pain display bursts of consumption interrupted by periods of “rest”, could lead to the occurrence of respiratory depression and subsequent involuntary overdose. Taken together, our results provide evidence that adaptations in the kappa opioid system within the NAc shell represent a functional target for therapeutic intervention in pain that could circumvent affective disorders and may prevent life threatening episodes.

Pain Enhances Spontaneous Opioid Withdrawal in the Rat

Michael Morgan

Washington State University Vancouver

An increase in the number of people taking opioids for pain has led to an increase in the number of people dependent on opioids. The withdrawal symptoms that occur upon termination of opioid use can cause patients to transition to opioid abuse. Animal studies provide a unique opportunity to systemically examine the factors (sex, type of opioid, chronic pain) that may contribute to opioid withdrawal. Although spontaneous opioid withdrawal is difficult to measure in animals, depression of home cage wheel running provides an objective and continuous measure of both the magnitude and duration of opioid withdrawal. Our data show that the duration of spontaneous opioid withdrawal is greatly enhanced in rats with hindpaw inflammation. This enhancement is evident in male and female rats following withdrawal from morphine or fentanyl. These enhanced withdrawal symptoms suggest that pain patients may be especially susceptible to opioid abuse as a means to avoid opioid withdrawal.

Ketamine in the treatment of opioid tolerance: does the choice of opioid play a role?

Tuomas Lilius, MD, PhD

Pharmacology, Clinical Pharmacology, HUSLAB University of Helsinki, Helsinki, Finland

The effects of N-methyl-D-aspartic acid (NMDA) receptor antagonists in enhancing opioid analgesia and attenuating opioid tolerance have been of great interest both in the laboratory and the clinic. Preclinical studies have mostly focused on the pharmacodynamic interactions between ketamine and morphine, whereas only scarce information about co-administration of ketamine and the widely used oxycodone exists. Pharmacokinetic interactions between ketamine and opioids have been only little studied. Further, the role of ketamine metabolites in opioid tolerance remains unclear.

Our recent studies in rats show that co-administration of low-dose ketamine or norketamine effectively attenuated morphine tolerance, whereas tolerance to oxycodone was less affected. This difference may be partly attributable to a pharmacokinetic interaction between morphine and ketamine leading to vastly increased concentrations of morphine, ketamine, and norketamine in the central nervous system. Co-administration of oxycodone and ketamine did not show changes in drug concentrations. Norketamine, the main active metabolite of ketamine, should be further studied in the treatment of opioid tolerance, as it attenuated morphine tolerance with minor adverse effects. In contrast, (2S,6S;2R,6R)-hydroxynorketamine, a major secondary metabolite with no NMDA receptor antagonist properties, did not have tolerance-attenuating effects.

In addition to pharmacodynamic differences, pharmacokinetic interactions between ketamine and different opioids may explain the wide variation in treatment response and adverse effects observed in clinical ketamine-opioid interaction studies. These results also warrant studies of possible pharmacokinetic ketamine-opioid interactions in humans.

Endogenous kappa opioid receptor inhibition of persistent pain

Bradley Taylor

University of Pittsburgh, Department of Anesthesiology

Injury triggers a sustained silent form of neuronal plasticity in spinal signaling (latent sensitization, LS) that engenders vulnerability to chronic pain. Intrathecal pharmacology studies using LY2456302 or nor-binaltorphimine revealed that LS in mice can be kept in remission by spinal kappa opioid receptors (KOR). This endogenous analgesic action lasts for over one year, attenuates pronociceptive cAMP receptor signaling, and is sex-dependent. Strategies that promote endogenous spinal KOR analgesia may prevent the transition from acute to chronic pain.

POSTER ABSTRACTS

1 - The effect of mu-opioid receptor activation on synaptic transmission within the orbital frontal cortex

Brittany Ambrose[1], Ben Lau[2], Stephanie Borgland[2]

[1]Cumming School of Medicine, University of Calgary, [2]Department of Physiology and Pharmacology, University of Calgary

The orbital frontal cortex (OFC) plays a critical role in estimating the likelihood of specific outcomes to guide future responses. Mu-opioid receptor (MOP) stimulation within selective regions of the OFC has been shown to enhance feeding and hedonic responses to sucrose. Furthermore, the selective MOP agonist DAMGO activated Fos in the rostral, but not caudal OFC. MOP receptors are expressed on parvalbumin positive basket cells in other parts of the prefrontal cortex. In other regions of the prefrontal cortex, mu-opioid agonists act pre-synaptically suppress GABAergic synaptic transmission. However, their precise cellular mechanism of action across the different sub-regions of the OFC remains unknown. Due to the functional heterogeneity and differences in inputs, it is important to characterize possible differences in mu-opioid actions between these sub-regions. Thus, we investigated the cellular actions of mu-opioids within the medial and lateral OFC. Immunohistochemistry revealed the presence of the endogenous opioid, β-endorphin, throughout both the medial and lateral OFC of adult male C57BL/6 mice. Using patch clamp electrophysiology, we show that DAMGO produced a significant suppression of evoked GABAergic inhibitory post synaptic currents (IPSCs) onto layer 2/3 pyramidal neurons within the medial, but not lateral OFC. This effect was inhibited by pre-application of the MOP antagonist, CTAP. CTAP application after DAMGO-mediated inhibition of IPSCs did not washout the effect, indicating that DAMGO-induces a long-term depression of medial OFC inhibitory synapses. Our results suggest that mu-opioids are present in the OFC and MOP inhibition of inhibitory synaptic transmission in regionally selective in the OFC.

2 - Fentanyl Reexamined

Jessica Anand[1], John Traynor[2]

[1]Univeristy of Michigan, [2]University of Michigan, Ann Arbor, USA

The number of drug seizures and overdose deaths involving fentanyl has risen steeply since 2016 and as a result emergency core scheduling for fentanyl analogs was put into place in late 2017. In light of the role fentanyl and its analogs play in the current opioid epidemic we chose to reexamine fentanyl and characterize its function in vitro and in vivo. Fentanyl is commonly known as a highly potent and efficacious mu opioid receptor (MOR) agonist with significant abuse potential. However, we have observed that fentanyl appears to have a delta opioid receptor (DOR) agonist component in vivo. Morphine and fentanyl both display dose dependent antinociception in the mouse warm water tail withdrawal assays, as expected for prototypical MOR agonists. Pretreatment with naltrindole (3.2 mg/kp ip), a DOR antagonist, did not shift the dose response curve for morphine; surprisingly naltrindole at this same dose produced a significant right-ward shift in the dose response curve for fentanyl. We decided to further explore this apparent DOR agonist activity of fentanyl in vitro and in vivo. Binding affinities and efficacy were determined at MOR, DOR, the kappa opioid receptor (KOR), and the nociception receptor (NOP) in vitro. The effects of MOR and DOR selective antagonists were examined in vitro to determine what effect, if any, receptor cross-talk has on the overall agonist activity of fentanyl. The contribution of DOR to the in vivo antinociceptive effects of fentanyl was examined using both antagonists and knock out mice. Funded by DA 033397.

3 - Labeling endogenous opioid receptors in living neurons via ligand-directed chemistry

Arttamangkul S[1], Placzek A[1], Birdsong W[1], Farrens D[1], Williams JT[1]
[1]Oregon Health Science University

Locating endogenous opioid receptors in brain tissues has been difficult even when antibodies are available. Due to large molecular weight and size, the penetration of antibody is often limited and thin slices from fixed tissues are necessary. To visualize the endogenous receptors in thicker living slices, we took a chemical approach where a small antagonist, naltrexamine is used to direct Alexa 594 dye to reside in proximity and form covalent bond with nucleophilic side chain of an amino acid on extracellular face of the receptor. The key functional group acyl imidazole reported to successfully label AMPA and GABA receptors is placed in the linker arm of the compound NaI-AI-594 and thus allows naltrexamine to be free from the binding pocket while leaving the dye on the receptor. With this strategy, the receptors can be visualized and electrophysiologically recorded. In this study, NaI-AI-594 was synthesized and used to label endogenous mu opioid receptors of mouse and rat locus coeruleus neurons. It also identified mu opioid receptors in other areas of the brain such as midbrain, habenula and parabrachial nucleus. Detailed characterization for specificity and labeling efficiency will be presented.

4 - Investigating the anti-addiction and chemotherapy induced neuropathic pain effects of MP1104, a novel mixed acting kappa, delta opioid receptor agonist in rodents

Diana Atigari[1], Rajendra Uprety[2], Susruta Majumdar[3], Bronwyn Kivell[1]
[1]School of Biological Sciences, Centre for Biodiscovery, Victoria University of Wellington, New Zealand, [2]Department of Neurology, Memorial Sloan Kettering Cancer Centre, New York, USA, [3]Center of Clinical Pharmacology, St. Louis College of Pharmacy and Washington University School of Medicine, St. Louis, MO, USA

Introduction: Development of effective non-addictive pain therapies are urgently needed. Kappa-opioid receptor (KOPr) activation reduces drug-seeking behaviour in preclinical models of drug use and also attenuates pain and inflammation. In this study, we utilised a chemotherapy-induced neuropathic pain (CINP) model to evaluate the effects of both acute and chronic treatment of MP1104, a mixed opioid agonist, on mechanical and cold allodynia. We also investigated the anti-cocaine effects of MP1104 in rats trained to self-administer cocaine.

Methods: To induce neuropathic pain, male C57BL/6J mice (n=60) were administered paclitaxel, a chemotherapy drug to give a cumulative dose of 16 mg/kg. Mechanical and cold allodynia measured using von Frey filaments and by placing a drop of acetone to hind paw and recording reaction time duration. For acute treatment, on day 15 mice were administered cumulative doses of MP1104 (or morphine), mechanical & cold allodynia evaluated. Chronic daily administration of MP1104 or morphine from day 16 to 38. Male Sprague-Dawley rats (n=20) were used to investigate the anti-cocaine effects of MP1104 in cocaine self-administration models.

Results: MP1104 dose-dependently reduced mechanical ($p<0.0001$) and cold allodynia ($p<0.0001$) to pre-paclitaxel levels. Non-linear regression analysis revealed MP1104 was more potent than morphine at reducing mechanical allodynia (EC_{50} = 0.45 mg/kg) and cold allodynia (EC_{50} =0.47 mg/kg).In rats trained to self-administer cocaine, MP1104 reduced cocaine prime reinstatement of drug-seeking behaviour at doses of 0.3 & 1mg/kg ($p<0.001$), caused significant downward shift ($p<0.0001$) in cocaine self-administration dose-response curve (two-way ANOVA with repeated measures).

Conclusion: MP1104 has potent effects in reducing both mechanical and cold allodynia in CINP following both acute and chronic treatment in mice. Moreover, MP1104 has no rewarding properties, thus making it suitable for developing better pain medications.

5 - Computational modeling and analysis of Oxycodone metabolism suggest high-risk drug-drug interactions with commonly co-administered benzodiazepines

Insiya Y. Attarwala[1], Zhaojia Zhang[1,2], Xiaoqing Tan[1,2], Lirong Wang[1,2], Xiang-Qun Xie[1,2,3]

[1]NIH National Center of Excellence for Computational Drug Abuse Research, [2]Department of Pharmaceutical Sciences and Computational Chemical Genomics Screening Center, School of Pharmacy, [3]Department of Computational Biology and Structural Biology, School of Medicine, University of Pittsburgh

In the past decade, prescription opioids have risen to be the leading cause of drug overdoses in the United States. Oxycodone, indicated for the treatment of moderate to severe pain, is a commonly used opioid with an unfortunately high abuse potential and risk of death due to overdose. Adding to this crisis are drug-drug interactions (DDIs) that occur when patients are simultaneously taking additional medications. Analysis of FDA Adverse Event Reporting Systems for oxycodone shows that most fatal outcomes involve concomitant use of other drugs, of which nearly half result from co-administration with benzodiazepines, commonly prescribed sedative-anxiolytics. This study examined the interaction between oxycodone and Diazepam, as a result of their shared Phase I metabolism by the CYP450 3A4 enzyme. The benzodiazepine diazepam is also a competitive inhibitor of CYP3A4. We hypothesized that concomitant administration of diazepam may affect the metabolism of oxycodone and may lead to increased or prolonged adverse effects of oxycodone. Using pharmacokinetic (PK) data of oxycodone at different clinical doses, we obtained a PK simulation of oxycodone using a one-compartment model with first-order dosing and linear elimination. We then simulated the oxycodone concentration-time profile in the presence of diazepam. Our simulation showed that in the presence of diazepam, levels of oxycodone and active metabolite Oxymorphone (from CYP2D6) accumulate. Our quantitative simulations with clinical doses show potential DDI and overdose risk thresholds for concomitant oxycodone diazepam use. Such information raises awareness for healthcare professionals prescribing opioids with other medications in the current climate of the opioid crisis.

6 - Differential G-Protein Expression Alters Pharmacological Properties of Kappa Opioid Receptor

Miriam E. Barnett[1], Brian I. Knapp[1], Jean M. Bidlack[1]

[1]Department of Pharmacology and Physiology, University of Rochester

In the canonical model, the kappa opioid receptor (KOR) signals through the $G\alpha_i$ and $G\alpha_o$ proteins. However, the involvement of other G proteins to downstream signaling events has not been rigorously characterized. The milieu of G proteins within a particular cell type may greatly influence the pharmacological properties of opioid ligands. Characterization of G-protein alpha subunit expression at both the mRNA and protein level in HEK 293 and CHO cells revealed that each line had distinct expression profiles. Notably, HEK 293 cells expressed the pertussis-insensitive $G\alpha_z$ and did not express $G\alpha_o$, while CHO cells expressed $G\alpha_o$ and not $G\alpha_z$. Changes in cyclic AMP levels were measured in HEK 293 and CHO cells stably expressing the human KOR after administration of classical kappa-selective agonists and antagonists. Like U50488, a Kappa-selective agonist, antagonists inhibited cyclic AMP levels in HEK 293 cells, but not in the CHO cells. G-protein specific toxins and overexpression systems were used to selectively measure signaling through specific G proteins in each cell line. The individual G proteins had different effects on the pharmacological properties of opioid ligands resulting in functionally biased KOR signaling. (Supported by NIH T32GM068411 and the Margo Cleveland Fund)

7 - Pharmacological Properties of Buprenorphine and Samidorphan Alone and in Combination Being Developed as an Adjunctive Treatment for Major Depressive Disorder

Jean M. Bidlack[1], Brian I. Knapp[1], Daniel R. Deaver[2], Margarita Plotnikava[2], Derrick Arnelle[2], Angela M. Quinn[2], May Fern Toh[2], Sokhom S. Pin[2], Mark N. Namchuk[2]
[1]University of Rochester, [2]Alkermes

A combination of buprenorphine and samidorphan at a 1:1 dose ratio (mg/mg), is being investigated as an adjunctive treatment for major depressive disorder (BUP/SAM, ALKS 5461). This study characterized the receptor and [^{35}S]GTPγS binding properties of buprenorphine and samidorphan alone and in combination. [^{3}H]Buprenorphine had a slow dissociation from the μ opioid receptor (MOR). In the [^{35}S]GTPγS binding assay, buprenorphine was a partial agonist at all three opioid receptors. Samidorphan was an antagonist at the MOR and a partial agonist at the κ and δ opioid receptors (KOR and DOR, respectively). A 1:5 molar ratio of buprenorphine:samidorphan, similar to steady-state plasma levels in humans, showed that samidorphan attenuated buprenorphine's efficacy without affecting its potency at the MOR. At the KOR, the same ratio did not change buprenorphine's efficacy. Similar results were obtained when MOR and KOR signaling, mediated by $G\alpha_i$ proteins, was evaluated in a BRET assay. When the MOR signaled through $G\alpha_o$ and $G\alpha_z$ proteins, buprenorphine had E_{max} values between 87 and 92% and samidorphan had E_{max} values between 27 and 33%. At the KOR, buprenorphine and samidorphan were partial agonists when the KOR signaled through $G\alpha_i$ and $G\alpha_o$ and full agonists signaling through $G\alpha_z$ proteins. Buprenorphine produced some β-arrestin recruitment mediated by the MOR, while a 1:3 molar ratio of buprenorphine:samidorphan produced no β-arrestin recruitment. The collective data show that samidorphan attenuates the *in vitro* efficacy of buprenorphine at the MOR but, did not alter buprenorphine's activity at the KOR.

8 - Mu- and Delta-opioid Modulation of Striatal Glutamate Release

William Birdsong[1], Bart Jongbloets[1], Kim Engeln[1], Dong Wang[2], Gregory Scherrer[2], Tiany Mao[1]
[1]Oregon Health & Science University, Portland, USA, [2]Stanford University, Stanford, USA

Affective/ motivational pain processing involves the medial thalamus, anterior cingulate cortex (ACC) and striatum. Opioid analgesics are known to modulate this circuitry and relieve the negative emotional effects of pain. However, the identity of the opioid receptors, their cellular locations, and their physiological effects on this affective circuitry remain uncharacterized. In the present study, synaptic connections between the three brain regions; thalamus, striatum, and ACC, were characterized based on the effect of opioid agonists to modulate glutamatergic transmission between these regions. Using optogenetic and electrophysiological approaches, it was determined that single striatal projection neurons in the dorsomedial striatum received direct input from both thalamic and cortical afferents. It was found that opioid receptors differentially modulated thalamic and cortical glutamate release in striatum. Additionally, μ and *∂*-opioid receptors were found to play distinct roles in modulating thalamo-cortical and intracortical synaptic transmission in the ACC. Imaging revealed that *∂*-opioid receptors are expressed on nearly all parvalbumin positive interneurons in the ACC. These data identify opioid receptor subtype-dependent inhibition of specific pathways within the thalmao-cortico-stiatal circuitry, whereby μ-opioids inhibited striatal glutamate release while *∂*-opioid activation facilitated release, providing insights into how opioid analgesics may modulate affective pain at the synaptic and circuit level.

9 - The neuropeptide system, BigLEN-GPR171, interacts with the opioid system to relieve pain and decrease tolerance

Leela Afrose[1], Ivone Gomes[2], Lakshmi Devi[2], Erin Bobeck[1]

[1]Utah State University, [2]Icahn School of Medicine at Mount Sinai

Recently BigLEN was identified (a ProSAAS derived peptide) as the endogenous peptide for the orphan receptor GPR171. BigLEN and GPR171 are both highly expressed in the descending pain modulatory pathways, including the periaqueductal gray (PAG). In addition, we find that GPR171 is co-localized with mu opioid receptors (MOPr) in the ventrolateral PAG in a portion of vGLut2 and GAD67 positive neurons. Given the expression pattern of GPR171, we hypothesized that GPR171 contributes to antinociception and tolerance. To test this, mice were injected with a GPR171 antagonist (MS21570; 5 mg/kg, i.p.) followed by the tail flick test which produced an increase in antinociception compared to controls. However, this compound is not as efficacious as morphine and pretreatment with MS21570 10 min prior to morphine (10 mg/kg, s.c.) did not alter morphine-induced antinociception. To assess the role of GPR171 in morphine tolerance, mice were injected with morphine twice daily for 5 days. A subset of mice were pretreated with MS21570 10 min prior to each morphine injection. Those mice pretreated with MS21570 showed a decrease in the development of morphine tolerance. To understand how these systems may be working together, GPR171 was knocked down in Neuro2A cells which led to a decrease in MOPr-mediated GTPγS signaling by DAMGO, but had no effect on GTPγS signaling by the delta opioid receptor agonist, deltorphin. Taken together, this data shows that GPR171 and MOPr interact with one another and GPR171 antagonist may be a novel target to treat pain or opioid tolerance.

10 - Morphine-induced condition place preference activates alpha-CaMKII but does not require its phosphorylation at Thr286.

Jannike M. Andersen[1], Siri H. Opdal[2], Christian P. Müller[3], Fernando Boix[1]

[1]Section for Drug Abuse Research, Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway, [2]Section for Pediatric Forensic Medicine, Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway, [3]Department of Psychiatry and Psychotherapy, University Hospital, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

Background: Conditioned Place Preference (CPP) is an association learning procedure mediated by synaptic changes in striatal neurons. Activation of Calcium/calmodulin-dependent protein kinase II (CaMKII), by phosphorylation at Thr286, has been shown to be important for synaptic plasticity processes to occur. In this study, we examined the contribution of alpha-CaMKII (αCaMKII) and its autophosphorylation at Thr286 (p-αCaMKII) on CPP induced by morphine. **Methods:** The effects of 10 or 30 µmol/kg morphine on αCaMKII, p-αCaMKII, and β-actin levels in dorsal and ventral striatum and hippocampus were examined in mice after acute administration, morphine-induced CPP, and a subchronic treatment mirroring the drug administrations during CPP. The acquisition of CPP was also tested in autophosphorylation deficient αCaMKIIT286A mice (Giese, Science 279, 1998) using the same morphine doses. **Results:** After morphine-induced CPP, the levels of αCaMKII and β-actin, but not p-αCaMKII, were significantly increased in the three brain areas. These protein levels were also increased after the acute morphine administration, but only in striatum. No changes were found after the subchronic morphine treatment. Besides, no differences were observed between αCaMKIIT286A and wild type mice in the expression of CPP induced by morphine. **Conclusions:** Morphine-induced CPP is accompanied by increases in the levels of β-actin and overall αCaMKII in striatum, but does not require phosphorylation of αCaMKII at Thr286. This is in contrast to cocaine-induced CPP (Easton, Transl Psychiatry 4, 2014). These changes were also observed in hippocampus after morphine-induced CPP, but not after acute morphine, indicating that this area is recruited during CPP acquisition.

11 - The active heroin metabolite 6-acetylmorphine has reinforcing properties and can sustain self-administration in the rat

Riccardo Avvisati[1], Jannike M. Andersen[2], Vigdis Vindenes[2], Inger Lise Bogen[2], Jørg Mørland[3], Aldo Badiani[1], Fernando Boix[2]

[1]Sussex Addiction Research and Intervention Centre (SARIC), University of Sussex, Brighton, UK, [2]Section for Drug Abuse Research, Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway, [3]Norwegian Institute of Public Health, Oslo, Norway

Introduction: 6-Acetylmorphine (6-AM) is the main active metabolite found immediately after heroin administration. However, 6-AM's reinforcing properties, which could be central for heroin's high drug abuse potential, have been sparsely examined. The present study examines the acquisition, extinction, relapse, and retraining of self-administration of 6-AM in rats compared with equimolar doses of heroin. **Methods**: Sprague-Dawley rats were submitted to an intravenous self-administration procedure with 6-AM or heroin encompassing: 1. acquisition (five FR1 sessions and two FR2 sessions) using 0.135 µmol/kg (44.3 µg/kg 6-AM, 50 µg/kg heroin) per infusion; 2. extinction (five sessions); 3. relapse (one session) using a single 0.068 µmol/kg (22.15 µg/kg 6-AM, 25 µg/kg heroin) priming infusion; and 4. retraining (one session) using 0.068 or 0.135 µmol/kg. **Results**: Whereas the rate of self-administration was similar for 6-AM and heroin during FR1, it was significantly higher for 6-AM during FR2. No differences were observed during extinction. Priming with 0.068 µmol/kg reinstated lever presses for both drugs during the first hour of the relapse session. Retraining with the dose used in acquisition (0.135 µmol/kg) restored lever presses for both drugs at a level similar to the last acquisition session. Retraining with half the dose (0.068 µmol/kg) significantly increased self-administration in animals receiving 6-AM, but not in animals receiving heroin. **Conclusions**: Intravenous 6-AM shows similar reinforcing properties as heroin, supporting its proposed involvement in the rewarding and abuse properties of its parent drug. However, some observed disparities also indicate the existence of some differences in their rewarding qualities after systemic administration.

12 - Cell type specific loss of striatal $G\alpha_{olf}$ signaling regulates morphine-induced behaviors in mice

Gloria Brunori[1], Anna Stauffer[1], Kathleen Sheridan[2], Janet Robishaw[1]

[1]Department of Biomedical Science, College of Medicine, Florida Atlantic University, Boca Raton, FL, USA, [2]Weis Center for Research, Geisinger Clinic, Danville, PA, USA

G protein signaling pathways are essential for the translation of neurotransmitter activity into behavior. Within the striatum, the $G\alpha_{olf}$ isoform mediates stimulation of adenylyl cyclase (AC) upon activation of dopamine D_1 receptors (D_1R) and adenosine $A_{2A}R$. The $G\gamma_7$ protein is also enriched in the striatum, where it orders the assembly and sets the functional level of the striatal-specific $G\alpha_{olf}\beta_2\gamma_7$ heterotrimer. Activation of opioid receptors and inhibition of AC in the striatum are key mechanisms for psychomotor and rewarding effects of morphine. Preliminary data obtained in *Gng7* gene (encoding $G\gamma_7$) global knock-out mice indicated that G_{olf} signaling is required to mediate the locomotor-enhancing effects of morphine. To dissect the role of G_{olf} signaling pathway in the main subpopulations of striatal medium spiny neurons, we generated new mouse models in which *Gng7* gene is deleted specifically in D_1R- or D_2R (also expressing $A_{2A}R$)-expressing neurons. Analysis of striatal membranes showed reduced levels of $G\alpha_{olf}$ protein and decreased levels of forskolin-stimulated AC activity in both strains. AC activity was also reduced following stimulation of D_1R and $A_{2A}R$ in D_1R- and D_2R-*Gng7* deleted mice, respectively. Notably, mice lacking the $G\gamma_7$ subunit in D_1R-positive neurons showed reduced sensitivity to the acute locomotor-enhancing effects of morphine, although they developed normal behavioral sensitization and conditioned place preference in response to chronic morphine. In contrast, loss of $G\gamma_7$ subunit in D_2R-positive neurons produced increased locomotor response to acute morphine. Collectively, these findings provide novel evidence regarding specific contributions of the striatal-specific G_{olf} signaling to opioid-mediated behaviors.

13 - Investigating agonist-dependent changes in the membrane organization of the μ-opioid receptor using fluorescence correlation spectroscopy

Arisbel Batista-Gondin[1,2], Michelle L Halls[2], Stephen J Briddon[1], Meritxell Canals[1,2]
[1]Centre of Membrane Proteins and Receptors (COMPARE), and School of Life Sciences, University of Nottingham, UK, [2]2 Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Differential regulation of the MOR by morphine and other agonists has been linked to opioid tolerance and dependence. At a cellular level, MOR regulation occurs via multiple mechanisms including receptor desensitization, internalization, recycling and plasma membrane redistribution. Here we have used advanced imaging techniques (Fluorescence Correlation Spectroscopy, FCS; Fluorescence Recovery After Photobleaching, FRAP) to monitor agonist-dependent changes in MOR organization within living cells.

SNAP-hMOR was stably expressed in HEK293 cells in the basal state and upon stimulation with the antagonist naloxone, the low internalizing agonist morphine and the high internalizing agonist DAMGO. FCS measurements were taken on the upper cell membrane and diffusion co-efficients (D), receptor number (N) and molecular brightness (e) were determined using autocorrelation and photon counting histogram analysis as previously descirbed[1]. Immobile MOR fraction and macro-D were determined using FRAP on the lower cell membrane.

DAMGO, but not morphine, caused a time- and concentration-dependent MOR clustering on the upper cell membrane, as indicated by a decreased D, reduced receptor number and an increased proportion of very bright receptor species. These effects were prevented by pre-incubation with naloxone. Similarly addition of DAMGO, but not morphine, caused a reduction of the proportion of mobile receptors. Interestingly, these changes are independent of MOR internalization, but dependent on GRK2/3-mediated phosphorylation. The role of Gβγ and PKC was also investigated.

Overall, these results demonstrate agonist-specific changes in the micro-organization of MOR at the plasma membrane, probably occurring prior to internalization. They provide insights into another molecular event differentially regulated by distinct opioids.

14 - Risk factors attributing to severity of pressure ulcers in adult palliative care patients with cancer.

Minkyung Cho[1], Jiwon Yoon[1], Hwanseok Choi[1]
[1]Seoul St. Mary's Hospital, Catholic Medical Center, Korea

Background Pressure ulcers are one of the most common medical problems in palliative care patients with cancers. A number of studies about predictors of formation of pressure ulcers have been conducted but there have been few studies about risk factors affecting severity of pressure ulcers. We analyzed factors associated with the severity of pressure ulcers in patients with cancers hospitalized in a palliative care ward setting. **Methods** This study was retrospective analysis of 186 patients with pressure ulcers with a mean age (±standard deviation) of 70.22±13.48 years (range: 22-96 years, median 73.0 years; 50.5% women), who were admitted in Seoul St. Mary's hospital palliative care ward in 2015. **Results** Patients were hospitalized for mean 20.69±17.10 days (1-91 days, median 21.5days). 159 patients (85.5%) had low grade (stage 1-2) and 27 patients (14.5%) had high grade (stage 3-6) pressure ulcers. Chi-square test was used to examine the differences of grade according to categorical variables and independent sample t-test was used for continuous variables; PPS (Palliative Performance Scale) and Braden scale appeared to have significantly associated to grade of pressure ulcers. Two factors assessed at admission appear to predict the severity of pressure ulcers in the multivariate logistic regression model; PPS (odds ratio [OR]=0.600 (95% CI=0.390-0.925,) P=0.021), Braden scale (OR=0.783 (95% CI=0.655-0.935), P=0.007). **Conclusion** PPS and Braden scale may contribute to severity of pressure ulcers in patients with cancers in palliative care ward.

15 - Neuroadaptations at nicotinic acetylcholine receptors and behavioral responses to nicotine in neuropathic rats

Andrea Cippitelli[1,2], Gloria Brunori[1,2], Jennifer Schoch[1,2], Akihiko Ozawa[1,2], Lawrence Toll[1,2]

[1]Biomedical Science Department, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, Florida, USA, [2]Torrey Pines Institute for Molecular Studies, Department of Neuropharmacology, Port St Lucie, Florida, USA

Tobacco addiction and chronic pain represent highly prevalent and comorbid conditions that engender considerable burdens upon individuals and systems. This complex relationship is poorly understood at both molecular and behavioral levels. Here we describe experiments aimed at understanding whether a chronic pain state induces neuroadaptations at nicotinic acetylcholine receptors (nAChRs) that lead to increased vulnerability to nicotine addiction or to the development of coping strategies to relieve pain symptoms. We found dramatic downregulation in the mRNA expression levels of α3, α4, α5, α7, β2 and β4 nAChR subunits in dorsal root ganglia and a time-dependent downregulation of discrete subunits in the cingulate cortex and the amygdala of rats subjected to ligation of the L5 spinal nerve, a procedure that models neuropathic pain, as compared to sham-operated rats. In contrast, expression of the nAChR subunits examined in brain regions important for rewarding processes was comparable in these rat groups. Spinal nerve ligated (SNL) and sham-operated rats showed no changes in patterns of acquisition, motivation for nicotine taking, extinction and cue-induced relapse into nicotine seeking, although the ligated group failed to reinstate lever pressing when relapse was induced by nicotine priming. Nicotine-experienced SNL rats did not show anxiogenic-like activity following administration of a nicotine dose that produced anxiety in control animals. SNL and sham rats were equally sensitive to nicotine-induced antinociception; however, nicotine produced a potent and long-lasting antiallodynic effect in SNL rats. Altogether, these results contribute to explain epidemiological studies demonstrating increased cigarette consumption in chronic pain individuals.

16 - Dynamic encoding of aversive pain perception within hierarchical neural ensembles

Gregory Corder[1], Biafra Ahanonu[1], Benjamin Grewe[1], Mark Schnitzer[1], Gregory Scherrer[2]

[1]Stanford University, Palo Alto, USA, [2]Stanford University, Stanford, USA

An unpleasant percept dominates the affective dimension of pain, which provides a motivational drive to initiate protective behaviors that limit exposure to noxious stimuli. While detailed mechanisms underlying the sensory detection and spinal processing of nociception have been uncovered, it remains unclear how brain circuits transform this emotionally inert information into an affective pain perception. Injury-induced plasticity within affective circuits, such as the basolateral amygdala (BLA), may lead to a miscoding of sensory information concomitant with the emergence of chronic pain. To identify the principles of nociceptive information coding in the BLA, we used a head-mounted miniature microscope to monitor the calcium activity dynamics of individual BLA neurons in freely behaving mice presented with a diverse set of painful and innocuous stimuli. We tracked the longitudinal dynamics of BLA neural coding across 9,777 cells before and after the development of neuropathic allodynia from a peripheral nerve injury. We found that prior to nerve injury, multidimensional and population vector analysis of Ca^{2+} transients revealed that a unique nociceptive neural ensemble in the BLA, distinct from positive valence ensembles, encodes a diverse array of painful stimuli. Silencing of this ensemble alleviated pain affective-motivational behaviors without altering the detection of noxious stimuli, withdrawal reflexes, anxiety, or reward. After the establishment of neuropathic pain, the ensemble representations of prior innocuous and noxious stimuli became more similar. Collectively, our results identify a neural representation of nociception in the amygdala that is necessary for the instantiation of the negative affective qualities of acute and chronic pain.

17 - Nociceptin/orphanin FQ in the central nucleus of the amygdala selectively reduces oxycodone self-administration in rats with high addiction-like behaviors

de Guglielmo G[1], Kallupi M[1], Maturin L[1], Schweitzer P[1], George O[1]

[1]Department of Neuroscience, The Scripps Research Institute, La Jolla, CA, USA

Over the past 15 years, oxycodone consumption has increased nearly 500%, and opioid-related overdose deaths have quadrupled. Recent work found that the dysregulation of the nociceptin/orphanin FQ (N/OFQ) receptor, may be important after chronic exposure to opioids. We investigated the role of N/OFQ on oxycodone addiction in heterogeneous stock (HS) rats, a unique outbred strain with high genetic variability that mimics genetic diversity in humans. We used oxycodone self-administration (150 µg/kg/inj, 12 h/session) combined with progressive-ratio responding and withdrawal-induced hyperalgesia. We measured robust individual differences and established an Addiction Index as a comprehensive evaluation of addiction-like behaviors for each individual, identifying rats with high (HA) and low (LA) addiction-like behavior. We found that HA rats, compared with LA rats, exhibited a significant increase in spontaneous g-aminobutyric acid (GABA) ergic transmission, reflected by isolated spontaneous inhibitory postsynaptic currents (sIPSCs), in the central nucleus of the amygdala (CeA). Superfusion of the CeA slice with N/OFQ (500 nM) decreased spontaneous GABA sIPSCs more in HA rats, suggesting that low levels of N/OFQ in the CeA may be responsible for high GABA release and high oxycodone intake. Finally, intra-CeA N/OFQ infusions (1 µg/site) significantly reduced oxycodone intake in HA rats, but not in LA rats suggesting that the downregulation of N/OFQ levels in the CeA may be responsible for the hyperGABAergic tone in the CeA of HA rats. The development and repurposing of small molecules that target the N/OFQ system may have therapeutic efficacy in the treatment of opioid use disorder, particularly in heavy users.

18 - Role of mu-opioid receptors in indirect-pathway striatal neurons in cocaine reward

Lauren K. Dobbs[1], Carolyn Bowering[1], Aya Matsui[1], Veronica A. Al[1]

[1]National Institute on Alcohol Abuse and Alcoholism, NIH

Cocaine-induced enhancement of dopamine levels within the striatum is important for cocaine's rewarding and stimulating effects. Interestingly, though, accumulating evidence from constitutive knockout models and pharmacological manipulations indicates that striatal opioid peptides and receptors also contribute to cocaine seeking and taking. Within the striatum, the mu-opioid receptor (MOR) is expressed in both the indirect- and direct-pathway medium spiny neurons (iMSNs and dMSNs, respectively), and thus the specific population of MORs contributing to cocaine reward remains unknown. Here, we generated mice with a selective deletion of MORs from iMSNs (iMSN-MORKO) and tested cocaine-mediated reward and locomotion. iMSN-MORKO mice had a 48% reduction of MOR mRNA in the striatum, with no change in expression in the periaqueductal gray. iMSN-MORKO mice were slower to acquire a cocaine conditioned place preference, suggesting MORs expressed in iMSNs facilitate conditioned cocaine reward. We next used slice electrophysiology to probe the functional role of MORs in iMSNs on striatal synaptic transmission. Application of met-enkephalin or the MOR-agonist DAMGO inhibited spontaneously-evoked $GABA_A$-mediated inhibitory post-synaptic currents (IPSCs) when measured in dMSNs, as well as IPSCs evoked by optogenetic stimulation of iMSN axon collaterals. These findings suggest that activation of MORs in iMSNs has an effect similar to dopamine D2 receptor activation in iMSNs on restraining collateral GABA transmission. Preliminary data suggest iMSN-MORKO mice are basally hyperactive and have an enhanced cocaine locomotor response. Future experiments will follow-up on this finding and also determine whether MORs in iMSNs are functionally lost in iMSN-MORKO mice using slice electrophysiology.

19 - Synthesis of FGF21, a Hormone Which Modulates the Dopamine Reward Pathway, Is Increased by GSK3 and HDAC Inhibitors

Louben Dorval[1], Brian I. Knapp[2], Jean M. Bidlack[3]

[1]Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, [2]University of Rochester, [3]University of Rochester

Fibroblast growth factor 21 (FGF21), a regulator of glucose and lipid metabolism, acts as a global starvation signal to modulate energy expenditure and suppress growth. Expressed in liver, brown adipose tissue, glia, and neurons, FGF21 crosses the blood-brain barrier and acts centrally to induce the sympathetic nervous system. Previous studies have shown that FGF21 decreases alcohol and sweet preference in mice. Furthermore, the effects of FGF21 on taste preference correlated with a decrease in dopamine levels in the nucleus accumbens, a primary brain region in the reward pathway. Therefore, FGF21 may also modulate the effects of morphine and other opioids in the reward pathway. This current study explored the effects of histone deacetylase (HDAC) and glycogen synthase kinase3 (GSK-3) on FGF21 mRNA expression levels. C6 glioma cells were treated with HDAC inhibitors, trichostatin A (TSA), vorinostat (SAHA), and valproate (VPA) and GSK-3 inhibitors, LiCl, 1-azakenpaullone (1-aza), SB-216763, and TWS119. FGF21 mRNA was upregulated (10-70 fold) by HDAC inhibitors. GSK-3 inhibitors also upregulated FGF21 mRNA expression. Notably, the specific GSK-3binhibitors TWS119 and 1-aza induced a higher increase (5-20 fold) in FGF21 mRNA expression compared to the nonselective GSK-3(a-b) inhibitors (1.7-3 fold). The increase in FGF21 mRNA levels by the HDAC and GSK inhibitors was both time- and concentration- dependent. These results demonstrate that GSK-3 and HDAC are targets in the regulation of FGF21 expression, which will be key in understanding the different effects of FGF21 in the brain. (Supported by NIH grant DA044766)

20 - G-protein biased kappa opioid receptor agonist attenuates cocaine self-administration in male mice

Alexandra Dunn[1], Jose Erazo[1], Amelia Dunn[1], Kyle Windisch[1], Brian Reed[1], Mary Jeanne Kreek[1]

[1]The Rockefeller University, New York, USA

INTRODUCTION: Biased kappa opioid receptor (KOR) agonism, in which compounds selectively activate specific intracellular signaling pathways (e.g. G-protein but not β-arrestin mediated pathways), has emerged as a potentially viable therapeutic approach for the treatment of cocaine addiction. However, the specific effects of biased KOR agonists have not been fully elucidated *in vivo*. We investigated the effects of a recently characterized G-protein biased KOR agonist, *N*-butyl-*N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine (BPHA; Dunn et al. 2018), on cocaine self-administration in mice.

METHODS: Adult male C57BL6 mice (n=25) were initially trained to respond for 0.5mg/kg/infusion cocaine during daily 2-hour FR1 intravenous self-administration sessions. Following acquisition, mice were pretreated with 30mg/kg BPHA on two consecutive sessions, followed by 45mg/kg BPHA for a single session. On a subsequent day, mice received 3mg/kg LY2444296, a short-acting KOR antagonist, prior to 45mg/kg BPHA pretreatment of cocaine self-administration.

RESULTS AND DISCUSSION: 30mg/kg BPHA pretreatment did not significantly decrease cocaine intake, whereas pretreatment with 45mg/kg BPHA significantly decreased cocaine self-administration (49 +/- 11%) compared to vehicle ($p<0.05$). Attenuation of cocaine intake by 45mg/kg BPHA was not blocked by pretreatment with a single dose of LY2444296 (3mg/kg), indicating that the BPHA-induced reduction in cocaine intake may not be KOR-mediated. Further studies are needed to elucidate the mechanism(s) of action and involvement of the KOR system in BPHA-induced attenuation of cocaine self-administration.

These studies were supported by the Robertson Therapeutic Discovery Fund (BR, MJK) and the Dr. Miriam and Sheldon G. Adelson Research Foundation (MJK).

21 - Identifying kappa opioid receptor-protein interactions following activation by unbiased and G-protein biased ligands

Wenzhu Zhang[1], Amelia Dunn[1], Brian Reed[1], Brian Chait[1], Mary Jeanne Kreek[1,2]
[1]Rockefeller University, New York, USA, [2]Rockefeller University, New York, USA

Biased agonists selectively activate certain signaling pathways downstream of their receptor. They are being developed for several drug targets including the kappa opioid receptor (KOR), a potential target for addiction. Biased ligands differentially effect signaling by inducing distinct conformational changes in the receptor. It is poorly understood how these conformations lead to differential signaling. Distinct receptor conformations possibly lead to specific receptor-protein interactions, driving signaling differences. In this study, changes in KOR-protein interactions were identified following drug treatmenT and compared across treatment groups. HEK293 cells expressing tagged-KOR were treated with U69,593, MCBPHA (*N*-methylcyclobutyl-*N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine) or BPHA (*N*-butyl- *N*-phenylethyl-*N*-3-hydroxyphenylethyl-amine). MCBPHA and BPHA are recently described biased KOR agonists that selectively activate G-protein over arrestin signaling when compared to U69,593 (Dunn, et. al. 2018, *IJNP,* in press). Immunoprecipitation of the receptor with stable-isotope labeled amino acids in cell culture (SILAC) followed by mass spectrometry was used to identify changes in receptor-protein interactions after drug treatment. Significant correlations were found between treatment by the two biased agonists, BPHA and MCBPHA. There was no correlation between the changes in receptor-protein interactions caused by U69,593 and either biased agonist. This study demonstrates that changes in receptor-protein interactions can be identified by immunoprecipitation followed by mass spectrometry, and suggests that changes in receptor-protein interactions may be correlated to extent of observed ligand bias. Candidate receptor-protein interactions identified will be further characterized for their role in KOR signaling. Supported by the Robertson Therapeutic Development Fund and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

22 - Preserved functional selectivity of MOR ligands in HEK293 cells and MOR-Venus DRG neurons reveals the physiological reality of biased signaling

Aliza T. Ehrlich[1,2], Meriem Semache[3], Dillon Dafonte[1], Leonie Runtz[1], Christine Colley[1,4], Amina Mezni[1,4], Christian LeGouill[3], Florence Gross[3], Viktoriya Lukasheva[3], Emmanuel Darcq[1], Michel Bouvier[3], Brigitte L. Kieffer[1,2]

[1]Department of Psychiatry, McGill University, Douglas Hospital Research Center, Montréal, QC, Canada, [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch-Graffenstaden, France, [3]Department of Biochemistry, Université de Montréal, Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, QC, Canada, [4]Department of Biochemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom

Over-production and prescription of opioids has caused a major crisis of opioid abuse, addiction and overdose with a global burden most strongly impacting North America. Biased agonists for Mu Opioid Receptors (MORs) are emerging drugs that are proposed to produce the desired G protein-effect of pain relief without unwanted βarrestin2-effects such as euphoria or respiratory depression. In this study, *in vitro* and *ex vivo* assays were assessed by employing 10 preclinical (Met-Enkephalin, DAMGO, Endomorphin-1, TRV130, PZM21) and clinical MOR agonists, (Fentanyl, Loperamide, Oxycodone, Morphine, Buprenorphine) to examine which drugs possess biased properties and should be promoted in the clinic and which assays are useful for the development of safer opioids. We employed transfected MORs in HEK-293 cells and used resonance energy transfer (RET) biosensors to established signaling signatures for 10 compounds. To examine endogenous MORs, we generated and fully characterized new knock-in mice expressing physiological levels of functional MORs. Agonist-induced MOR-Venus internalization and endosome co-localization were examined in MOR-Venus neurons and correlated well with over-expressed MORs in HEK-293 cell profiling, despite significant differences in levels of receptors and effectors. In both systems, the partial MOR agonist, buprenorphine's profile was uniquely identifiable. This study demonstrates, that observations in transfected systems are indicative of native MOR function and buprenorphine may be a better alternative for naïve opioid, chronic pain patients.

23 - Nociception-evoked impulsivity in rats and its reversal with morphine

Nidia Espinoza[1], Javier Ibias[1], Arbi Nazarian[1]

[1]Western University of Health Sciences

According to the U.S. National Institute on Drug Abuse, about 21-29% of patients who are prescribed opioids for chronic pain misuse them and 8-12% develop an opioid use disorder. Opioid use disorders and addiction to opioids is believed to be significantly impacted by changes in impaired decision making, and impulsivity. The goal of this project was to establish a nociception-evoked model of impulsivity in rats; to determine if morphine reduces impulsivity; and examine whether impulsivity and/or morphine effects are sexually dimorphic. To measure impulsive choice, animals were trained in a delay discounting task for food pellets using a delay schedule of 0, 8, 16, and 32 seconds. After training was complete and baseline measurements were established, rats received intraplantar Complete Freund's Adjuvant (CFA). Animals were then subjected to the delay discounting task again under two treatment groups, saline and morphine for two weeks. The results showed that CFA treatment increased delayed discounting (i.e. more impulsive) and this effect was marginally more robust in female than male rats. Moreover, morphine treatment decreased nociception-evoked delayed discounting. The effects of morphine were gradually lost as the treatment continued during the two weeks testing period, suggestive of the development of morphine tolerance. The present findings demonstrate nociception-evoked impulsivity in rats that is sensitive to opioids and highlights the need for continued examination of impulsivity as a potential measure of the affective component of pain, as well as a factor in examining opioid abuse in cases of chronic pain conditions.

24 - Endomorphin analog treatment expedites recovery from chronic inflammatory pain and protects against latent sensitization

Amy Feehan[1], James E Zadina[1,2,3,4]

[1]Tulane Brain Institute, [2]Department of Medicine Tulane University, [3]Department of Pharmacology Tulane University, [4]SE Louisiana Veterans HCS, New Orleans, LA

Opioids are unrivaled for relief of moderate to severe pain, but in addition to well-known side effects, including abuse potential, opioids have also been shown to paradoxically cause pain sensitization over time, thus limiting their use. Morphine, for example, worsens and prolongs mechanical allodynia and thermal hyperalgesia after Complete Freund's Adjuvant (CFA) injection into the hind paw. ZH853 is a novel endomorphin analog that has reduced side effects, including abuse liability, and superior analgesia compared to morphine. For preclinical development, we wanted to test whether chronic drug administration, before or after inflammatory pain, would induce sensitization of pain, as morphine does. In the current study, adult male Sprague-Dawley rats were implanted with intrathecal catheters and administered drug via osmotic minipump for 5 days. Inflammation was induced by CFA injection to a hind paw and sensitivity was measured with von Frey and Hargreaves testing. Studies have also shown gait impairments in this paradigm, and we used the CatWalk XT to determine whether morphine and/or ZH853 made these impairments worse. When all animals had recovered to baseline, naltrexone was used to probe whether latent sensitization had developed. We found that morphine prolonged and intensified hypersensitivity in both paradigms, while ZH853 reduced the total time and intensity of hypersensitivity, and protected against CFA-induced latent sensitization versus vehicle and morphine treatment. This study suggests that, unlike currently used opioids, ZH853 can treat chronic inflammatory pain effectively without causing further sensitization of the pain system.

25 - Intrinsic G-protein efficacy, not ß-arrestin 2 dependent signaling, confers reduced opioid-induced respiratory depression

Alexander Gillis[1], Arsalan Yousuf[1], Preeti Manandhar[2], Setareh Sianati[1], Andrea Kliewer[3], Stefan Schulz[3], MacDonald J Christie[1]

[1]University of Sydney, [2]Macquarie University, [3]Friedrich Schiller University, Jena, Germany

ß-arrestin 2 (ß-arr2) dependent signaling via the μ-opioid receptor (MOPr) has been proposed to contribute to opioid analgesic side effects. We studied opioid-induced respiratory depression using whole-body plethysmography and antinociception in a hotplate assay. ß-arr2 knockout (KO) and phosphorylation deficient, arrestin non-recruiting MOPr knockin mice (11S/T-A), were used to study the contribution of ß-arr2 dependent mechanisms to opioid-induced respiratory depression. Morphine severely depressed respiration in wild-type, ß-arr2 KO and 11S/T-A mice, with no significant genotype effect on potency or maximal effect. G-protein biased agonists that do not strongly induce MOPr-ß-arr2 interactions have been developed and appear to have favourable therapeutic profiles in preclinical and early clinical trials. *In vitro* studies of G-protein activation using GIRK channels with receptor inactivation by partial irreversible antagonism showed that G-protein biased agonists PZM21 and TRV130 have lower intrinsic efficacy than morphine. *In vivo*, these compounds induced similar respiratory depression to morphine but with lower maximal effect. The very low efficacy agonist buprenorphine has been characterised as having a low ceiling effect in respiratory depression, leading to the hypothesis that the favourable profiles of PZM21 and TRV130 are due to their low efficacy. Heterozygous MOPr K.O. (+/-) animals have reduced receptor expression and are a reasonable genotypic model of low efficacy agonists activating a fraction of receptors. In MOPr +/- animals morphine produces full analgesia, but only partially depresses respiratory frequency compared to wild-type. Low intrinsic G-protein efficacy, rather than ß-arrestin 2 dependent signaling, may explain reduced respiratory depression induced by apparently G-protein biased agonists.

26 - Exploring rapid desensitization of mu-opioid receptors induced by G protein-biased agonists

Sam Groom[1], Alex Conibear[2], Alex Disney[1], Stephen Husbands[1], Yangmei Li[3], Lawrence Toll[4], Graeme Henderson[2], Eamonn Kelly[2], Chris Bailey[1]

[1]University of Bath, Bath, UK, [2]University of Bristol, Bristol, UK, [3]Torrey Pines Institute for Molecular Studies, Port St. Lucie, USA, [4]Florida Atlantic University, Boca Raton, USA

G-protein-biased agonists of mu-opioid receptors (MOPrs) have the potential to induce less receptor desensitization and tolerance than traditional opioids. The aim of this study was to investigate MOPr desensitisation induced by 2 potential G-protein-biased MOPr agonists: PZM21 (Manglik *et* al., (2016) Nature 537:185-190) and 'Compound 1' (Tyr-c[D-Lys-Phe-Tyr-Gly]) (Li *et al*., (2016) J Med Chem 59:1239-1245). In HEK293 cells, agonist-induced phosphorylation of Ser375 (a GRK substrate) on MOPr after agonist exposure was measured using Western blotting. Whole-cell voltage-clamp recordings were taken from rat locus coeruleus neurons. MOPrs couple to GIRK channels, with the amplitude of observed K^+ currents providing a measure of MOPr activation. Opioids (30μM) were perfused for 10 minutes, with reductions in evoked-current amplitude tracked as a measure of receptor desensitisation. Morphine and DAMGO both induced Ser375 phosphorylation. In contrast, PZM21 and Compound 1 induced negligible Ser375 phosphorylation, suggesting they are G-protein-biased. The amplitude of K^+ currents evoked by morphine, PZM21 and Compound 1 were broadly similar and significantly less than those evoked by Met-Enkephalin. This indicates that, like morphine, PZM21 and Compound 1 are partial MOPr agonists. While PZM21 induced less MOPr desensitization than morphine, Compound 1 induced robust MOPr desensitization of greater magnitude than morphine. These data indicate that although G protein-biased agonists at MOPrs induce minimal Ser375 phosphorylation and promote little arrestin recruitment, their abilities to induce rapid MOPr desensitization can be vastly different. Subsequent experiments are ongoing to investigate the intracellular mechanisms involved, and the *in vivo* consequences.

27 - Preclinical Characterization of Mitragyna Speciosa Alkaloids for the Treatment of Alcohol Use Disorder

Anna Gutridge[1], Meridith Robins[1], Rajendra Uprety[2], Robert J. Cassell[1], Gavril Pasternak[2], Susruta Majumdar[2,3], Richard van Rijn[1]

[1]Purdue University, Medicinal Chemistry and Molecular Pharmacology and Purdue Institute of Integrative Neuroscience, West Lafayette, IN, USA, [2]Memorial Sloan Kettering Cancer Center, Molecular Pharmacology and Chemistry Program, and Neurology, New York, NY, USA., [3]Center for Clinical Pharmacology, Washington University School of Medicine and St. Louis College of Pharmacy, St. Louis, MO, USA

Mitragyna speciosa (kratom) is an alkaloid containing plant gaining traction in opioid-dependent individuals to mitigate opioid withdrawal effects. Several alkaloids present in kratom target opioid receptors, but interact in a manner which does not recruit β-arrestin. Mu opioid receptor (μOR) drugs biased towards G-proteins over β-arrestin show advantages including reduced respiratory depression and dependence liability at equianalgesic doses to morphine. We have previously demonstrated delta opioid receptor (δOR) agonists with minimal β-arrestin2 recruitment decrease voluntary alcohol consumption in C57BL/6 mice; therefore, we hypothesized kratom-derived alkaloids will decrease alcohol consumption but not display robust conditioned place preference (CPP). We characterized kratom and the kratom-derived alkaloids mitragynine, 7-hydroxymitragynine, speciogynine and paynantheine for G-protein signaling and β-arrestin2 recruitment in vitro at δORs and μORs. Behavioral effects of alkaloid administration were investigated in a limited-access, 2-bottle choice 10% alcohol paradigm, locomotor test and acute CPP to assess potential reward. Kratom alkaloids showed G-protein signaling (inhibition of cAMP production), but undetectable β-arrestin2 recruitment at μOR and δOR. Kratom, mitragynine, paynantheine and 7-OH-mitragynine dose-dependently reduced alcohol intake in mice. Reduction of alcohol intake by 7-OH-mitragynine was attenuated in δOR-knockout mice. Mitragynine, kratom and 7-OH-mitragynine show attenuated place preference at therapeutic doses, compared to morphine. Our results indicate kratom alkaloids most likely reduce alcohol use by targeting δORs in addition to in a G-protein biased manner. We propose kratom alkaloids may be a useful chemical template to develop novel pharmacological interventions for the treatment of alcohol use disorder.

28 - Evaluation of two peripherally-restricted kappa-opioid receptor agonists for safer analgesia without CNS liabilities

Hannah Harris[1], Shyam Kamble[1], Shainnel O. Eans[1], Jessica M. Medina[1,2], Michelle L. Ganno[2], Marcello A. Giulianotti[2], Richard A. Houghten[2], Bonnie A. Avery[1], Jay McLaughlin[1]

[1]University of Florida, Gainesville, USA, [2]Torrey Pines Institute for Molecular Studies

Clinically-used mu opioid receptor (MOR) agonists produce robust analgesia, but central nervous system (CNS) action also results in liabilities such as sedation, respiratory depression and abuse. Peripherally-located kappa opioid receptors (KOR) are thought to mediate significant analgesia void of many side effects produced by centrally acting opioids. Two potential candidates previously identified from a library of 6,250,000 tetrapeptides, ffir-NH_2 and ff(nle)r-NH_2, demonstrated affinity (IC_{50} = ~2 nM) and selectivity for the KOR (Dooley et al., 1998). This study characterized ffir-NH_2 and ff(nle)r-NH_2 in mice after central (i.c.v.), intraperitoneal (i.p.) or oral (p.o.) administration for dose-dependent, peripherally-restricted opioid-receptor mediated antinociception and potential liabilities. In the 55°C warm-water tail-withdrawal test, each tetrapeptide produced robust antinociception after all routes of administration, with a response after i.p. administration that was 9.9- and 21.0-times more potent than morphine, respectively (ffir-NH_2 ED_{50}=0.53 (0.08-2.50) mg/kg; ff(nle)r-NH_2 ED_{50}=0.25 (0.05-1.08) mg/kg). Pretreatment with opioid antagonists and testing in KOR KO mice demonstrated KOR selectivity, and naloxone methiodide pretreatment suggested activity that was peripherally-restricted. Supporting this, pharmacokinetic analysis following i.v. administration of either tetrapeptide demonstrated plasma levels consistent with the duration of antinociception, but no detected tetrapeptide in brain tissue. The tetrapeptides further demonstrated dose-dependent antinociception and antiallodynia in mouse models of inflammatory and neuropathic pain. High peripheral doses of ffir-NH_2, but not ff(nle)r-NH_2, increased urine production, motor impairment and respiratory depression, but neither tetrapeptide demonstrated place conditioning. Together, these data show that ffir-NH_2 and ff(nle)r-NH_2 produce rapid, potent, peripherally-restricted antinociception with fewer side effects.

29 - Specific PKC isoform mediates ongoing pain in a mouse model of migraine

YING HE[1], Zaijie Jim Wang[2]

[1]University of Illinois at Chicago, Chicago, USA, [2]University of Illinois at Chicago, Chicago, USA

Migraine is one of the most common neurological disorders characterized by recurrent attacks of typically throbbing and unilateral headache, affecting up to 20 % of the population worldwide. Despite the high prevalence and severity of this primary headache disorder, limited progress has been made in understanding and treating migraine. By characterizing and validating a mouse migraine model, this study aimed to investigate the functional contribution of PKC isoforms in migraine. Systemic administration of NO donor, nitroglycerin (NTG) induced significant and prolonged mechanical hypersensitivity in female mice. We found the presence of migraine-like ongoing pain in mice after chronic intermittent treatment of NTG. The peptide antagonist of calcitonin gene related peptide (CGRP), αCGRP (8-37) effectively blocked ongoing pain and elicited pain relief-induced CPP in NTG-treated mice. Prominent activation of specific PKC isoform was observed in the superficial laminae of the spinal cord dorsal horn in chronic NTG-treated mice. Functional inhibition of the PKC isoform significantly attenuated ongoing spontaneous pain in chronic NTG-treated mice. Furthermore, we recapitulated the NTG-triggered migraine model in PKC isoform-null mice. In response to repeated administration of NTG, ongoing spontaneous pain was not developed in mice lacking the specific PKC isoform. This study is the first to identify the presence of ongoing pain in mice treated with nitroglycerin, a known human migraine trigger that closely resembles the common manifestation of spontaneous migraine attacks in humans. These findings demonstrated a critical regulatory role of spinal PKC in migraine pathophysiology, which may offer new pharmacological targets for anti-migraine treatment.

30 - Mechanisms of presynaptic suppression of GABA release by opioid receptors in the hippocampus

X. Jenny He[1], Matthew Banghart[1]

[1]University of California San Diego

Opioid receptors are prominent in interneurons of the hippocampus where they modulate GABAergic inhibition onto pyramidal cells. Mu opioid receptor (MOR) activation is known to suppress GABA release from parvalbumin (PV) basket cells in the CA1 region, which positions opioids to affect spike timing, oscillations, and synchronization of neuronal populations in the hippocampus. Although the delta opioid receptor (DOR) is also prominent in the hippocampus, its functional role in distinct interneuron classes has not been established, nor have the molecular mechanisms that underlie MOR and DOR-mediated suppression of synaptic transmission. Using a combination of electrophysiology and optogenetics, we discovered that both MOR and DOR strongly inhibit GABA release from PV+ basket cells in a mutually-occlusive manner. Although both receptors appeared to utilize a presynaptic mechanism, they were differentially prone to desensitization. We further characterized the molecular mechanisms of opioid receptor-mediated suppression using two-photon calcium imaging and pharmacology and found evidence for modulation of processes downstream of voltage-gated calcium channel activation. Because the small size of presynaptic terminals in central neurons makes them largely inaccessible to pharmacological manipulations with membrane-impermeant peptide probes, we also explored a genetic approach for identifying the molecular effectors downstream of receptor activation. Our results suggest a previously unappreciated role for DOR in modulating perisomatic inhibition in the hippocampus and reveal the molecular mechanisms by which both MOR and DOR regulate GABA release from PV basket cells in CA1.

31 - Fentanyl Depression of Respiration in Mice

R.Hill [1], W.L.Dewey [2], E.Kelly [1], G.Henderson [1]

[1]University of Bristol, [2]Virginia Commonwealth University

In 2016 more opioid overdose deaths in the USA involved fentanyl-based opioids than heroin or prescription opioids such as oxycodone. It has been suggested that fentanyl is resistant to naloxone reversal. This investigation examined fentanyl depression of respiration, reversal by naloxone and acute tolerance to fentanyl.

Experiments were conducted in male mice (CD-1). Respiration was measured by whole body plethysmography in mice breathing either 95% air/5% CO_2 or 100% air. All drugs were administered intraperitoneally.

Fentanyl (0.05-1.35 mg/kg) dose dependently depressed respiration (20-75%). Morphine (1-10 mg/kg) also dose dependently depressed respiration (15-40%). Acute administration of morphine (10 mg/kg) or fentanyl (0.15 mg/kg) produced equivalent levels of respiratory depression (~ 40% decrease in minute volume). Administration of naloxone (0.3 and 1 mg/kg) fully reversed morphine depression of respiration, but not that induced by fentanyl. Administration of 3 mg/kg naloxone fully reversed both morphine and fentanyl induced depression of respiration.

Administration of two doses of fentanyl (0.15 mg/kg) 2 hrs apart resulted in significantly less depression of respiration by the second dose of fentanyl compared to the first. Pre-treatment with the GRK2/3 inhibitor compound 101 (10 mg/kg) or the PKC inhibitor calphostin C (45 mg/kg) did not prevent this tolerance. Also, compound 101 did not decrease acute fentanyl depression of respiration suggesting that it did not involve GRK2/3 and arrestin binding.

These data indicate that fentanyl is more resistant to naloxone antagonism compared to a classical opioid agonist such as morphine.

32 - Co-administration of chemokine receptor antagonists with opioids: Potentiation of analgesic effects on incisional pain in rats

Saadet Inan[1], Mia N Watson[1], Menahem Doura[1], Christopher S Tallarida[1], Xiaohong Chen[1], Joseph J Meissler[1], Ellen B Geller[1], Scott M Rawls[1], Alan Cowan[1], Toby K Eisenstein[1], Martin W Adler[1]

[1]Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140

Our laboratories have shown that there is cross-desensitization between opioid and chemokine receptors and that chemokines can inhibit the analgesic activity of opioids. Here, we investigated whether co-administration of chemokine receptor antagonists (CRAs) with opioids would enhance the analgesic effect of opioids on incisional pain in rats. We chose morphine and oxycodone, which are the most commonly prescribed opioids for pain. Male Sprague-Dawley rats (200-230 g) underwent incisional surgery on the left hind paw. Pain responses were evaluated measuring mechanical allodynia at various times post-surgery from 15 to 360 minutes. Morphine, oxycodone, maraviroc (a CCR5 antagonist), AMD3100 (a CXCR4 antagonist), or their vehicles were injected s.c., alone or in combination, at 25 minutes post-surgery. We found that a) morphine and oxycodone significantly reversed mechanical allodynia in a time- and dose-dependent manner; b) neither maraviroc nor AMD3100 had an effect by themselves; c) addition of AMD3100 significantly shifted both morphine and oxycodone dose-responses to the left (1.8- and 2.0-fold, respectively); d) addition of maraviroc shifted the dose-response curves of morphine (2.4-fold) and oxycodone (1.5-fold) to the left; and e) addition of the two CRAs with morphine further shifted the dose-response curve to the left (3.3-fold). These results indicate that the combination of CRAs with opioids permits use of lower doses of the opioids to achieve an analgesic effect equal to that obtained with opioids alone. Using opioids in lower doses has the potential to reduce unwanted side effects and the development of tolerance and dependence. DoD grant W81XWH-15-1-0252; NIDA P30 DA013429

33 - Multidisciplinary Approach in the Optimization of a Highly Selective Sigma-1 Receptor Antagonist

Sebastiano Intagliata[1], Theresa A. Kopajtic[2], Jonathan L. Katz[2], Michelle L. Ganno[3], Abhisheak Sharma[4], Bonnie A. Avery[4], Shainnel O. Eans[3,5], Jay P. McLaughlin[5], Christopher R. McCurdy[1]

[1]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA, [2]Psychobiology Section, Intramural Research Program, Department of Health and Human Services, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA, [3]Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL 34987, USA, [4]Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA, [5]Department Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA

Chronic pain is one of the leading causes of adult disability in the United States, and it is estimated that about 20 million individuals suffer from some form of peripheral neuropathy. Chronic pain conditions represent a major health-care cost, due to both the medical expenses and the lost work days. Current treatments possess notable liabilities such as tolerance, respiratory depression, and addiction in relation to opioids; or sedation and dizziness in the case of antiepileptic drugs. Thus, novel analgesics which have fewer side effects than current pain treatments are still needed. The sigma-1 receptor (S1R) has been recently proposed as novel target, due to the ability of the S1R antagonists to modulate the pain effectively with no liabilities. Our research group has developed **CM304** (**FTC146**) the most potent and selective S1R ligand to date for this receptor, which has entered into clinical trials as a positron emission tomography and magnetic resonance imaging (PET/MRI) diagnostic agent for peripheral nerve injury. Despite **CM304** demonstrating antialloydinic activity in rodents, it possess a short half-life and poor oral bioavailability, rendering it unsuitable for therapeutic development. Herein, we present a multidisciplinary approach for the lead-optimization of a highly selective S1R antagonist. The methods include: synthesis and structure activity-relationship (SAR) investigation, *in vitro*/*in vivo* pharmacology evaluation, and *in vitro*/*in vivo* pharmacokinetic studies of novel **CM304** analogs. Synthetic pathways of new compounds along with preliminary binding data, and *in vitro* metabolic stability are reported being reported.

34 - Evaluation of mixed efficacy opioid ligands in drug discrimination assays

Emily Jutkiewicz[1], Ashleigh L Matthews[1], Henry I Mosberg[1]

[1]University of Michigan, Ann Arbor, USA

Mixed efficacy opioid ligands have been developed as alternatives to mu opioid receptor agonists for producing analgesia with potentially fewer side effects, such as tolerance, physical dependence, and abuse liability. To determine if mixed efficacy ligands produce similar interoceptive effects to receptor-selective opioid agonists, the current study evaluated the effects of mixed efficacy opioid ligands in rats trained to discriminate either a selective mu- or delta-opioid receptor agonist. Drug discrimination assays are useful for categorizing common drug stimulus effects, predicting potential abuse liability, and obtaining quantitative pharmacological analysis of drug effects in vivo. For these experiments, rats were trained to discriminate either 3 mg/kg morphine or SNC80 from saline on a fixed ratio 10 schedule of reinforcement in a food-reinforced, drug discrimination paradigm. In rats trained to discriminate morphine, hydrocodone and buprenorphine fully generalize to the discriminative stimulus effects of morphine. In rats trained to discriminate SNC80, the SNC80 analogs AZD2327 and DPI287 fully generalized to the discriminative stimulus effects of SNC80. The peptidomimetic MOR agonist/DOR antagonist AAH8 produces antinociception in rats but fails to generalize to either the discriminative stimulus effects of morphine or SNC80. Similarly, the MOR agonist/DOR agonist MMP2200 does not generalize to the discriminative stimulus effects of morphine but fully generalizes to the discriminative stimulus effects of SNC80. These data suggest that incorporating high affinity, DOR activity with a MOR agonist may alter the discriminative stimulus effects of MOR agonists. Supported by DA041565 and DA003910.

35 - An enzymatic approach reverses nicotine dependence, decreases compulsive-like intake and prevents relapse in rats

Marsida Kallupi[1,2], Song Xue[2], Bin Zhou[2], Kim Janda[2], Olivier george[1,2]

[1]The Scripps Research Institute, [2]The Scripps Research Institute, La Jolla, USA

Tobacco use disorder is the leading cause of disease and preventable death worldwide, but current medications that are based on pharmacodynamics have low efficacy. Novel pharmacokinetic approaches to prevent nicotine from reaching the brain have been tested using vaccines, but such efforts have failed because antibody affinity and concentration are not sufficient to completely prevent nicotine from reaching the brain. In the present study, we provide preclinical evidence of the efficacy of an enzymatic approach to reverse nicotine dependence, reduce compulsive-like nicotine intake, and prevent relapse in rats with a history of nicotine dependence. Chronic administration of NicA2-J1, an engineered nicotine-degrading enzyme that was originally isolated from *P. putida* S16, completely prevented nicotine from reaching the brain and reversed somatic signs of withdrawal, hyperalgesia, and irritability-like behavior in nicotine-dependent rats with a history of escalation of nicotine self-administration. NicA2-J1 also decreased compulsive-like nicotine intake, reflected by responding despite the adverse consequences of contingent footshocks, and prevented nicotine- and stress (yohimbine)-induced relapse. These results demonstrate the efficacy of enzymatic therapy in treating nicotine addiction in advanced animal models and provide a firm grounding for the development of biological therapies for smoking cessation in humans.

36 - Pharmacological effects of modifying the N-substituent of hydromorphone

Sophia Kaska[1], Rachel S. Crowley[1], Thomas C. Irvin[2], Meining Wang[2], Arthur E. Jacobson[2], Thomas E. Prisinzano[1], Kenner C. Rice[2]

[1]Department of Medicinal Chemistry, School of Pharmacy, The University of Kansas, Lawrence, KS, [2]Drug Design and Synthesis Section, Molecular Targets and Medications Discovery Branch, IRP, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism, NIH, Department of HHS, Bethesda, MD, United States

Hydromorphone (Dilaudid) is a potent and effective opioid analgesic for the treatment of pain. While opioid medications are effective analgesics for the treatment of pain through G-proteins, they present a number of undesired side effects, such as tolerance, dependence, and respiratory depression. These side-effects have been said to be mediated by beta-arrestin recruitment. Importantly, structural modification of hydromorphone has demonstrated the relationship of its substituents and the effect on biological activity. For instance, when the *N*-methyl substituent is replaced with an *N*-phenethyl substituent in some opioids, their activity can be transformed from agonist to antagonist. In this collaboration, a number of N-derivatives of opioids have been generated to explore their agonist and antagonist activity, as well as to evaluate beta-arrestin recruitment. The N-phenethyl derivative of hydromorphone is relatively inert, but when a para-nitro group is added, the agonist activity is transformed. Interestingly, other N-substituents have also converted weak mu agonists into more potent agonists. Furthermore, to evaluate the intracellular signaling mechanisms that lead to the negative side effects of opioid medications, beta-arrestin recruitment was investigated. Two of these compounds demonstrate some recruitment of beta-arrestin, while further evaluation of other compounds for bias towards this signaling pathway is in progress. Overall, these data suggest that the N-substituent of an opioid is important for dictating the agonist function and activity of a molecule and that elucidation of these structure-activity relationships will assist in the development of effective opioid analgesics with reduced side effects.

37 - A selective bivalent antagonist for the mu-delta opioid receptor heterodimer potentiates oxymorphone anti-nociception while sharply reducing morphine withdrawal

Attila Keresztes[1], Paul Nguyen[1], Keith Olson[1,2], Victor Hruby[2], John M. Streicher[1]

[1]Department of Pharmacology, College of Medicine, University of Arizona, Tucson AZ USA, [2]Department of Chemistry and Biochemistry, College of Science, University of Arizona, Tucson AZ USA

Experimental observations over decades have shown that the mu (MOR) and delta (DOR) opioid receptor systems interact. One hypothesis for this interaction is that the MOR and DOR heterodimerize to form a distinct signaling unit (MDOR). However, few MDOR-selective tools exist, and no MDOR-selective antagonists, creating controversy as to the role or even existence of the MDOR *in vivo*. We thus created a novel series of bivalent antagonists by linking MOR and DOR antagonist pharmacophores by a variable-length spacer. We found a lead compound D24M with 0.85 nM potency and ≥89 fold selectivity at the MDOR, which was selective for the MDOR in CD-1 mice up to intracerebroventricular (*icv*) doses of 10 nmol. We further tested D24M in mouse models of opioid anti-nociception and dependence. We found that 1 nmol of *icv* D24M strongly potentiated oxymorphone anti-nociception in the tail flick (AUC increase of 52.3%), paw incision (increase of 628%), and chemotherapy-induced peripheral neuropathy pain models (CIPN; increase of 249.6%). Importantly, the MOR antagonist CTAP and the DOR antagonist naltrindole could not replicate this increase, suggesting an MDOR mechanism. We further found that 1 nmol of *icv* D24M given 5 minutes prior to naloxone precipitation of withdrawal strongly blocked jumping behavior in acute and chronic models of morphine dependence, while D24M alone produced no withdrawal behavior. These results suggest that the MDOR has a role in blocking anti-nociception and promoting withdrawal. Our results also suggest that MDOR antagonists could be novel therapeutics to enhance opioid anti-nociception and treat opioid withdrawal.

38 - DPP4 inhibitors, Ile-Pro-Ile (IPI) and vildagliptin produce diverse antihyperalgesic effects following intrathecal administration in inflammatory pain models in rats

Kornél Király[1], Mihály Balogh[1], Bence Varga[1], Dávid Karádi[1], Mahmoud Al-Khrasani[1], Zita Puskár[2], Márk Kozsurek[2], Pál Riba[1]

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, [2]Department of Anatomy, Histology and Embryology, Semmelweis University

We have recently reported the ineffectiveness of Ile-Pro-Ile and vildagliptin following intrathecal administration in tail-flick test. They produced robust opioid-mediated antihyperalgesic action in carrageenan-induced inflammatory pain and a limited non-opioid analgesia in neuropathic pain. Further analysis suggested a different mechanisms, Ile-Pro-Ile acts exclusively through μ receptors while vildagliptin has a mixed action with δ receptor dominancy. The present study was aimed to assess their actions on CFA-induced monoarthritis model and formalin test.

Methods: male Wistar rats (160-220g) were used. CFA-induced hyperalgesia was measured by Randall-Selitto test and dynamic plantar aesthesiometer (DPA) on the 4th day. In formalin test pain behaviors were counted from 0 to 60 min.

The test compounds restored the prehyperalgesic threshold evoked by CFA when Randall-Selitto but not DPA test was applied. Ile-Pro-Ile showed pure μ-mediated effect while vildagliptin had mixed, δ-dominated action. In formalin test only Ile-Pro-Ile was able to significantly reduce pain reactions of the 2nd phase but not the 1st phase. This effect was abolished by μ selective or nonselective opioid antagonists and significantly, though partially, by a selective δ antagonist. Our results are in agreement with our previously published work proving that the two DPP4 inhibitors could activate different analgesic pathways. In both pathways endogenous opioid peptides are involved. Endomorphins or β-endorphin could be involved in the action of Ile-Pro-Ile whereas enkephalins could mediate that of vildagliptin. The Ile-Pro-Ile activated analgesic pathway might play a role in controlling pain transmission in formalin test. The molecular background of these diverse effects need further elaboration.

39 - Evaluation of the analgesic and side-effects of G-protein biased mu-opioid receptor Salvinorin A analogues kurkinorin and kurkinol

Bronwyn Kivell[1], Amy Alder[1], Rachel Crowley[2], Thomas Prisinzano[2]

[1]Victoria University of Wellington, Wellington, New Zealand, [2]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

Development of G-protein biased mu-opioid receptor agonists with reduced β-arrestin signaling are highly sought after for the development of safer pain medications with reduced abuse liability, tolerance, dependence, constipation and respiratory depression. Here we evaluate the analgesic and side-effects of novel G-protein biased mu-opioid agonists kurkinorin and kurkinol based on the structural scaffold of salvinorin A. Analgesic effects were evaluated in C57BL/6 mice using the 50°C hot-water tail withdrawal assay using cumulative administration of kurkinorin or kurkinol and analgesic effects were compared to morphine. Kurkinorin showed equal potency to morphine (ED_{50} 6.43 ± 0.27 (mg/kg)), whereas kurkinol was more potent (ED_{50} values of 2.35 ± 0.25; $p<0.001$). Tolerance was determined following daily administration of morphine (10 mg/kg), kurkinorin (10 mg/kg) or kurkinol (5 mg/kg) and dose-response tail-withdrawal effects evaluated on Day 9. Unlike morphine, which showed significant tolerance from days 5-9 with an ED_{50} on day 9 of 16.35 ± 0.75 (mg/kg), kurkinorin showed reduced tolerance and kurkinol showed no significant tolerance effects (ED_{50} values on day 9 of (7.9 and 3.60 (mg/kg))) respectively. Effects on motor co-ordination were also evaluated using the accelerating rotarod, and latency to fall recorded. Kurkinorin and kurkinol showed a shorter duration of motor impairment effects compared to morphine ($p<0.001$). Morphine significantly inhibited gastrointestinal transit via transit of charcoal meal ($p<0.0001$), whereas kurkinorin shows no change. The G-protein biased mu-opioid agonists kurkinorin and kurkinol have potent analgesic effects and reduced side-effects and support the development of biased agonists for development of analgesics with reduced side-effects.

40 - Enhanced opioid analgesia and loss of tolerance but exacerbated side effects in mice expressing G-protein biased, phosphorylation-defic

Andrea Kliewer[1], Frank Schmiedel[1], Setareh Sianati[2], Alexis Bailey[3], Jordan T Bateman[4], Erica S Levitt[4], John T Williams[5], MacDonald J Christie[2], Stefan Schulz[1]

[1]Friedrich Schiller University, Jena, Germany, [2]2Discipline of Pharmacology, School of Medical Sciences, University of Sydney, NSW 2006, Australia., [3]Institute of Medical and Biomedical Education, St George's University of London, London SW17 ORE, UK, [4]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL 32608, [5]The Vollum Institute, Oregon Health and Science University, 3181S.W. Sam Jackson Pk. Rd., Portland, OR, 97239, USA

Opioid analgesics are powerful pain relievers, however, over time pain control is diminished due to development of analgesic tolerance and life threatening side effects limit utility. Until now the initial molecular mechanisms causing tolerance remain unresolved. *In vitro* evidence indicates that rapid μ-opioid receptor desensitization and ß-arrestin interaction is controlled by hierarchical phosphorylation of multiple intracellular serine (S) and threonine (T) residues. To assess the contribution of phosphorylation to μ-receptor signaling *in vivo*, we created three lines of knockin mice with a series of carboxyl-terminal S/T to alanine mutations that are increasingly unable to recruit regulatory arrestin proteins. Here we show that desensitization is inhibited in locus coeruleus neurons from mice with phosphorylation-deficient μ-opioid receptors. Consistent with this, acute fentanyl- and morphine-induced analgesia are both strongly enhanced. Upon chronic administration analgesic tolerance to fentanyl and morphine is either abrogated or greatly diminished, while signs of physical dependence persist. Unexpectedly, opioid side effects including constipation and respiratory depression are unchanged or exacerbated, indicating that arrestin recruitment does not contribute to severity of these side effects. Our findings identify carboxyl-terminal multi-site phosphorylation as the key step that drives acute μ-opioid receptor desensitization and long-term tolerance. They also predict that G protein-biased opioid agonists will produce more effective analgesia and reduced tolerance but also severe adverse effects.

41 - The complex roles of μ-opioid receptor phosphorylation and ß-arrestins in mediating opioid analgesia with fewer side effects

Andrea Kliewer[1], Frank Schmiedel[1], Stefan Schulz[1]

[1]Friedrich Schiller University, Jena, Germany

The efficiency of μ-opioid receptor signaling is regulated by coordinated receptor phosphorylation and subsequent interaction with ß-arrestin-1 and ß-arrestin-2. We have recently reported that tolerance to fentanyl and morphine is either abrogated or greatly diminished in total phosphorylation-mutant μ-opioid receptor knockin (TPD) mice. This study was designed to examine whether residual morphine tolerance in TPD mice can be abolished by genetic deletion of ß-arrestin-2. A head-to-head comparison of the two mouse lines revealed that ßarr2-KO mice exhibit only a very modest analgesic phenotype compared to TPD mice. Unlike TPD mice, the analgesic efficacy of fentanyl or morphine was not enhanced or prolonged in ßarr2-KO mice. Moreover, analgesic tolerance as a result of chronic administration of fentanyl or morphine in ßarr2-KO mice was indistinguishable from wild-type mice. Combination of phosphorylation- and ß-arrestin-2-deficiency did not result in any additional inhibition of morphine tolerance. Notably, opioid side effects such as respiratory depression and constipation induced by subanalgesic doses of fentanyl or morphine were reduced in ßarr2-KO mice but exacerbated in TPD mice. Thus, these findings do not support role of a ß-arrestin-2 in regulating μ-opioid receptor responsiveness *in vivo*. We conclude that a rigorous re-examination of the physiological functions of ß-arrestin proteins will require novel animal models in that μ-opioid receptor neurons are devoid of both ß-arrestin-1 and ß-arrestin-2.

42 - Contribution of β-arrestin and ERK signaling in the anxiolytic-like effects of the δ-opioid receptor (δOR) agonist SNC80

Mee Jung Ko[1,3,4], Terrance Chiang[1], Arbaaz M. Mukadam[1], Grace E. Mulia[1], Marcus M. Weera[2,3,4], Julia A. Chester[2,3,4], Richard M. van Rijn[1,3,4]

[1]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, Indiana 47907, [2]Department of Psychological Sciences, College of Health and Human Sciences, Purdue University, West Lafayette, Indiana 47907, [3]Interdisciplinary Life Science PhD Program (PULSe), Purdue University, West Lafayette, Indiana 47907, [4]Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, Indiana 47907

Opioids have been considered as a therapeutic approach for anxiety disorders. Although it has been suggested that β-arrestin proteins may contribute to the side effect profile of many opioids, the role of β-arrestin in δ-opioid receptor (δOR)-mediated anxiolysis has not been fully understood. Previous studies have identified that the selective δOR agonist, SNC80, which strongly recruits β-arrestin, has an anxiolytic-like profile in rodents. Here, we further characterized the distinctive role of β-arrestin-biased δOR signaling in anxiety-like behavior and conditioned fear responses. In wild-type mice, systemic administration of SNC80 (s.c. 20 mg/kg) demonstrated anxiolytic-like effects in the elevated plus maze (EPM) and the dark-light transition box, whereas these effects were abolished in global β-arrestin-2 knockout mice. We also discovered that SNC80 caused strong extracellular signal-regulated kinase1/2 (ERK1/2) phosphorylation in CHO cells (overexpressing δOR and β-arrestin-2) as well as in the amygdala and the striatum of wild-type mice. The anxiolytic-like effects of SNC80 were blocked by the MEK (MAPK/ERK kinase, an upstream kinase of ERK1/2) inhibitor, SL327. We further expanded our study by exploring conditioned fear, and found that SNC80 also decreased conditioned fear-potentiated startle (FPS) responses in wild-type mice. These effects, however, were not impacted by a global knockout of β-arrestin-2. It is possible that the lack of β-arrestin 2 requirements for δOR-mediated FPS responses may be indicative of low β-arrestin-2 expression in the brain regions responsible for the conditioned fear responses. Taken together, we suggest that β-arrestin-2-biased δOR signaling modulates anxiety-like behavior through the downstream ERK1/2 phosphorylation.

43 - Interruption of continuous morphine administration in mice negatively impacts brain and behavior

Emilia Lefevre[1], Marc Pisansky[1], Lauren Bystrom[1], David Leipold[1], Thomas Kono[1], Federico Buffaldi[1], Marco Pravetoni[1], Patrick Rothwell[1]

[1]University of Minnesota, Minneapolis, USA

Clinical use of opiate-based analgesics can lead to negative consequences like dependence and addiction. We propose that maladaptive changes in brain function occur when otherwise continuous opioid exposure is interrupted by short periods of withdrawal. To test this hypothesis, male and female mice were implanted with osmotic minipumps to continuously deliver morphine (63 mg/kg/day), and administered twice-daily naloxone injections (10 mg/kg) to interrupt opioid receptor stimulation. Continuous morphine exposure produced tolerance of psychomotor activation. In contrast, interruption of morphine exposure with naloxone caused psychomotor sensitization, an addiction-related behavior related to adaptations in the nucleus accumbens (NAc). We next performed RNA-seq analysis of NAc tissue, to analyze differential gene expression after continuous or interrupted morphine exposure. After controlling for false discovery rate, we found no genes that were significantly regulated by continuous morphine exposure alone, whereas interrupted morphine exposure significantly regulated 1,328 genes. Genes that were differentially regulated by continuous and interrupted morphine exposure included GABA-A receptor and ion channel subunits, prompting further analysis of cellular properties using ex-vivo slice physiology. We recorded from NAc medium spiny neurons (MSNs) identified by expression of the D1 or D2 dopamine receptor. Intrinsic excitability was significantly, and selectively, increased in D2 MSNs after interrupted morphine. Continuous morphine exposure increased the amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) onto D1 MSNs, whereas interrupted morphine exposure tended to increase sIPSC amplitude onto D2 MSNs. This study illustrates dramatic and divergent adaptations in cellular function that occur with different patterns of opiate administration.

44 - Ketamine in the treatment of opioid tolerance: does the opioid have a role?

Tuomas O. Lilius[1,2,3], Emilia Kangas[1], Hanna Viisanen[1], Viljami Jokinen[1], Mikko Niemi[2,3], Pekka V. Rauhala[1], Eija A. Kalso[1,4]

[1]Pharmacology, University of Helsinki, Helsinki, Finland, [2]Clinical Pharmacology, University of Helsinki, Helsinki, Finland, [3]HUSLAB, Helsinki University Hospital, Helsinki, Finland, [4]Department of Anaesthesiology, Intensive Care Medicine, and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

The effects of *N*-methyl-D-aspartic acid (NMDA) receptor antagonists in enhancing opioid analgesia and attenuating opioid tolerance have been of great interest both in the laboratory and the clinic. Preclinical studies have mostly focused on the pharmacodynamic interactions between ketamine and morphine, whereas only scarce information about co-administration of ketamine and the widely used oxycodone exists. Pharmacokinetic interactions between ketamine and opioids have been only little studied. Further, the role of ketamine metabolites in opioid tolerance remains unclear. Our recent studies show that co-administration of low-dose ketamine or norketamine effectively attenuated morphine tolerance in rats, whereas tolerance to oxycodone was less affected. This difference may be partly attributable to a pharmacokinetic interaction between morphine and ketamine leading to vastly increased concentrations of morphine, ketamine, and norketamine in the central nervous system. Co-administration of oxycodone and ketamine did not show changes in drug concentrations. Norketamine, the main active metabolite of ketamine, should be further studied in the treatment of opioid tolerance, as it attenuated morphine tolerance with minor adverse effects. In contrast, (2S,6S;2R,6R)-hydroxynorketamine, a major secondary metabolite with no NMDA receptor antagonist properties, did not have tolerance-attenuating effects. In addition to pharmacodynamic differences, pharmacokinetic interactions between ketamine and different opioids may explain the wide variation in treatment response and adverse effects observed in clinical ketamine-opioid interaction studies. These results also warrant studies of possible pharmacokinetic ketamine-opioid interactions in humans.

45 - Roles of Protein Kinase C in Kappa Opioid Receptor-Mediated Effects in vivo: Pharmacological and Phosphoproteomic Approaches

Jeffrey J. Liu[1], Yi-Ting Chiu[2], Chongguang Chen[2], Matthias Mann[1], Lee-Yuan Liu-Chen[2]

[1]Max Planck Institute of Biochemistry, Martinsried, Germany, [2]Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

Kappa opioid receptor (KOR) agonists possess the capacity to become anti-pruritic drugs as well as the next generation of analgesics, but are limited by their dysphoric, psychotomimetic and hallucinogenic properties. Recent evidence demonstrated that the KOR-mediated adverse effects are reduced in some class of KOR agonists, suggesting that distinct downstream biochemical pathways, such as protein kinase C (PKC), may be responsible for different behavioral outcomes. Here, we showed that PKC inhibition preserved the beneficial antinociceptive and antipruritic effects induced by the KOR agonist U50,488H, but attenuated the adverse condition placed aversion (CPA), sedation and motor incoordination. We also found that PKC inhibition diminished phosphorylation of KOR at S369. Using a large-scale shotgun mass spectrometry-based phosphoproteomics, we investigated the downstream phosphorylation changes induced by U50,488H with and without PKC inhibition, in a temporal- and brain region-specific fashion. At five-minute, we observed PKC-dependent modulation of GRKs and Wnt Signaling pathways, whereas at thirty-minute, we observed PKC-dependent modulation of calcium signaling, stress signaling, cytoskeleton changes and mTOR signaling. We previously showed that the mTOR pathway was involved in KOR agonist-promoted CPA. Altogether, we investigated behavioral effects and biochemical signaling pathway changes as a result of PKC inhibition during KOR signaling. We also further demonstrated that adverse behavioral effects from KOR activation can be separated through downstream effector inhibition. (supported by NIH grants R01 DA041359 and P30 DA013429)

46 - Correlation of KOR phosphorylation in mouse brains with sedation, motor incoordination and aversion induced by agonists

Chongguang Chen[1], Peng Huang[1], Jeffrey Liu[2], Yi-Ting Chiu[1], John Logue[1], Brigitte Kieffer[3], Matthias Mann[2], Lee-Yuan Liu-Chen[1]

[1]Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA, [2]Max Planck Institute of Biochemistry, Martinsried, Germany, [3]Douglas Mental Health Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada

Selective KOR agonists are promising analgesics and antipruritic agents. However, clinical applications of KOR agonists are limited by side effects, including dysphoria, sedation, motor incoordination and psychotomimetic effects in humans, except for nalfurafine in most patients. Nalfurafine has been used in Japan for treatment of pruritus in hemodialysis patients. We have previously found that in male CD-1 mice, nalfurafine produced analgesic and anti-scratch effects dose-dependently, like U50,488H. However, at doses effective for analgesic and anti-scratch effects, U50,488H, but not nalfurafine, caused aversion, anhedonia, sedation and motor incoordination. In this study, we demonstrated that U50,488H, but not nalfurafine, promoted KOR phosphorylation at T363 and S369 in mouse brains as detected by immunoblotting with phospho-KOR specific antibodies. In addition, U50,488H, but not nalfurafine, caused KOR internalization in the brain of a mutant mouse line expressing a fusion protein of KOR conjugated at the C-terminus with tdTomato (KtdT). Furthermore, in phosphoproteomic studies, U50,488H, but not nalfurafine, promoted phosphorylation of GRK5/6 at S484/T485 in the striatum and cortex, which was shown to enhance GRK5 activities. Taken together, these data reveal a correlation between agonist-promoted KOR phosphorylation and behaviors considered to be detrimental side effects, such as sedation, aversion and motor incoordination. These results also suggest that agonist-induced KOR phosphorylation in mouse brains may be used to screen for KOR agonists devoid of these side effects. (Supported by NIH grants R01 DA041359 and P30 DA013429)

47 - Identification of local GPCR protein interaction networks using proteomics and proximity labeling

Braden T. Lobingier[1], Ruth Huttenhain[2], Brian Shoichet[3], Nevan J. Krogan[2], Mark von Zastrow[1,2]

[1]Department of Psychiatry, University of California San Francisco, [2]Department of Cellular and Molecular Pharmacology, University of California San Francisco, [3]Pharmaceutical Chemistry, University of California San Francisco, [4]Pharmaceutical Chemistry

Cellular activity is inherently organized in space and in time. Here we describe a method to capture information about these dimensions and define the protein networks underlying rapidly evolving cellular processes. The approach builds on proximity labeling, combined with quantitative proteomics and spatial references, to obtain temporally resolved 'snapshots' of the environment around a protein of interest with sub-organelle spatial resolution. We apply this method with labeling periods of 30 seconds to interrogate the local protein networks engaged by G protein-coupled receptors upon ligand-induced stimulation. We demonstrate that the methodology provides a quantitative readout of receptor engagement with previously known protein partners, and that it allows discovery of new network components. We now apply this approach to examine the protein interaction networks engaged by the mu-type opioid receptor when stimulated by full, partial, and G protein biased agonists.

48 - Sex differences in kappa opioid receptor activated brain networks implicated in the response to stress and drugs of abuse

Qianhan Ma[1], Sue Wonnacott[1], Sarah J. Bailey[1], Chris P. Bailey[1]

[1]Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, UK

Introduction: Kappa opioid receptors (KOPrs) are involved in both stress responses and addiction-related behaviour (Bruchas et al (2010) Brain Research 1314:44-55). It has been shown that the role of KOPrs in motivated behaviour is sex dependent (Russell et al (2014) Biol Psychiatry 76:213-222). This study aimed to investigate the influence of sex on brain regions that are activated by stress and compare the effect of in vivo stressors and KOPrs activation.

Methods: Adult (8-13wks) male and female C57BL/6 c-Fos-GFP transgenic mice, in which expression of green fluorescent protein (GFP) is driven by the activation of c-Fos (Barth et al (2004) J Neurosci 24:6466-75). Mice were administered either KOPr agonist (U50,488; 20mg/kg) or a physical stressor – forced swim stress (FSS). Following transcardial perfusion fixation, brain sections were taken and immunolabelled to assess cFos and cFos-driven GFP expression, to indicate brain regions activated by the *in vivo* treatments.

Results: In male mice, both cFos and cFos-driven GFP expression in the nucleus accumbens (NAcc) and prelimbic prefrontal cortex (PFC) were increased by both U50,488 and FSS. In contrast, U50,488 did not increase cFos or cFos-driven GFP expression in either NAcc or PFC in female mice, FSS increased expression in NAcc not PFC. The effects of U50,488, but not FSS, were blocked by pre-administration of the KOPr antagonist norBNI (10mg/kg).

Conclusion: Together these data suggest that sex differences in brain regions activated by KOPr agonists and physical stressors, potentially reflecting the sex differences seen in the behavioural effects of KOPrs.

49 - Dissecting Agonist-induced Nociceptin/Orphanin FQ Receptor Phosphorylation in vitro and in vivo

Anika Mann[1], Thomas Guenther[1], Lionel Moulédous[2], Carine Froment[3], Patrick R. O´Neill[4], Gloria Brunori[5], Nurulain T. Zaveri[6], Michael R. Bruchas[4], Brigitte L. Kieffer[7], Lawrence Toll[5], Stefan Schulz[1]

[1]Institute of Pharmacology and Toxicology, Jena Universtiy Hospital, Friedrich Schiller University Jena, Drackendorfer Str. 1, 07747, Jena, Germany, [2]Research Center on AnimalCognition, Center for Integrative Biology, Toulouse University, CNRS, UPS, France, [3]Institut de Pharmacologie et de Biologie StructurateUniversité de Toulouse, CNRS, UPS, France, [4]Departments ofAnesthesiology, Neuroscience, andPsychiatry, Washington University School ofMedicine, St. Louis, Missouri 63110, [5]Torrey Pines Institute forMolecular Studies, Port St. Lucie, FL, USA, [6]Astraea Therapeutics, LLC, Mountain View, California, [7]Douglas Research Center, Department ofPsychiatry, FacultyofMedicine, McGill University, Canada

The nociceptin/orphanin FQ opioid peptide (NOP) receptor is the fourth member of the opioid receptor family and a promising target for the development of novel analgesics. The detailed molecular events underlying its agonist-dependent regulation remain incompletely understood. Here, we generated a series of phosphosite-specific antibodies and examined agonist-induced NOP receptor phosphorylation. We found that agonist-induced phosphorylation occurs primarily at four carboxyl-terminal serine and threonine residues in human NOP receptor, namely S346, S351, T362 and S363. Agonist-induced NOP receptor phosphorylation proceeds with a temporal hierarchy, in which S346 appears to be the primary site of phosphorylation. In NOP-eGFP mice, NOP receptor multisite phosphorylation and internalization occurred in a dose-dependent and agonist-selective manner that could be blocked by specific antagonists. A comparison of chemically distinct NOP receptor agonists revealed dissociation between G protein signaling and receptor phosphorylation. We also show that G protein-coupled receptor kinases 2 and 3 (GRK2/3) cooperate during agonist-induced phosphorylation, which in turn facilitates NOP receptor desensitization and internalization. Together, we showed for the first time agonist-selective NOP receptor phosphorylation, *in vitro* as well as *in vivo*.

50 - A Stable Heroin Analog Vaccine Formulation that Induces Long Duration Antibody Titers that Block the Antinociceptive Effects of Heroin and Hydromorphone

Gary Matyas[1], Oscar Torres[1,2], Agnieszka Sulima[3,4], Zoltan Beck[1,2], Arthur E. Jacobson[3,4], Kenner C. Rice[3,4]

[1]Walter Reed Army Institute of Research, [2]Henry M. Jackson Foundation for the Advancement of Military Medicine, [3]National Institute on Drug Abuse, [4]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health

We have recently described a new heroin hapten, 6-AmHap, when conjugated to tetanus toxoid (TT), mixed with Army Liposome Formulation (ALF) as an adjuvant, and used to immunize mice protected both mice and rats from both subcutaneous and intravenous heroin challenge (Sulima et al., J. Med Chem 2018). The vaccine induced antibodies bound to heroin, its degradation products and cross-reacted with other opioids. In separate studies, the duration of the antibody response has been monitored in both mice and rats. Although the experiments are still ongoing, the antibody titers to 6-AmHap have remained high for 6 months following the last immunization. Challenge of the animals with heroin indicates that vaccine-induced efficacy is still maintained in tail immersion and hot plate assays. Based on the strong cross-reactivity of the antibodies to hydromorphone from competition ELISA, mice were immunized with the 6-AmHap-TT conjugate mixed with ALF and aluminum hydroxide adjuvants. The animals were challenged with heroin or hydromorphone at 1 mg/kg by the subcutaneous route every 20 min. Control animals reached 100% MPE in a tail-flick assay after only 1 dose of heroin or hydromorphone. Immunized animals challenged with heroin required 2-3 doses to reach 100% MPE, while those challenged with hydromorphone required 3-4 doses. The average EC_{50} of the immunized animals was 1.7 ± 0.27 and 1.0 ± 0.15 mg/kg for heroin and hydromorphone, respectively. Based on this data, the 6-AmHap-based heroin vaccine also is expected to have efficacy against hydrocodone and other opioids.

51 - Morphine and HIV-1 Tat-induced calcium flux in hippocampal neurons is dependent on L-type calcium channels and NMDAR signaling

Virginia D. McLane[1], Pamela E. Knapp[1,2,3], Kurt F. Hauser[1,2,3]

[1]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, USA, [2]Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA, USA, [3]Institute for Drug & Alcohol Studies, Virginia Commonwealth University, Richmond, VA, USA

Opiates exacerbate HIV-induced neuropathogenesis within vulnerable regions of the central nervous system, such as the hippocampus. Prolonged (72 h) exposure to neurotoxic HIV-1 protein *transactivator of transcription* (Tat, 100 nM) reduced primary murine hippocampal neuron survival *in vitro*, particularly in GAD67+, GABAergic cells ($p<0.001$). Acute Tat exposure increased neuronal intracellular calcium concentration ($[Ca_{2+}]_i$) by 193.9 ± 53.9% compared to controls, which was exacerbated by morphine (312.0 ± 31.5%) and reversed by $GABA_A$ agonist muscimol (100 nM, three-way interaction, $p<0.001$). $[Ca_{2+}]_i$ returned to baseline within 20 min in Tat-exposed cells (114.4 ± 30.3%); however, $GABA_A$ antagonist bicuculline (20 µM) prolonged Tat-induced elevation of $[Ca_{2+}]_i$ in the presence of morphine (208.0 ± 22.8%, $p<0.001$). To identify key sources of Tat-induced calcium influx, potential extracellular [NMDAR (MK-801), AMPAR (CNQX), L-type Ca^{2+} channels (nimodipine, isradipine)] and intracellular [$RyR_{1,3}$ (dantrolene), σ_1R (BD-1063)] calcium sources were blocked pharmacologically. Tat-induced $[Ca_{2+}]_i$ was significantly reduced by nimodipine (10 µM, $p<0.001$) and MK-801 (20 µM, $p<0.001$), but not BD-1063 (1 µM, $p=0.381$). Morphine-induced calcium influx was reduced by isradipine (5 µM, $p=0.009$) and BD-1063 ($p=0.038$). Bicuculline and morphine interactive effects within Tat-exposed cells were reduced by CNQX (500 nM, $p<0.001$). Overall, these data indicate morphine, bicuculline, and HIV-1 Tat elevate $[Ca_{2+}]_i$ within hippocampal neurons through both independent (i.e., NMDAR, RyR, σ_1) and shared (i.e., $Ca_v1.2$) calcium sources. Thus, $GABA_A$ signaling appears to ameliorate aspects of Tat-induced overexcitability. The potential loss of vulnerable GABAergic interneurons within the hippocampus may exacerbate opiate- and HIV-induced neuropathology. Support: NIH R01 DA018633.

52 - Diverse cell types within the vlPAG exhibit unique adaptations to membrane firing properties after inflammation.

Kylie McPherson[1], Katherine Suchland[1], Susan Ingram[1,2]

[1]Oregon Health & Science University, [2]Oregon Health & Science University, Portland, USA

The periaqueductal grey (PAG) is an important integration site within the descending pain modulatory pathway that receives diverse inputs. The vlPAG is a highly heterogeneous region with diverse cell types that have yet to be characterized fully in terms of their response to noxious stimuli and opioids. Using whole-cell patch-clamp recordings of membrane firing properties in naïve rats we defined 5 distinct cell-types within the vlPAG: onset-spiking, fast-spiking, transiently fast-spiking, random-spiking, and dopamine neurons. Animals that experienced inflammation (2 h), induced by complete Freund's Adjuvant (CFA) injected into the hindpaw, have neurons that exhibit hyper-excitable characteristics including increased firing frequency and altered firing patterns. Fos expression is robustly increased 2 h after CFA, with a significant reduction 6 d after CFA that remains significantly greater than the naïve animals—suggesting altered levels of neuronal activity after CFA that change between early and persistent inflammation. Studies are examining whether inflammation-induced Fos expression labels sensitized vlPAG neurons at acute (2 and 24 hours) and after persistent inflammation (6 days) using Fos-GFP expression in Long-Evans rats. Additionally, the studies use retrograde fluorescent CTb labeling from the rostral ventromedial medulla (RVM) to examine intrinsic properties of vlPAG neurons that project to the RVM. The effects of opioids on the intrinsic membrane properties of these cell types will also be examined.

53 - Mobility of the mu opioid receptor as an effector independent assay for alterations in signaling state

Marissa Metz[1,2], Reagan Pennock[1], Shane Hentges[1,2], Diego Krapf[3,4]

[1]Department of Biomedical Sciences, Colorado State University, [2]Molecular, Cellular, and Integrative Neurosciences Program, Colorado State University, [3]Electrical and Computer Engineering, Colorado State University, [4]School of Biomedical Engineering, Colorado State University, [5]Colorado State University

Assays for opioid receptor activity typically exploit the signaling of a relevant effector, such as potassium channel currents or cyclic AMP generation. However, these assays do not directly sample events occurring at the receptor itself, potentially masking important processes that happen upstream of effector activation. One way to better understand opioid receptor activity is to directly observe receptor mobility using single particle tracking. Flag-tagged mu opioid receptors were tracked using spinning disk and TIRF microscopy in response to application of DAMGO. In early signaling stages as desensitization commenced but before internalization begins, receptors exhibited an increase in overall mobility with a decrease in mobile state dwell times within compartmentalized membrane areas. However, in late signaling stages when internalization begins, Flag-MORs exhibited decreases in overall mobility with increases in dwell times of remaining mobile receptors. Further analysis revealed that free portions of mobile states were in part a result of g protein activation. Association with clathrin accounted for a small portion of both mobile and immobile tracks, with a larger proportion of immobile receptors colocalized with clathrin when they were treated with DAMGO for 10 minutes. Understanding of which mobility states correspond to different signaling states will allow for better, more direct assays of receptor signaling, and may pose an interesting, effector-free avenue for assaying drugs with different efficacy profiles for opioid signaling pathways.

54 - Multi-site phosphorylation is required for sustained interaction with GRKs and arrestins in mediating rapid mu-opioid receptor desensitization.

Elke Miess[1], Arisbel B Gondin[2], Arsalan Yousuf[3], Ralph Steinborn[1], Nadja Mösslein[4], Yunshi Yang[4], Martin Göldner[4], Julia G. Ruland[4], Moritz Bünemann[4], Cornelius Krasel[4], MacDonald J. Christie[3], Michelle L Halls[2], Stefan Schulz[1], Meritxell Canals[2]

[1]Department of Pharmacology and Toxicology, Jena University Hospital - Friedrich Schiller University Jena, Germany, [2]Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia, [3]Discipline of Pharmacology, University of Sydney, Sydney, Australia, [4]Department of Pharmacology and Toxicology, Philipps-University Marburg, Germany

G protein-coupled receptor kinases and β-arrestins are key regulators of μ-opioid receptor (MOP) signaling and trafficking. We have previously shown that high-efficacy opioids such as DAMGO stimulate a GRK2/3-mediated multisite phosphorylation of conserved C-terminal tail Ser and Thr residues, which facilitate internalization of the receptor. In contrast, morphine-induced phosphorylation is limited to Ser^{375} and it is not sufficient to drive substantial receptor internalization. Here, we report how specific multisite phosphorylation controls the dynamics of GRK and β-arrestin interactions with MOP and show how such phosphorylation mediates receptor desensitization. We show that the kinetics of GRK2/3 recruitment to a DAMGO-activated MOP are faster than the kinetics of β-arrestin recruitment. β-arrestin recruitment requires GRK2 activity and MOP phosphorylation, but surprisingly, GRK recruitment is also dependent on the integrity of the phosphorylation sites in the C-terminus. Translocation of both regulatory proteins and their stable interaction with MOP are dependent on phosphorylation of Ser and Thr residues within the ^{375}STANT^{379} motif of the C-tail. Our results also suggest that other residues outside this motif participate in the initial and transient recruitment of GRK and β-arrestins. Finally, using complementary patch clamp approaches, we show that high efficacy agonist desensitization of MOP has two components; a sustained component, which requires GRK2-mediated phosphorylation and a potential soluble factor, and a rapid component likely mediated by GRK2 but independent of receptor phosphorylation. Elucidating these complex receptor-effector interactions represents an important step towards a mechanistic understanding of MOP desensitization that leads to the development of tolerance and dependence.

55 - Chronic Pain Enhances Spontaneous Fentanyl Withdrawal in Rats

Andrea T. Lee[1], Tammy N. Hilgendorf[1], Ram Kandasamy[1], Michael M. Morgan[1]

[1]Washington State University, [2]WA

Overdose deaths caused by fentanyl and its analogs have increased dramatically in recent years. Despite over 20,000 deaths caused by fentanyl overdose in the US in 2016, almost all animal research focuses on morphine dependence. Although morphine and fentanyl produce antinociception and dependence by binding to the mu-opioid receptor, the distinct signaling mechanisms engaged and short half-life of fentanyl may induce more severe withdrawal symptoms. This hypothesis was tested by examining the magnitude and duration of spontaneous fentanyl withdrawal in rats with and without inflammatory pain. Dependence was induced by continuous subcutaneous fentanyl infusion (1 mg/kg/day) for 3 days. CFA was injected into the hindpaw to induce inflammation while rats were anesthetized for implantation of the fentanyl pump. Withdrawal was assessed by measuring depression of home cage wheel running as described previously (Kandasamy et al., 2017). Withdrawal was evident as a 50% reduction in wheel running during the 24 hours following removal of the fentanyl pump in pain-free rats. Running had recovered to near baseline levels of running by Day 2. In contrast, a near complete inhibition of wheel running occurred following removal of the fentanyl pump in rats with hindpaw inflammation. Although there was a gradual increase in wheel running across days, wheel running was significantly depressed throughout the 6 days of withdrawal assessment. These data indicate that chronic pain significantly enhances spontaneous fentanyl withdrawal. Such pronounced and prolonged withdrawal likely contributes to the continued use and abuse of fentanyl and fentanyl analogs.

56 - Exposure of early life stress reduces the expression of μ-opioid receptor in the PAG of adult mice brain and morphine analgesia

Kazuo Nakamoto[1], Asami Imanishi[1], Natsuki Kyoya[1], Masataka Kasuga[1], Shogo Tokuyama[1]

[1]Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University

Early life stress relates to the pathogenesis of psychiatric disorders and chronic pain in adult patients. However, information about the mechanisms for chronic pain which induced by early life stress exposure is limited. In this study, to elucidate the mechanism underlying early life stress-induced increase of pain sensitivity, we investigated the changes of each opioid receptors mRNA expression in some brain area of mice which subjected to maternal separation combined with social isolation (MSSI) as an early life stress. Furthermore, we tested the analgesic effect of morphine on thermal stimulation in tail flick test. In the periaqueductal gray (PAG) area, a region that is implicated in the opioid control of nociception, μ-, δ-and κ-opioid receptor (MOR, DOR and KOR) mRNA expression were significantly decreased in MSSI model mice compared to control mice. In the amygdala, KOR mRNA is significantly increased in MSSI model mice, but not control mice. In the medial prefrontal cortex and rostral ventral medulla area, MOR mRNA showed tendency to decrease in MSSI model mice. However, there are no significant difference between control and MSSI model mice in other area of the brain. In the tail flick test, MSSI model mice showed significantly decrease of morphine induced analgesia compared to control mice. On the other hand, morphine induced hyper-locomotion did not change between two groups. Finally, we conclude that MSSI induced decrease of MOR mRNA expression in the PAG area, suggesting that these phenomenon could be induced the increase of pain sensitivity in MSSI model mice.

57 - Inside the Net: morphine-induced conditioned place preference and effects on perineuronal nets in the ventral tegmental area

Toni A.E. Nigro[1,2], Dolores Beatriz Vazquez Sanroman[1]

[1]Oklahoma State University Center for Health Sciences, [2]Oklahoma State University College of Osteopathic Medicine

The ventral tegmental area (VTA) is the origin of the brain dopamine-rewarding pathway implicated in morphine reinforcing properties. VTA dopaminergic neurons activity is modulated by VTA-GABA neurons. Indeed, GABAergic neurons are important substrates for morphine effects and are surrounded by perineuronal nets (PNNs). PNNs are aggregations that form net-like structures surrounding GABAergic interneurons and are important for plasticity during critical periods, such as adolescent neurodevelopment, and during drug-associated learning and memory. The present experiments examined whether morphine conditioned place preference (CPP) in adolescent rats lead to changes in the PNNs and neuronal activity within the VTA. Twenty-four Sprague Dawley adolescent rats were used. In the present set of experiments, we explored the acquisition, extinction, and reinstatement of a morphine-induced CPP using an 'unbiased' apparatus for all stages, rats were given 2-daily 20-minutes sessions for a total of eight pairings for each phase. Rats received 5 mg/kg or saline during conditioning sessions. Further, 8-extintion sessions (drug-free). Followed by a drug-primed (2.5 mg/kg) or saline session. Morphine significantly increased the percentage time on the drug-paired floor [One way ANOVA: $F(1,22)=4.65$, $p< 0.05$; mor-sal 60%; sal-sal 50%]. Morphine-prime induced reinstatement was effective only for 40% of the animals. No sex differences were found regarding morphine preference score [$X2(1) = 0.85$, $p=0.68$]. At the cellular level, morphine preference decreased PNN expression [$X2(1) = 7.91$, $p<0.01$]. Our results showed that morphine inducing PNN-dependent plasticity in the brain reward system of adolescent rats.

58 - Cannabinoid Type-1 Receptors can Mediate the Opioid-Sparing Effects of Delta-9-tetrahydrocannabinol in Nonhuman Primates

Mark R. Nilges[1], Zachary Bondy[1], Joseph A. Grace[1], Peter J. Winsauer[1,2]

[1]Louisiana State University Health Sciences Center, [2]Alcohol and Drug Abuse Center of Excellence

Combinations of opioids and cannabinoids can produce synergistic antinociceptive effects. These "opioid-sparing" effects could potentially reduce some of the adverse effects produced by opioids. In this experiment, thermal antinociception was assessed using a warm-water tail-withdrawal procedure in nonhuman primates (rhesus macaques). The acute antinociceptive effects of cumulative doses of heroin were studied in the presence or absence of the cannabinoid receptor ligands delta-9-tetrahydrocannabinol (Δ^9-THC), rimonabant, cannabidiol (CBD), or cannabinol (CBN). We also studied the effects of a serotonin-noreprinephrine reuptake inhibitor, amitriptyline, as a positive control. When CBN or CBD was administered prior to cumulative doses of heroin, no shift was evident in the dose-effect curve compared to heroin alone. However, a combination of Δ^9-THC (1 mg/kg) and heroin shifted the ED_{50} for heroin 3.4-fold leftward compared to heroin alone. A selective cannabinoid type-1 receptor (CB1R) antagonist, rimonabant, was then tested to determine if the enhanced antinociceptive effects produced by Δ^9-THC could be antagonized. When rimonabant (0.32 – 1 mg/kg) alone was administered prior to heroin, the dose-effect curve was shifted rightward; however, this shift was not evident 15 minutes after the cumulative heroin injections. When these doses of rimonabant preceeded Δ^9-THC, the leftward shift of the Δ^9-THC-heroin dose-effect curve was dose-dependently antagonized. This is consistent with the notion that Δ^9-THC can produce opioid-sparing effects in a CB1R-dependent manner, and rimonabant can produce antagonist/inverse agonist effects in nonhuman primates.

59 - Growth hormone and Insulin-like growth factor-1 promotes the recovery of neurons after methadone exposure

Erik Nylander[1], Sofia Zelleroth[1], Fred Nyberg[1], Alfhild Grönbladh[1], Mathias Hallberg[1]
[1]Uppsala University, Sweden

Background:

Accumulating data suggest that long-term use of methadone may be linked to reduced cognitive function. These effects are associated with increased neuronal cell death and reduced neurogenesis. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are suggested to act pro-cognitive and neuroprotective in the CNS and may counteract some of these effects. The aim for present study was to examine if GH/IGF-1 acts protective or increases the recovery of the mitochondrial function or membrane integrity in methadone-treated cells.

Methods:

Primary cortical cells from rats were harvested and grown for seven days *in vitro*. Cells were then either *a)* exposed to a repeated methadone treatment for three days with or without GH/IGF-1 or *b)* exposed to methadone for 24h, washed, and later exposed to GH/IGF-1 for 48h.
The mitochondrial function, as assessed in the tetrazolium-bromide (MTT) assay, and membrane integrity, as assessed in the lactate dehydrogenase (LDH) assay, were evaluated at the end of the experiments.

Results:

Growth hormone, but not IGF-1, significantly decreased LDH-release when co-treated with methadone but no effects was seen in the MTT-assay. Furthermore, both GH and IGF-1 significantly reduced LDH release and increased the mitochondrial function after cells had been treated for 24h with methadone.

Conclusion:

Growth hormone protects cells when co-treated with methadone for three days by stabilizing the membrane integrity. No protective effects were seen using IGF-1. However, both GH and IGF-1 improved the recovery of the mitochondrial function and the membrane integrity after acute 24h methadone treatment.

60 - The non-selective opioid diprenorphine produces delta-opioid receptor-mediated rapid antidepressant-like effects in mice

Keith M Olson[1], Todd M. Hillhouse[1], James E Hallahan[1], Evan Schramm[1], John Traynor[1]
[1]University of Michigan, Ann Arbor

Major depressive disorder (MDD) is the most common mood disorder worldwide with a lifetime prevalence of ~15%. However, current FDA approved treatments are limited by a multi-week delayed onset of action and minimal efficacy in some patients. Preclinical evidence indicates delta-opioid receptor (DOR) agonists and kappa-opioid receptor (KOR) antagonists may produce rapid onset antidepressant effects and provide a novel depression target in treatment resistant patients [^{3}H]-diprenorphine saturation binding experiments in membranes expressing the mu-opioid receptor (MOR); (K_{Dm}= 0.31 +/- 0.04); DOR (K_{Dd}= 1.1 nM +/- 0.16); and KOR (KOR K_{Dk}= 0.36 +/- 0.09) revealed non-selective affinity, as expected. *In vitro*[^{35}S]GTPgS assays in the same cell lines revealed diprenorphine is a DOR and KOR partial agonist (EC_{50d}= 4.1 nM +/- 2.0, E_{MAXd}= 40 % +/- 5.0; EC_{50k}= 0.71 nM +/- 0.21, E_{MAXk}= 41 % +/- 5.3); it also has potent MOR antagonist against DAMGO (K_B= 0.18 +/- 0.05). *In vivo*diprenorphine produced anti-depressive-like effects in the tail suspension test and the novelty-induced hypophagia test that were blocked by the DOR-selective antagonist naltrindole. While classical DOR agonists, such as SNC80, produce seizure activity, diprenorphine did not produce seizures and blocked SNC80 mediated seizures indicating that higher DOR efficacy is needed to produce seizures than anti-depressant-like effects. Alternatively, the DOR/KOR partial agonist and MOR antagonist profile of diprenorphine may mitigate against seizures. Future directions include the development of diprenorphine analogues to determine if modulating DOR/KOR agonist activity improves diprenorphine anti-depressant-like efficacy.

61 - Inhibition of alpha7 nicotinic receptors in heroin-primed reinstatement of conditioned place preference

Josephine C Palandri[1,2], Sharon Smith[3], David J Heal[3], Susan Wonnacott[2], Chris Bailey[1]

[1]Department of Pharmacy & Pharmacology, University of Bath, Bath BA2 7AY, UK, [2]Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, UK, [3]RenaSci, Nottingham, UK

Drug addicts form associative memories in the context of drug use, which can trigger cravings and prompt relapse (Kauer JA & Malenka RC (2007) Nat Rev Neurosci 8:844-858). We and others have shown that methyllycaconitine (MLA), an α7-nicotinic acetylcholine receptor (α7nAChR) antagonist attenuates the reinstatement of morphine-primed conditioned place preference (CPP) in rodents (Feng B et al (2011) Behav Brain Res 220:100-105; Wright VL et al (2018) Addict Biol in press). The aim of this study is to investigate the effects of MLA on CPP with a more clinically relevant opiate, heroin. All rats (male, Wistar, 6-7 weeks) underwent heroin CPP (1 injection daily over 4 days, alternating between saline and heroin (1mg/kg) (Cordery SF et al. (2014) Addict Biol 19:575-86). To test the effects of MLA on heroin CPP acquisition, rats were given MLA (4mg/kg) 20 minutes prior to each conditioning injection. To test the effects on reinstatement, animals underwent CPP extinction and received either saline or MLA (4mg/kg) 20 minutes prior to heroin-priming (1mg/kg). Although MLA pre-treatment had no effect on the acquisition of heroin CPP, a single MLA dose pre-reinstatement completely prevented heroin-primed reinstatement. These data show that MLA does not affect heroin reward per se, but can selectively affect heroin-related memories, suggesting a role for α7nAChRs in opiate relapse. The ability to pharmacologically distinguish between the different stages of addiction is exciting for potential therapies targeted at opiate relapse. Analysis of the effect of MLA on intravenous self-administration of heroin is ongoing.

62 - Clinical Characteristics of Opioid Use Disorder

Sarah A. Palumbo[1], Vanessa Troiani[2], Kayleigh M. Adamson[2], Sarah Robishaw[2], Sarathbabu Krishnamurthy[2], Raghu Metpally[2], Laney Jones[2], Sarah Pendergrass[2], Joseph Chronowski[2], Christopher Lam[2], Richard C. Crist[3], Glenn A. Doyle[3], Thomas Ferraro[4], Mingyao Li[3], Wade H. Berrettini[3], Janet Robishaw[1]

[1]Florida Atlantic University, Boca Raton, FL, [2]Geisinger, Danville, PA, [3]University of Pennsylvania, Philadelphia, PA, [4]Cooper Medical School of Rowan University, Camden, NJ

The Opioid Epidemic affects 100 million people in the United States with 115 people per day dying from an opioid overdose. The US Burden of Disease Collaborators lists Pennsylvania as 4th in the nation for drug abuse deaths at 708 per 100,000 in 2016; and, CDC data reports Pennsylvania as showing the largest increase in Emergency Department visits for opioid overdoses from July 2016 to September 2017. The goal of this NIH-funded study is to investigate clinical and genetic predictors of Opioid Use Disorder (OUD) among chronic pain patients served by the Geisinger health system in Pennsylvania.

Using electronic health record data, we identified two groups: 1) MUA, participants with a medication use agreement for opioids; and 2) TMUA, participants with a terminated MUA resulting from rule violations indicative of OUD. Notably, the TMUA group showed a higher prevalence of International Classification of Diseases (ICD) pain codes for lumbar/sacral region ($p = 10^{-13}$), musculoskeletal system ($p = 10^{-9}$), and multiple systems ($p = 10^{-11}$). In addition, the TMUA group exhibited a higher frequency of ICD10 codes for polysubstance use disorders, including nicotine ($p = 10^{-67}$), alcohol ($p = 10^{-21}$), and other substances ($p = 10^{-78}$). Finally, the TMUA group revealed a higher incidence of ICD10 codes for mental health disorders, including anxiety ($p = 10^{-18}$) and major depression ($p = 10^{-12}$). Having identified one group of stable users and another of presumptive OUD patients, we will conduct genome-wide association studies to identify a polygenic risk score for development of OUD.

63 - Development of vaccines to treat heroin and prescription opioid abuse suitable for clinical trials

Baruffaldi F[1], Raleigh M[1], Peterson S[1], Laudenbach M[1], Winston S[2], Runyon S[3], Carroll FI[3], Pentel PR[1,4], Pravetoni M[1,4]

[1]Minneapolis Medical Research Foundation, MN, [2]Winston Biopharmaceutical Consulting, CO, [3]RTI International, NC, [4]University of Minnesota Department of Medicine, MN

Vaccines for the treatment of opioid use disorders and prevention of fatal overdoses have shown promising pre-clinical efficacy. However, readying vaccine candidates for human testing presents unique quality and regulatory challenges. This study first focused on optimization and characterization of a conjugate vaccine targeting oxycodone, and then applied this technology to formulate analogous heroin and fentanyl vaccines. To this end, several batches of an oxycodone hapten conjugated to GMP-grade keyhole limpet hemocyanin (OXY-KLH) carrier protein adsorbed on alum adjuvant were produced to test the effect of coupling reaction temperature, time, buffer composition, and protein:alum ratio on vaccine immunogenicity and efficacy in rats. Small conjugates could be evaluated by gel electrophoresis and size exclusion chromatography but were not immunogenic while larger conjugates were immunogenic but their size could only be measured by dynamic light scattering (DLS). OXY-KLH purification using tangential flow filtration (TFF, scalable) was compared to ultrafiltration (non-scalable) and both elicited equivalent oxycodone-specific serum IgG antibody titers and blockage of brain oxycodone compared to control. Following TFF, levels of residuals were negligible. OXY-KLH formulated in alum was stable over 6 months at 4°-37°C as confirmed by immunogenicity and reduced brain oxycodone levels by 66 – 73% compared to control in rats. An analogous heroin/morphine vaccine (M-KLH) was formulated under conditions similar to the oxycodone vaccine. An analogous fentanyl vaccine (F-KLH) showed efficacy in blocking fentanyl distribution to the brain in mice and rats. These data suggest that KLH-based opioid vaccines adsorbed on alum adjuvant are suitable for pharmaceutical manufacturing.

64 - In vivo EEG signatures during chronic fentanyl exposure, spontaneous withdrawal, and protracted abstinence

Corey Puryear[1], Angelica Patino[1], Connie Sanchez-Morillo[1]

[1]Alkermes, Waltham, USA

Preclinical studies in rodent models of opioid addiction are commonly used to characterize long-term effects on brain function. However, understanding the effects opioid agonists on cortical circuit activity may better elucidate the functional impact of opioids. Chronic opioid exposure and withdrawal has been shown to produce dynamic changes in oscillatory activity in brain areas central to the pathophysiology of drug addiction. Therefore, it is important to understand whether these dynamic changes can be observed in skull-level EEG recordings, which hold greater translational potential to clinical EEG studies. As such, we characterized the effects of chronic fentanyl exposure, withdrawal and abstinence on cortical EEG patterns.

Fentanyl-treated rats exhibited significant physical dependence, as evidenced by transient weight loss and somatic phenotypes during withdrawal. Cortical EEG dynamics displayed distinct oscillatory signatures that varied according to the phase of fentanyl exposure or withdrawal. Fentanyl exposure caused a transient increase in delta (1-4Hz) and alpha (8-12Hz) power, and a transient decrease in gamma (30-80Hz) power. In contrast, spontaneous withdrawal was characterized by acute, transient increases in multiple frequency bands. Interestingly, alpha power remained increased above baseline levels during the subsequent three weeks of abstinence.

These results demonstrate the profound impact of fentanyl on cortical circuit function. Normalization of opioid-induced changes in oscillatory dynamics, and subsequent rebound during withdrawal suggests the engagement of allostatic mechanisms that may compensate for the persistent activation of mu-opioid receptors. The persistent elevation of alpha power during protracted abstinence suggests that chronic fentanyl exposure has a long-term impact on cortical circuitry.

65 - Differential Activation and Cellular Localization of Protein Kinases in the Periaqueductal Gray Following Morphine Treatment

Akila Ram[1], Erin N. Bobeck[1]

[1]Department of Biology, Utah State University

Morphine is one of the oldest, and one of the most commonly prescribed painkillers for acute and chronic pain despite detrimental side effects after long-term use. Morphine functions as an agonist of the m-opioid receptor (MOPr), which is an inhibitory G-protein coupled receptor. In this study, we examine the molecular consequences of chronic morphine treatment in the mouse ventrolateral periaqueductal gray (vlPAG) which is a key brain region known to be involved in the development of tolerance. The purpose of this research was to investigate activation of different kinases within the vlPAG following repeated morphine treatment. Wild-type mice were administered 9 boluses of morphine (10 mg/kg, s.c.) or saline over 5 days, following which the brains were harvested. We performed fluorescence immunohistochemistry to map the localization and phosphorylation of extracellular signal-regulated kinase (ERK), protein kinase-C (PKC), and protein kinase-A (PKA). We observed significantly greater activated ERK in the vlPAG of morphine-treated animals. We also note that phosphorylated PKC tends to localize to the plasma membrane following morphine treatment. Further, we observed that phosphorylated PKA tends to localize to the nucleus and that there is a significant reduction in nuclear punctae of pPKA following morphine treatment. In addition, we observed putative differential activation patterns of all three proteins along the rostral-caudal axis. Taken together, this study demonstrates a differential activation and localization of ERK, PKC and PKA, and opens avenues to explore the role of chronic morphine treatment on G-protein or β-arrestin signaling and kinase nuclear transport.

66 - Sex differences in plasticity and stress-related genes in the rat following oxycodone conditioned place preference

Matthew Randesi[1], Yan Zhou[1,2], Jason Gray[3], Batsheva Reich[4], Bruce S. McEwen[3], Teresa Milner[4], Kreek [1]

[1]The Rockefeller University, New York, USA, [2]Rockefeller University, [3]The Rockefeller University, New York, USA, [4]Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY

Oxycodone abuse is a pressing public health issue. This study was conducted to determine if there are sex differences in gene expression in the hippocampus of rats following oxycodone conditioned place preference. Rat brains were harvested and the medial (dentate gyrus/CA1) and lateral (CA2/CA3) dorsal hippocampus were isolated. Following total RNA isolation, cDNA was prepared for gene expression analysis using a RT^2 Profiler PCR expression array (Qiagen, Inc.). This custom designed qPCR expression array contained a total of 25 genes; including those genes coding for opioid peptides and receptors, as well as candidate genes involved in synaptic plasticity, including those upregulated following oxycodone self-administration in mice, and genes involved in stress-responses. The gene expression levels of male and female oxycodone exposed rats were compared to male and female saline controls.

Gene expression in the medial hippocampus saw twice as many significant changes in gene expression than in the lateral hippocampus. Within the dentate gyrus/CA1 region, there were nearly twice as many sex (male/female) differences found than there were condition (saline/oxycodone) differences. We found a significant Sex x condition interaction for the expression levels of the gene that codes for protein kinase B (*Akt1*) in the medial region, with oxycodone exposed females having significantly greater expression that both control females as well as oxycodone males ($P=0.0497$ and $P=0.0032$, respectively). In conclusion, we found modest sex- and regional-differences in expression of opioid, stress response, plasticity and kinase/signaling related genes in the rat hippocampus following oxycodone exposure.

67 - The effects of remifentanil dose on the acquisition and persistence of responding for drug-paired cues.

Stephen H. Robertson[1], Emily M. Jutkiewicz[1]

[1]University of Michigan

Drug-paired cues take on reinforcing properties that can promote drug seeking and taking. Previous research has shown that, following response-independent infusions of remifentanil paired with a cue, rats learned to make a novel response for presentations of a drug-paired cue to a greater extent than control rats. In the current experiment, we first established a cue as a conditioned reinforcer by pairing i.v. infusions of remifentanil (1.0, 3.2, or 10.0 ug/kg/infusion) with the cue. Twenty infusions of remifentanil were paired with 20 cue presentations that were delivered according to a variable time 3-min schedule each day for 5 days. The control group received the same number of drug infusions and cue presentations, but they were not explicitly paired. For the next 28 sessions, rats were allowed to freely respond on a nose poke manipulandum for presentations of the drug-paired cue, which were delivered according to a random ratio 2 schedule. We found that responding to produce drug-paired cues was elevated in the experimental group relative to the control group. The number of responses emitted was dose-dependent such that following 3.2 and 10 ug/kg/infusion, but not 1 ug/kg/infusion, animals in the experimental group responded more than controls. For animals conditioned with 3.2 ug/kg/infusion, responding persisted for 24 days; whereas responding following conditioning with 10 ug/kg/infusion persisted for 7 days. These findings demonstrate that drug-paired cues sustain drug seeking behavior, suggesting that Pavlovian conditioning processes contribute to prolonged drug use.

68 - Local mu-opioid receptor antagonism blunts evoked phasic dopamine release in rat nucleus accumbens

Alexander Gómez-A[1], Tatiana A. Shnitko[1], Haley Barefoot[1], Eleanor L. Brightbill[1], Leslie A. Sombers[2], Saleem M. Nicola[3], Donita L. Robinson[1]

[1]University of North Carolina - Chapel Hill, [2]North Carolina State University, Raleigh, NC, [3]Albert Einstein College of Medicine, Bronx, New York

The present study addressed the hypothesis that mu-opioid receptors (MORs) within the nucleus accumbens (NAc) regulate dopamine release. Specifically, we infused the MOR antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2) into the NAc core while evoked dopamine release was measured by fast-scan cyclic voltammetry. We report that CTAP dose-dependently inhibited electrically-evoked dopamine release, with full blockade achieved with the 8 µg dose. In contrast, evoked dopamine release increased after nomifensine infusion and was unchanged after vehicle infusion. These findings demonstrate profound local control of dopamine release by MORs within the NAc core, which have implications for regulation of reward processing.

69 - The Trends of Opioid Prescription Patterns Among Patients with Fibromyalgia

CVM Sarmento[1], M Maz[1], T Pfeifer[1], W Liu[1]

[1]The University of Kansas Medical Center

Although opioid medications may be effective for the management of acute pain, several studies have been reporting a lack of effectiveness of opioid treatment in chronic pain conditions such as fibromyalgia (FM) and Rheumatoid Arthritis (RA), yet previous studies speculated that opioid prescriptions for patients with FM have been increasing in the past years. *Aim:* This study aimed to identify patterns of prescribing opioid medications for patients with FM in the past eight years at the University of Kansas Medical Center. *Methods:* Fibromyalgia, chronic pain, narcotics, opioids, medication, and drugs were used as search terms on the Healthcare Enterprise Repository for Ontological Narration (HERON) database to identify patients at our institution with a diagnosis of FM who had received opioid prescriptions from January 1st, 2010 to December 31st, 2017. The collected data were analyzed descriptively. In addition, a Chi-square test for trend was used to analyze a possible linear relationship between the proportions over time *Results:* Although opioid medications were prescribed more frequently in 2010 (40%) and 2011 (42%), the number of opioid prescriptions have decreased since 2012 and reached the lowest number in 2016 (27%). The Chi-square test for trend shows that from 2010 to 2017 the prescriptions of opioids had a statistically significant decrease, $p < 0.0001$. *Conclusion*: Contrary to the previous literature and the hypothesis that opioids had been increasingly prescribed for FM patients in the past decade, the findings of this analysis indicated that the frequency of opioid prescriptions has decreased since 2012.

70 - HA-epitope tag knockin mice demonstrate lack of μ-opioid receptor splice variants in mouse brain

Sebastian Fritzwanker[1], Andrea Kliewer[1], Stefan Schulz[1]

[1]Friedrich Schiller University, Jena, Germany

GPCRs are notoriously difficult to detect in native tissues. In an effort to resolve this problem, we have developed a novel mouse model with insertion of HA-epitope tag sequence fused to the amino-terminus of the μ-opioid receptor. Here, we provide proof-of-principle that this approach provides a powerful means for highly efficient immunodetection of low abundant GPCR targets in their tissues of origin. We show that the HA-tag facilitates both super-resolution imaging of endogenous receptors and quantitative mass spectrometry of biochemically isolated receptors. Mass spectrometric analyses confirmed post-translational modifications and protein-protein-interactions of MOP previously observed in cultured cells, most notably agonist-selective phosphorylation of carboxyl-terminal serine and threonine residues and activity-dependent interaction with Ga_{i1} and Ga_{o} proteins. Mass spectrometry also unequivocally identified the carboxyl-terminal ^{387}LENLEAETAPLP^{398} motif, which is part of the canonical sequence. At least 14 isoforms have been proposed to arise from alternative splicing of the MOP carboxyl-terminus such that this sequence is specifically replaced by various unrelated sequences while the rest of the receptor protein remains unchanged. Unexpectedly, our mass spectrometric analysis failed to detect any of the alternative sequences. The ^{387}LENLEAETAPLP^{398} motif is the epitope of the rabbit monoclonal antibody UMB-3. The use of UMB-3 for multiple successive rounds of immunodepletion revealed that mouse brain homogenates contain >98% of HA-tagged MOP which also contain the UMB-3 epitope. We conclude that nearly all MOP receptors in mouse brain contain the canonical amino acid sequence and that alternative spliced variants may represent heteronuclear mRNA that is either not or poorly translated.

71 - The association of long-term opium use with overall and cause-specific mortality: Results from a long-term, prospective population-based study

Ramin Shakeri[1], Farin Kamangar[2], Hossein Poustchi[3], Akram Pourshams[3], Shahin Merat[3], Arash Etemadi[4], Siavosh Nasseri Moghaddam[1], Masoud Sotoudeh[5], Masoud Khoshnia[6], Abdolsamad Gharavi[6], Amir Ali Sohrabpour[5], Farhad Islami[7], Atieh Rahmati[5], Christian C Abnet[4], Sanford M Dawsey[4], Nicholas E Day[8], Paul D Pharoah[8], Paolo Boffetta[9], Paul Brennan[10], Neal D Freedman[4], Reza Malekzadeh[5]

[1]Digestive Oncology Research Center, Digestive Disease Research Institute, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran, [2]Department of Biology, School of Computer, Mathematical, and Natural Sciences, Morgan State University, Baltimore, Maryland, USA, [3]Liver and Pancreaticobiliary Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran, [4]Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland, USA, [5]Digestive Disease Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran, [6]Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran, [7]Surveillance and Health Services Research, American Cancer Society, Atlanta, Georgia, USA, [8]Departments of Oncology and Public Health and Primary Care, University of Cambridge, Cambridge, UK, [9]The Tisch Cancer Institute, Ichan School of Medicine at Mount Sinai, New York, USA, [10]Genetic Epidemiology Group, International Agency for Research on Cancer, Lyon, France

Background: Over 35 million people use opioids for medicinal or recreational purposes. Opioid overdose is well known to cause death. However, there is a dearth of data on the long-term health effects of opioids, including opium itself. Therefore, we compared overall and cause-specific mortality of long-term opium users to non-users.

Methods: Detailed and validated data on opium use were obtained at baseline from 50,045 participants of the Golestan Cohort Study. Data were also collected on other covariates, including sex, age, tobacco and alcohol use, education, and income.

Results: At baseline, 17% (n=8,487) of the study participants reported ever using opium, of whom 89.8% were current users. During 500,718 person-years of follow-up, 6,466 of the participants died. Ever use of opium was associated with higher risk of all-cause mortality, with an adjusted HR (95% CI) of 1.68 (1.58-1.79). It was also significantly associated with higher risk of death from ischemic heart disease (1.92; 95% CI 1.66-2.22), cerebrovascular disease (1.41; 1.17-1.71), cancers of the esophagus (1.47; 1.07-2.02) and stomach (1.44; 1.04-1.98), COPD (4.38; 2.95-6.51), asthma (2.96; 1.39-6.30), liver disease (2.83; 1.72-4.63), and infectious diseases (1.72; 1.22-2.42). The associations persisted after stringent sensitivity analyses. Overall, 40% of deaths among opium users, and 10% of all deaths in this population were attributable to opium use.

Conclusions: Together, these results strongly suggest that chronic opium use causes numerous chronic diseases, including cardiovascular disease and several forms of cancer. Future studies in other populations using these and other forms of opioids are urgently needed.

72 - Predictive factors for quality of life and impact of physical activity in Korean breast cancer survivors

Hyun Hwa Shin[1], Chul-Min Kim[1], Yoon Jee Oh[1]

[1]The Catholic University of Korea, Seoul, Republic of Korea

Background Improving long term life quality after cancer management is a growing concern for breast cancer survivors. We aimed to investigate the associated factors and the impact of regular physical activity on life quality in breast cancer survivors.

Methods Using the data from 2007 – 2012 Korea National Health and Nutrition Examination Survey, 155 subjects with a history of breast cancer were included. Life quality was assessed by EQ-5D index, and EQ-5D index level above mean value was regarded as high life quality. Association between various physical activities and life quality was analyzed.

Results Among inclusion, 104 (67.1%) had high life quality (EQ-5D index ≥0.87) and 51 (32.9%) had low life quality (EQ-5D index <0.87). Of those, 13.7%, 16.4%, and 60.2% were regularly doing high, moderate, and light intensity physical activity. Young age, current economic activity, no bereaved spouse, favorable subjective health status, absence of arthritis or myocardial infarction or angina, and the presence of walking and stretching exercise were significantly associated with high life quality. Presence of high or moderate intensity exercise did not affect life quality. In multivariate analysis, subjective health status, arthritis, walking, and stretching exercise were independently associated with high life quality.

Conclusion Light intensity exercise including walking and stretching is associated with high life quality in breast cancer survivors. Comprehensive lifestyle management considering subjective health status, musculoskeletal disease, and encouraging light intensity exercise are important to maintain favorable life quality.

73 - A genetically encoded biosensor reveals location bias of opioid drug action

Miriam Stoeber[2], Damien Jullie[2], Braden Lobingier[2], Jan Steyaert[3], Peter W. Schiller[4], Aashish Manglik[2], Mark von Zastrow[2]

[1]UCSF, Department of Psychiatry, [2]University of California San Francisco, San Francisco, USA, [3]Structural Biology Brussels, Vrije Universiteit Brussel, Belgium, [4]Clinical Research Institute of Montreal

Opioid receptors (ORs) precisely modulate behavior when activated by native peptide ligands but distort behaviors to produce pathology when activated by non-peptide drugs. A fundamental question is how drugs differ from peptides in their actions on target neurons. Here we show that drugs differ in the subcellular location at which they activate ORs. We develop a genetically encoded biosensor that directly detects ligand-induced activation of ORs and uncover a real-time map of the spatiotemporal organization of OR activation in living neurons. Peptide agonists produce a characteristic activation pattern initiated in the plasma membrane and propagating to endosomes after receptor internalization. Drugs produce a different activation pattern by additionally driving OR activation in the somatic Golgi apparatus and Golgi elements extending throughout the dendritic arbor. These results establish an approach to probe the cellular basis of neuromodulation and reveal that drugs distort the spatiotemporal landscape of neuronal OR activation.

74 - The effects of opioid sensitization on incentive sensitization and dopamine neurotransmission

Catherine Thomas[1], Cassandra Klune[1], Alicia Zumbusch[1], Una Mrdja[1], Stephanie Borgland[1,2], Vedran Lovic[1,2,3]

[1]University of Calgary, [2]Hotchkiss Brain Institute, [3]ACHRI

Through repeated pairings with a drug, drug-associated stimuli or cues (e.g., needles, syringes, pill bottles) can become imbued with incentive (motivational) value. This incentive property empowers drug cues to instigate and intensify drug seeking, consumption, and relapse. Repeated drug exposure produces enduring changes that can intensify the physiological and behavioural effects of further drug exposure (sensitization). All drugs of abuse potentiate dopamine (DA) neurotransmission in the nucleus accumbens (NAcc), a key node in the brain's reward circuitry thought to be necessary for the attribution of motivational value to drugs and drug-cues. The effects of opioid sensitization on attribution of incentive value to an opioid-cue and cue-evoked DA neurotransmission remain unknown. *We found that an opioid cue (compound light-tone) gained incentive value after a single Pavlovian cue-drug pairing session.* This effect was potentiated by a 10-day opioid sensitization regimen (i.e., escalating doses of remifentanil in the absence of any discrete cues). Furthermore, *opioid sensitization leads to significantly greater cue-evoked DA neurotransmission, compared to non-sensitized controls*. The relationship between opioid pre-exposure, incentive value attribution and cue- and drug-evoked DA neurotransmission may offer a unique opportunity to predict the ability of drug cues to spur on drug seeking, consumption, and relapse.

75 - Modulation of opiate reward and intake, as well as antinociceptive effects by the OPRM1 A118G SNP.

A. Thorsell[1], L. Holm[1], M. Heilig[1]

[1]CSAN/IKE, Linköping University, Sweden

Both the therapeutic response to mu-opioid receptor (MOR) antagonists, as well as sensitivity to the rewarding effects of MOR-agonists may be moderated by a variation at the mu-opioid receptor gene locus (OPRM1: Oprm-A118G). We have previously generated and described a "humanized" mouse carrying the respective human OPRM1 A118G alleles (i.e.118AA and 118GG). Here, we examine the role of the OPRM1 A118G variation in reward sensitivity and opiate intake, using animal models of drug reinforcement and reward. Reward-related behavior for opiates (including morphine, oxycodone) was measured using the Conditioned Place Preference (CPP) model. Drug intake was measured using an oral limited access model as well as a continuous access paradigm. Pain sensitivity was evaluated using the tail-flick and hot plate tests, and anxiety-related behavior during acute withdrawal following opiate-administration was measured on the elevated plus-maze (EPM). The OPRM1-A118G's as well as C57Bl6/J(Rj) were used in the experiments.

The OPRM1 118AA carriers displayed a blunted anxiogenic behavioral response following acute withdrawal from opiates compared to the 118GG's. Additionally, the 118GG's overall displayed more pronounced CPP, and furthermore at doses not inducing CPP in the C57Bl6/J for morphine. Oxycodone conditioning generated strong CPP in all animals used. A higher consumption of oral morphine was seen in the 118GG's compared to the 118AAs. Pain responses were also differentially modulated by morphine, but not by oxycodone.

In conclusion, for morphine and oxycodone, the OPRM1 A118G modulates reward sensitivity in a manner not easily related to the antinociceptive effects of the respective compounds.

76 - Interaction between spinal high-mobility group box-1 and glial cells in global cerebral ischemia-induced mechanical allodynia

Shogo Tokuyama[1], Wataru Matsuura[1], Shinichi Harada[1], Keyue Liu[2], Masahiro Nishibori[2]

[1]Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University, [2]Department of Pharmacology, Graduate School of Medicine, Dentistry and Pharmacological Sciences, Okayama University

Central post-stroke pain (CPSP) is one of the complications of cerebral ischemia and neuropathic pain. We have previously shown that spinal high-mobility group box-1 (HMGB1) plays an important role in the induction of CPSP. It has been reported that HMGB1 exacerbates inflammation and pain condition through its receptor as toll-like receptor 4 (TLR4) or receptor for advanced glycation end-products (RAGE). Furthermore, the glial cells also involve in pain exacerbation. In this study, we investigated whether the interaction between spinal glial cells and HMGB1 signaling is involved in the induction of CPSP. Male ddY mice were subjected to 30 min of bilateral carotid artery occlusion (BCAO). The development of hind paw mechanical hyperalgesia was measured using the von Frey test. Spinal HMGB1, microglia and astrocyte expression were clearly increased on day 3 after BCAO. Although HMGB1 colocalized with NeuN (marker of neurons), but not with Iba1 (marker of microglia) and GFAP (marker of astrocyte) on day 3 after BCAO. Intrathecal (i.t.) injection of lipopolysaccharides from *Rhodobacter sphaeroides* (LPS-RS, a TLR4 antagonist) and low-molecular-weight heparin (LMWH, a RAGE antagonist) significantly suppressed mechanical allodynia on day 3 after BCAO. In addition, BCAO-induced increase of spinal microglia and astrocyte were suppressed by i.t. injection of anti-HMGB1 monoclonal antibody and LPS-RS administration, but not LMWH administration. These results suggest that the interaction between spinal glial cells and HMGB1/TLR4 may be involved in the induction of CPSP. On the other hand, HMGB1/RAGE signaling may relate to other mechanisms instead of spinal glial cells activation for CPSP.

77 - Identifying a role for gonadotropin-releasing hormone and kisspeptin neurons in the development of opioid-induced hypogonadism

Karen J. Tonsfeldt[1], Laura J. Cui[1], Pamela L Mellon[1]

[1]University of California San Diego

One understudied aspect of opioid use is the development of opioid-induced hypogonadism, which occurs in the vast majority of opioid users regardless of sex, route of administration, or duration of use. Hypogonadism is characterized by low levels of estrogen and testosterone. The hypothalamus-pituitary-gonad axis consists of Kisspeptin (Kiss1) neurons that stimulate gonadotropin-releasing hormone (GnRH) neurons. Pulses of GnRH stimulate pulses of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary to act on the gonads to promote the production of estrogen and testosterone. We are exploring the actions of opioids and mu-opioid receptor signaling in Kiss1 and GnRH neurons. We have identified the mu opioid receptor (MOR) in the immortalized kisspeptin neurons, KT-aRs, and GnRH neurons, GT1-7s, using qPCR and dermorphin-A594 binding. We have also found that morphine or DAMGO treatment decreases Kiss1 and GnRH transcriptional activity. GT1-7 cells transfected a GnRH-luc showed reduced GnRH transcription following morphine treatment. KT-aR cells treated with DAMGO show a marked decrease in Kiss1 mRNA using qPCR. These studies suggest a direct effect of MOR on Kiss1 and GnRH transcription, which may contribute to the development of opioid-induced hypogonadism. Responsiveness of GnRH neurons to kisspeptin can be measured by the increase in serum LH following exogenous kisspeptin challenge. The increase in LH following kisspeptin administration was abolished in acutely morphine-treated mice, indicating interference of GnRH neuron responsiveness by morphine. Overall, our preliminary findings are beginning to establish the roles of the kisspeptin and GnRH neurons in the etiology of opioid-induced hypogonadism.

78 - Further evidence for the utility of central μ-opioids for cancer patients.

Kazuhiro Torigoe[1], Yusuke Hamada[2,3], Michiko Narita[2], Yoshiaki Machida[1], Tomohisa Mori[2], Naoko Kuzumaki[2], Minoru Narita[2,3]

[1]Div. Practical Edu., Hoshi Univ., Tokyo, Japan, [2]Dept. Pharmacol., Hoshi Univ., Tokyo, Japan, [3]L-StaR, Hoshi Univ., Tokyo, Japan

In a clinical setting, μ-opioid agonists are widely used for pain relief of cancer patients. Generally, it is thought that the peripheral μ-opioid receptor is involved in the occurrence of side effects such as constipation. Although centralμ-opioid receptors are thought to be important for the development of analgesic effects, a little is known about the effects of peripheral and central μ-opioids on tumor growth. Here we investigated the effect of μ-opioid receptor stimulation on tumor growth in the peripheral or central region by activating the peripheral μ-opioid receptor or artificially activating the hypothalamic μ-opioidergic neurons. Lewis lung carcinoma cells were transplanted subcutaneously into the right hip of the mouse, and tumor-bearing animal were prepared.Quantitative analysis of tumor volume was performed in saline-treated, loperamide, a peripheral μ-opioid receptor agonist, (10 mg/kg/day, b.i.d, i.p.) - treated or methylnaltrexone (MNTX), a peripheral μ-opioid receptor antagonist, (10 mg/ kg/day) - treated mice at 4,7,11 and 14 days after tumor transplantation. Treatment of loperamide resulted in tumor growth. On the other hand, treatment ofMNTX inhibited tumor growth. Furthermore, the activation of pro-opiomelanocortinneurons in the arcuate nucleus significantly suppressed tumor growth via the activation of cell-mediated immunity. These findings suggest that the stimulation of central μ-opioid receptors may enhance antitumor immunity via cell-mediated immunity and the use of μ-opioids that selectively activate central μ-opioid receptors may produce not only more effective analgesic effect but also antitumor effect.

79 - Antinociceptive and Locomotor Effects of Fentanyl-Related Substances in Mice

Neil B. Varshneya[1], D. Matthew Walentiny[1], Lea T. Moisa[1], Teneille D. Walker[2], Luli R. Akinfiresoye[2], Patrick M. Beardsley[1]

[1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA, [2]Diversion Control Division, Drug Enforcement Administration, United States Department of Justice, Springfield, VA, USA

In 2016, drug overdose was the leading cause of death for Americans under the age of 52 and resulted in more than 42,000 opioid-related fatalities. A five-fold increase in deaths involving fentanyl or fentanyl analogs between 2013 and 2016 contributed to this problem. In the present study, fentanyl-related substances were tested in adult male Swiss Webster mice for their effects on locomotion and antinociception and compared to those of fentanyl and morphine. In locomotor activity tests, fentanyl (1 and 10 mg/kg), morphine (100 and 180 mg/kg), crotonyl fentanyl (10 mg/kg), and para-fluorobutyrylfentanyl (10 and 100 mg/kg) produced significant ($p \leq 0.05$) dose-dependent increases in locomotion. However, valeryl fentanyl was without effects on locomotion up to 100 mg/kg. In warm-water tail-withdrawal tests, fentanyl (0.1 and 1 mg/kg), morphine (10 and 32 mg/kg), crotonyl fentanyl (0.01-10 mg/kg), para-fluorobutyrylfentanyl (1-10 mg/kg), and valeryl fentanyl (10 and 32 mg/kg) produced significant ($p \leq 0.05$) dose-dependent increases in antinociception with increasing ED50s (CI) for doing so of fentanyl [0.08 (0.04-0.16)] > crotonyl fentanyl [0.23 mg/kg (0.18-0.29)] > para-fluorobutyrylfentanyl [0.91 mg/kg (0.46-1.58)] > valeryl fentanyl [6.43 mg/kg (3.91-10.46)] > morphine [7.82 mg/kg (5.42-11.02)]. Naltrexone pretreatment (1 mg/kg) increased antinociceptive ED50s several fold with decreasing magnitude of valeryl fentanyl (11.90x) > para-fluorobutyrylfentanyl (10.87x) > crotonyl fentanyl (6.27x) > fentanyl (3.95x) > morphine (1.48x). Our data show that within some fentanyl-related substances doses eliciting hyperlocomotion and antinociception are separable, and suggest that their antinociceptive effects can be even more susceptible to antagonism than those of morphine.

80 - AT-312 (1-(1-((cis)-4-isopropyl cyclohexyl) piperidine-4-yl)-1H-indol-2-yl) methanol) a novel therapeutic compound for opioid relapse in an adolescent rat model

Vazquez Sanroman D[1], Bardo M.T.[2], Nurulain Zaveri[3]

[1]Oklahoma State University, [2]University of Kentucky, [3]Astraea Therapeutics

Nociceptin opioid peptide (NOP) receptor activation translates into inhibition of opioid-mediated reward. The ventral tegmental area (VTA) is not only the origin of the brain dopamine reward pathway, but also an important substrate for NOP receptor effects. Indeed, VTA-GABAergic neurons are surrounded by perineuronal nets (PNNs), which consist of extracellular matrix elements necessary for the modulation of nociception and synaptic plasticity involved in drug addiction. To construct a model of opioid relapse in adolescent rats, we evaluated the effect of the NOP receptor full agonist AT-312 on the acquisition, extinction and reinstatement of morphine-induced conditioned place preference (CPP) in adolescent female and male (PND34) Sprague Dawley rats. In Experiment 1, we examined the effects of AT-312 on morphine (5 mg/kg) CPP. In Experiment 2, we analyzed the effect of AT-312 on extinction. After conditioning, rats underwent daily AT-312 (3 mg/kg, s.c.) administration and extinction sessions. In Experiment 3, we tested the effect of AT-312 on the reinstating effects of a priming dose of morphine (2.5 mg/kg). Our results showed that systemic administration of AT-312 in adolescent rats blocked morphine-induced CPP [$F(1, 5) = 25.36$, $p < .05$], facilitated morphine-conditioned extinction [$F(1, 5) = 25.36$, $p < .05$], and remarkably reduced drug-primed reinstatement [$X2(1) = 10.53$, $p<0.05$]. At the cellular level, AT 312 also decreased PNN expression [$X2(1) = 7.91$, $p<0.01$]. Our results showed that the NOP agonist AT-312 altered morphine reward and reinstatement, while also inducing PNN-dependent plasticity in the brain reward system of adolescent rats.

81 - Physical association between μ opioid and dopamine D1 receptors : Implications for modulation of locomotor sensitization in dopamine-independent manner

Yi-Min Tao[1], Chuan Yu[1], Yu-Jun Wang[1], Jing-Gen Liu[1]

[1]Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai 201203, China;

Introduction: Exposed to opiates induces locomotor sensitization in rodents, which may be related to the development of compulsive drug-seeking behavior. Numerous studies have demonstrated that locomotor sensitization can occur in a dopamine transmission-independent manner, however, the underlying mechanisms are unclear.

Methods: The coimmunoprecipitation, BRET and cross-antagonism assays were used to demonstrating the existence of receptor heterodimers. The function of heterodimers was evaluated by behavioral studies of locomotor sensitization.

Results/Conclusion: We demonstrated that dopamine D1 receptor (D1R) antagonist SCH23390 antagonized the signaling originated by stimulation of μ opioid receptor (μOR) with agonists in transfected cells expressing two receptors and in wild type but not D1R KO mouse striatal tissues, suggesting that D1R antagonist SCH23390 was able to modify μOR function via receptor heteromers, since the ability of an antagonist of one of the receptors to inhibit signals originated by stimulation of the partner receptor was a biochemical characteristic of receptor heteromers. The existence of D1R/μOR heterodimers was further supported by biochemical and biophysical assays. Moreover, in vivo we demonstrated that, on condition that dopamine release was absent (e.g. destruction of the dopaminergic projection from the ventral tegmental area to the striatum), SCH23390 still significantly inhibited μ agonist-induced behavioral responses in rats. Additionally, we demonstrated that D1R or μOR KO mice, which were unable to form D1R/μOR heterodimer, failed to express locomotor sensitization to morphine. These results suggest that D1R/μOR heterodimers may be involved in dopamine independent expression of locomotor sensitization to opiates.

82 - AMPA Receptor Positive Allosteric Modulators Attenuate Opioid Tolerance and Dependence

Xiaoyu Hu[1], Xuebi Tian[1], Xiao Guo[1], Ying He[1], Haijun Chen[2], Jia Zhou[2], Zaijie Jim Wang[1]

[1]University of Illinois, [2]University of Texas Medical Branch, Galveston, TX

Development of opioid tolerance and dependence hinders the use of opioids for the treatment of chronic pain. In searching for the mechanism and potential intervention for opioid tolerance and dependence, we studied the action of two positive allosteric modulators of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR PAMs). In mice treated with morphine (100 mg/kg, s.c.), acute morphine tolerance and dependence developed in 4-6 h. Treatment with aniracetam, a well-established AMPAR PAM, was able to completely prevent and reverse the development of acute antinociceptive tolerance to morphine. Partial, but significant, effects of aniracetam on acute morphine induced-physical dependence were also observed. Moreover, aniracetam significantly reversed the established morphine tolerance and dependence in a chronic model of morphine tolerance and dependence produced by intermittent morphine (10 mg/kg, s.c. for 5d). In addition, HJC0122, a new AMPAR PAM was found to have similar effects as aniracetam but with a higher potency. These previously undisclosed actions of AMPAR PAMs are intriguing and may shed lights on understanding the APMA signaling pathway in opioid addiction.

Supported by R01DA041809, P30DA28821, R01DA038446.

No conflict of interest.

83 - Characterization of JVA 4001, a novel mixed opioid agonist/ kappa opioid receptor antagonist that attenuates drug and stress-induced reinstatement of extinguished morphine-conditioned place preference

Lisa Wilson[1], Ariana Brice[1], Shainnel O. Eans[1], Grant Simpson[1], Mike Ferrecane[1], Jane V. Aldrich[1], Jay McLaughlin[1], Heather Stacy[1]

[1]University of Florida, Gainesville, USA

Mixed opioid agonist/kappa opioid receptor (KOR) antagonists such as the macrocyclic tetrapeptide CJ-15,208 (*cyclo*[Phe-D-Pro-Phe-Trp) show promise as treatments for both pain and drug abuse. Accordingly, we hypothesized that a mixed mu/kappa opioid receptor agonist/KOR antagonist analog of CJ-15,208, JVA 4001, would produce potent antinociception while also preventing both drug- and stress-induced reinstatement of extinguished cocaine and morphine conditioned place preference (CPP). Characterized in the mouse 55ºC warm-water tail withdrawal assay, JVA 4001 produced short-acting but potent antinociception after both central (ED_{50} and 95% C.I. value = 0.11(0.05-0.21) nmol, i.c.v.) and peripheral (i.p. and p.o.) administration mediated by MOR and (to a lesser extent) KOR agonism. Furthermore, JVA 4001 demonstrated potent KOR-selective antagonism, preventing U50,488-induced antinociception for up to 4 h after administration through central (1 pmol, i.c.v.) or peripheral (1 mg/kg, i.p. or 10 mg/kg, p.o.) routes. Although JVA 4001 demonstrated hyperlocomotion and respiratory depression consistent with MOR agonism, these effects were less than those produced by traditional MOR agonists, and JVA 4001 did not produce the CPP demonstrated by morphine. In contrast, JVA 4001 dose-dependently blocked both stress- and drug-induced reinstatement of extinguished place-preference responses of cocaine- and morphine-CPP mice in a time-dependent manner. Overall, although additional pharmacokinetic studies are needed, the promising antinociception with reduced liabilities and the ability to prevent both drug- and stress-induced reinstatement of multiple abused substances highlight JVA 4001 as an excellent lead candidate for the treatment of pain and drug abuse.

84 - Comparative Characterization of Operant Oxycodone Self-Administration in Male BALB/c and C57Bl/6 Mice

Kyle Windisch[1], Brian Reed[1], Amelia Dunn[1], Alexandra Dunn[1], Marco Pravetoni[2], Mary Jeanne Kreek[1]

[1]Rockefeller University, New York, USA, [2]Minneapolis Medical Research Foundation, and Department of Medicine and Pharmacology, University of Minnesota, Minneapolis, MN, USA

INTRODUCTION: The BALB/c mouse strain is commonly used in the development of addiction vaccines due in part to the strain's robust immunological response. However, use of BALB/c mice for the development of opioid vaccines is challenged by the limited behavioral outputs demonstrated with these mice. BALB/c mice have been shown to acquire heroin, but not morphine, intravenous self-administration (IVSA) but only for very low unit doses. Here we investigated BALB/c oxycodone IVSA. METHODS: For acquisition, separate groups of male BALB/cAnNCrl had eight daily 1-hr oxycodone IVSA sessions (56, 112, or 250 ug/kg/inf), followed subsequently by dose response sessions (28, 56, 112, 250 and 500 ug/kg/inf; 2 days/dose). For maintenance, separate groups of BALB/cAnNCrl and C57Bl/6NCrl mice had 14 daily 2-hr oxycodone sessions (28 or 250 ug/kg/inf). RESULTS: All mice met criteria for acquisition by the final acquisition session. A standard inverted U dose response was observed with no significant difference between groups. BALB/c mice had lower initial responding for 250 ug/kg/inf oxycodone (day 1) compared to C57Bl/6 mice; however, a similar pattern of escalated intake was observed between the BALB/c and C57Bl/6 mice. CONCLUSION: This is the first report of BALB/c mice stably self-administering an opioid with unit doses commensurate to those used with other mouse strains. Further research is needed to determine if this is unique to oxycodone or the BALB/cAnNCrl substrain.

Support: Gary R. Helman Postdoctoral Research Fellowship (KAW), Miriam and Sheldon Adelson Medical Research Foundation (MJK), and NIH-NIDA R01 DA041730 (MP).

85 - Generation of conditional Oprm1 knockout rat models using Easi-CRISPR with long ssDNA donors

Jin Xu[1], Valerie P. Le Rouzic[1], Rolen Quadros[2], Gavril W. Pasternak[1], Miyasaka Yoshiki[3], Tomoji Mashimo*[3], Thomas L. Saunders*[4], Channabasavaiah B. Gurumurthy*[2], Yingxian Pan*[1]

[1]Memorial Sloan-Kettering Cancer Center, New York, USA, [2]Mouse Genome Engineering Core Facility, University of Nebraska Medical Center, Omaha, NE 68198 USA, [3]Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University, Osaka 565-0871 Japan, [4]Transgenic Animal Core, University of Michigan, Ann Arbor, MI 48109 USA

A single-copy mu opioid receptor (*OPRM1*) gene generates a vast array of mu opioid receptor splice variants through extensive alternative pre-mRNA splicing that is conserved from mouse to rat to human. Two distinct promoters associated with either exon 1 (E1) or exon 11 (E11) control the expression of these splice variants. E1-associated variants mainly encode full-length carboxyl (C-) terminal, 7-transmembrane (7-TM) domain receptors, whereas most E11-associated variants are truncated, 6-TM domain receptors. Using gene targeted mouse models, we have demonstrated the functional relevance and difference of the E1-associated and E11-associated variants. While mouse models are valuable, rats have many advantages both in behavioral modeling and in vivo manipulation. In the present study, we have generated two conditional Oprm1 knockout (KO) models in Sprague Dawley rats using a newly developed Easi-CRISPR with long ssDNA donors approach. The first rat model is the conditional E11 KO model in which E11 codon and adjacent intron sequences were flanked by two loxPs. Cre-mediated E11 deletion was confirmed through breeding a transgenic CAG-Cre rat. The second rat model is the conditional E1 KO model with a similar strategy. Currently, these rat models are being bred and used for initial pharmacological studies. These conditional Oprm1 KO rat models will provide valuable resources for the research community, and would expand our ability explore mu opioid pharmacology with approaches and techniques not feasible in mice. *Correspondent authors. Supported by grants from NIH (DA029244, DA06241, DA042888 and CA08748)

86 - The roles of alternatively spliced mu opioid receptor intracellular C-termini encoded by exon 7 on fentanyl actions

Jin Xu[1], Valerie P. Le Rouzic[1], Zhigang Lu[2], Gavril Pasternak[1,3], Yingxian Pan[4]

[1]Memorial Sloan-Kettering Cancer Center, New York, USA, [2]Nanjing University of Chinese Medicine, Nanjing, China, [3]Memorial Sloan-Kettering Cancer Center, New York, USA, [4]Memorial Sloan-Kettering Cancer Center, New York, USA

The mu opioid receptor gene (*OPRM1*) undergoes extensive alternative pre-mRNA splicing, generating multiple splice variants that are conserved from rodents to humans. One type of the splice variants is full-length 7-transmembrane (TM) C-terminal variants that are identical except for the sequences at the tip of the intracellular C-terminal tail. Increasing evidence supports the pharmacological importance of these 7TM C-terminal variants. Particularly, exon 7-associated C-terminal truncation in C57BL/6J mice (mE7M-B6) diminished morphine tolerance and reward without altering physical dependence, like those seen in a β-arrestin2 KO mouse. Together with the results from cell-based studies, it suggests a physical and functional interaction of E7-associated C-terminal tails with β-arrestin2 that contributes to morphine desensitization and tolerance. In the present study, we further investigate the roles of E7-associated C-terminal tails on fentanyl actions in mE7M-B6 mice. In mE7M-B6 mice in which a stop codon was inserted at the beginning of E7 and all E7-associated C-termini were abolished, fentanyl analgesia determined by dose-response curve in a radiant heat tail-flick assay was not affected. However, fentanyl-induced tolerance was significantly reduced, while fentanyl-induced physical dependence determined by naloxone-precipitated withdrawal was not changed. These data further support the functional relevance of E7-associated splice variants in opioid pharmacology.

Supported in part by grants from NIH (DA029244, DA06241, DA042888 and CA08748), the Peter F. McManus Charitable Trust, Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research.

87 - The Relation between Serum Uric acid and Spirometric Values and the Risk of Respiratory Disease

Jiwon Yoon[1], Minkyung Cho[1], Chulmin Kim[1]

[1]Seoul St. Mary's Hospital, Catholic Medical Center, Korea

Background : Serum uric acid (UA), the final product of purine degradation, has been shown to be increased in the tissue hypoxic state. Several studies have been reported that patients with chronic obstructive pulmonary disease (COPD) have high serum levels of UA. However, the relationship between UA levels and spirometric values has not been investigated.

Methods: The subjects of this study were 3,037 individuals aged 19 years and or older who underwent pulmonary function tests (PFT), using the data from the Korea National Health and Nutritional Survey(KNHANES) conducted in 2016. Chi-square test was used to evaluate baseline characteristics. Logistic regression and linear regression analysis were used to evaluate associations between UA and spirometric values

Results: Elevated levels of UA were significantly increased in males (90.5%) than in females (9.5%). Percent predicted forced vital capacity (FVC %), forced expiratory volume in 1 s (FEV1 %) and FEV1/FVC were inversely correlated with UA levels. Linear regression analysis showed that FVC % and FEV1 % were predictive for UA levels, independently of sex, age, smoking, drinking. Logistic regression analysis indicated that Elevated levels of UA were significantly associated with risk of COPD and Obstructive pulmonary diseases, after adjustment for sex, age, smoking, drinking, physical activity (odds ratio, 1. 480; 95% confidence interval, 1.004-2.182)

Conclusion: FVC % and FEV1 % were significantly associated with serum uric acid. Elevated levels of UA were increased with risk of respiratory disease in a general population of Korea

Key words: uric acid, spirometry, COPD

88 - Increasing G-protein signalling bias for μ-opioid receptor agonists enhances protein kinase C dependent desensitization.

Arsalan Yousuf[1], Setareh Sianati[1], Marco Avena[1], MacDonald J. Christie[1]

[1]University of Sydney, Australia

μ-Receptor agonists have the ability to differentially activate distinct signalling pathways such as G-protein signalling versus β-arrestin-2-dependent regulation, a phenomenon termed biased signalling. Biased signalling has been suggested to influence side effect profile, as well as tolerance and dependence on opioids (Schmid et al., 2017). We and others have established that morphine, which weakly recruits β-arrestin-2, induces desensitization of the μ-receptor via a protein kinase C (PKC) dependent mechanism but peptides such as met-enkalphalin that strongly recruit β-arrestin-2 desensitise via G-protein receptor kinase/β-arrestin-2 dependent mechanisms (Yousuf et al., 2015). To determine the role of G-protein versus arrestin bias in PKC-dependent regulation of desensitization we examined a spectrum of biased opioids. We first established the G-protein versus arrestin bias of the series using perforated patch clamp recordings of GIRK channel conductance (G-protein signalling) and β-arrestin-2 recruitment to the receptor (BRET), as well as endocytosis (immunohistochemically) in AtT20 cells stably expressing μ-receptors. Acute desensitization was then measured in the presence and absence of the potent PKC blocker, calphostin–C, to resolve the proportion of desensitization mediated by PKC. Our results showed that endomorphin-2, the most strongly arrestin biased opioid in the series, showed the smallest effect of calphostin-C (7 ± 3 % reduction of desensitization) with a progressive increase in PKC-dependence for met-enkephalin, morphine, to bilorphin, a novel, strongly G-protein biased peptidic agonist, which showed strong dependence on PKC (80 ± 4 % inhibition by calphostin-C). In conclusion, our results show that increasing bias towards G-protein versus arrestin signalling increases PKC-mediated receptor desensitization.

89 - Profile of a Novel bifunctional NOP/MOP receptor agonist for Opioid Use Disorder and Non-addicting Analgesia: Efficacy and lack of opioid side effects in nonhuman primates

Nurulain Zaveri[1], Mei-Chuan Ko[3], Huiping Ding[3], Norikazu Kiguchi[3,4], Dennis Yasuda[1], Pankaj R. Daga[1], Willma E. Polgar[1], James J. Lu[1], Paul W. Czoty[3], Shiroh Kishioka[4]

[1]Astraea Therapeutics, [2]Wake Forest University, [3]Department of Physiology and Pharmacology, Wake Forest School of Medicine, [4]Department of Pharmacology, Wakayama Medical University

The opioid crisis, declared as a public health emergency, is in essence a double epidemic of opioid addiction and chronic pain. Over 25 million people have daily pain and over 2 million are addicted to opioids. Since opioids provide the most effective pain relief, efforts to treat pain with opioids have fueled the opioid use disorders characterized by physical dependence and craving, potentially leading to serious consequences such as abuse and respiratory depression. New pharmacological approaches that provide alternatives for safe, effective analgesia and address opioid use disorders are urgently needed, but are, as yet, unavailable to patients. Agonists for the nociceptin opioid receptor (NOP) have been known to modulate the pharmacology of mu opioid (MOP) agonists, particularly antinociception and opioid-induced reward. We have hypothesized that bifunctional NOP/MOP agonists may have an attractive profile of potent analgesic effects and lack opioid rewarding effects. Here we report such a profile for a novel NOP/MOP bifunctional agonist AT-121, discovered using structure-based drug design and medicinal chemistry optimization. AT-121 possesses partial agonist activity at the NOP and MOP receptor in a single molecule. Detailed characterization in nonhuman primates shows that AT-121 has “dual” pharmacological efficacy such that it suppresses oxycodone’s reinforcing effects and also shows potent analgesia (with lower ED50 than morphine). AT-121 lacks the side effects of opioids, such as respiratory depression, abuse potential, opioid-induced hyperalgesia, physical dependence and tolerance. Bifunctional NOP/MOP agonists with an appropriate balance of NOP and MOP agonist efficacy may provide an innovative solution for the opioid crisis.

90 - Structurally different Anabolic Androgenic Steroids induce neurotoxic effects in primary cortical cell cultures

Sofia Zelleroth[1], Erik Nylander[1], Fred Nyberg[1], Alfhild Grönbladh[1], Mathias Hallberg[1]

[1]Uppsala University, Uppsala, Sweden

Anabolic Androgenic steroids (AASs) are suggested as a gateway to opioid abuse and dependence of other drugs of abuse. The AASs are used to enhance physical appearance and performance in sports, which have become a growing problem among non-athletes. Many of the individuals abusing AAS also use opioids and several heroin-abusers have a history of previous AAS misuse. This raises the question, how does AASs affect the brain resulting in increased sensitivity to other narcotics? The aim was to investigate the toxic effects of structurally different AASs to gain more knowledge about how the brain is affected by AAS abuse.

The toxicity of testosterone, nandrolone, stanozolol and trenbolone was studied in primary cortical cell cultures. The cells were exposed to increasing concentrations of the AASs for 24 hours or 3 days. The cells were co-treated with the androgen receptor (AR) antagonist, flutamide, to determine if the effects were mediated by the AR. Thereafter, cellular toxicity was determined by measuring mitochondrial activity, lactate dehydrogenase release and caspase activity.

All AASs studied induced toxic effects in primary cortical cells, in a time-dependent and dose-dependent manner. Testosterone, nandrolone and trenbolone caused their toxic effects by induction of apoptosis. In contrast, stanozolol seemed to cause necrosis. Flutamide were able to abolish or diminish the AAS-induced effects.

In conclusion, supraphysiological concentrations of the AASs studied induce neurotoxic effects, but to different extent and possibly by different mechanisms. However, the main effects seem to be mediated by activation of the classical AR-pathway.

91 - Morphine induced alteration in gut microbiome contributes to analgesic tolerance by modulating the Gut-Immune-CNS Axis

Li Zhang[1,2], Jingjing Meng[2], Ramakrishnan Sundaram[2], Sabita Roy[1,2]

[1]University of Minnesota, [2]University of Miami

Opioids are the most prescribed analgesic agent for pain relieve clinically. However its clinical use is limited by co-morbidities such as tolerance and dependence. We have previously shown that chronic morphine treatment results in significant sustained inflammation as a consequence of gut barrier disruption and bacterial translocation that is mediated by increased gut epithelial expression of TLR2 and TLR4 proteins. A significant body of literature shows that chronic inflammation is correlated with morphine analgesic tolerance. Our recent study demonstrates that morphine treatment results in significant gut microbial disruption, a significant expansion of gram-positive pathogenic bacteria. The purpose of this study was to investigate the role of gut microbiome in the development of morphine induced analgesic tolerance. We show that morphine treatment of germ free mice and pan antibiotic treated mice results in significantly attenuated morphine induced anti-nociceptive tolerance, bacterial translocation, TLR2 and TLR4 expression, and reduced systemic inflammation. Moreover, morphine induced analgesic tolerance was significantly decreased in in TLR2KO and TLR4KO mice. Notable treatment of mice with the probiotics, VSL#3, which consisted of eight beneficial bacteria, reduced morphine induced analgesic tolerance, TLR2 and TLR4 expression and chronic inflammation by restoring gut homeostasis. In conclusion, we demonstrated that morphine-induced dysbiosis modulated analgesic tolerance through gut-immune-CNS axis. Maintaining gut homeostasis is a therapeutic target for morphine tolerance, which extends the effectiveness of opioids in clinical pain management.

92 - Diarylurea-based allosteric modulators of the cannabinoid CB1 receptor: Structure-activity relationship studies on the pyrrolidinylpyridinyl group

Thuy Nguyen[1], Thomas F. Gamage[1], Ann M. Decker[1], Nadezhda German[1], Charlotte E. Farquhar[1], Tiffany L. Langston[1], Jenny Wiley[1], Brian F. Thomas[1], Terry Kenakin[2], Yanan Zhang[1]

[1]Research Triangle Institute, [2]University of North Carolina Chapel Hill

Allosteric modulators have attracted significant interest in the modulation of the CB1 signaling for therapeutic benefits while avoiding the adverse effects associated with orthosteric ligands. In our previous studies on the CB1 receptor allosteric modulator PSNCBAM-1 we have reported that the pyrrolidinyl ring is not required for CB1 modulation and the pyridinyl moiety could be replaced by a simple phenyl or substituted phenyl rings. Here we extended the structure-activity relationships of these diarylureas by introducing heterocyclic groups at the place of the pyridinyl ring of PSNCBAM-1. These novel compounds showed good potencies and binding affinities in calcium mobilization, GTP-γ-S binding and radioligand binding assays. Most compounds possessed low nanomolar IC_{50} values at CB1 receptor without any significant activities at the CB2 receptor. These modulators reduced the Emax of the orthosteric CB1 receptor agonist CP55940, as expected with negative allosteric modulators (NAMs). Interestingly, this new series of compounds displayed signaling pathway bias and variations in cooperativities with the orthosteric agonist CP55,940 and antagonist SR141716A. These results suggest that the CB1 signaling could be fine-tuned based on the orthosteric ligands and signaling pathways.

Support: NIH grant DA040693 (YZ).

Notes

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