BRITISH PHARMACOLOGICAL SOCIETY

INRC

TODAY'S SCIENCE TOMORROW'S MEDICINES

INTERNATIONAL NARCOTICS RESEARCH CONFERENCE

11–14 JULY 2016

Bath Assembly Rooms
Bath, United Kingdom

Programme

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Information for participants

KEY TIMINGS

Sunday 10 July

15.00–17.00	Registration (Bath Assembly Rooms)
20.00–21.30	Welcome reception (Pump Rooms)

Monday 11 July

08.00	Registration
08.30	Plenary Lecture
09.30	Opioid receptor structure and function
10.35	Coffee
10.55	Opioid receptor structure and function
12.30	Lunch
13.30	New Roles for opioids
14.35	Coffee
14.55	New Roles for opioids
16.30	Datablitz Session
17.00	Poster Session A
19.00	Close
	Pub of the night – Bath Brew House

Tuesday 12 July

08.00	Registration
08.15	Plenary Lecture
09.15	Opioids, craving and addiction
10.20	Coffee
10.40	Opioids, craving and addiction
12.15	Lunch
13.15	Opioids: from itch to cancer
14.20	Coffee
14.40	Opioids: from itch to cancer
16.15	Datablitz Session
16.45	Poster Session B
18.45	Close
	Pub of the night – The Bell

Wednesday 13 July

08.00	Registration
08.15	Founders' Lecture
09.15	Opioid receptor regulation and crosstalk
10.45	Coffee
11.05	Opioid receptor regulation and crosstalk
12.40	Lunch
13.40	Free time or grant-writing and careers workshop

Thursday 14 July

08.15	Plenary Lecture
09.15	Neuronal plasticity: pain and addiction
10.20	Coffee
10.40	Neuronal plasticity: pain and addiction
12.15	Lunch
13.15	Young Investigator Symposium: Pain and perception
14.40	Coffee
15.00	Young Investigator Symposium: Pain and perception
16.15	INRC Business Meeting
16.40	Conference Close
19.00	Conference Dinner

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POSTER PRESENTERS

Posters should be A0 portrait format. Posters for Monday's poster session should be put up first thing on Monday morning and taken down after the poster session that evening. Posters for Tuesday's poster session should be put up first thing on Tuesday morning and taken down after the poster session that evening. Please check the programme for the timings of the poster sessions. Authors with odd poster numbers will present for the first hour of each poster session and authors with even numbers will present for the second hour of each poster session. Velcro fastenings will be provided. Posters not taken down after the poster sessions will need to be removed from the boards, and if not collected, will be disposed of.

ORAL COMMUNICATION PRESENTERS

Please bring your presentation on a USB stick and load it on the laptop in the lecture theatre in the refreshment/ lunch break that takes place before your session starts. If you are a Mac user, you will need to provide your own laptop and cabling/adaptors.

CERTIFICATES OF ATTENDANCE/ CPD CERTIFICATES

If you require a certificate of attendance or a CPD certificate, please ask at the registration desk. *Please note that if you do not sign for these at the meeting, we will be unable to issue them.*

INTERNET ACCESS

Complimentary Wi-Fi access will be available throughout the meeting at the venue.
Network name: **Assembly Rooms Guest.**
Simply click 'Join' (no password required).

REFRESHMENTS

Scheduled tea and coffee breaks will take place during both days of the conference and will be held in the breakout areas. Lunch is also included on both days and will be served in the breakout areas.

SOCIAL PROGRAMME

The Welcome Reception will take place at the Pump Rooms from 8pm to 9.30pm on Sunday 10 July. The address for the Pump Rooms is as follows:

The Pump Rooms
Abbey Chambers
Church St
Avon
Bath
BA1 1LZ

The Conference Dinner will take place at the Assembly Rooms from 7pm on Thursday 14 July.

PHOTOGRAPHIC POLICY

Please note that photographs taken at this meeting may be used on our website, social networking sites, and in other publications. If you do not wish to have your image used for this purpose, please speak to a BPS staff member or email meetings@bps.ac.uk.

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Bath Tourist Office
Abbey Churchyard
Bath
BA1 1LY

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BRITISH JOURNAL OF PHARMACOLOGY

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HEPTARES therapeutics

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NIDA is the lead federal agency supporting scientific research on drug use and its consequences. Our mission is to advance science on the causes and consequences of drug use and addiction and to apply that knowledge to improve individual and public health through:

- Strategically supporting and conducting basic and clinical research on drug use (including nicotine), its consequences, and the underlying neurobiological, behavioral, and social mechanisms involved
- Ensuring the effective translation, implementation, and dissemination of scientific research findings to improve the prevention and treatment of substance use disorders and enhance public awareness of addiction as a brain disorder

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PFIZER

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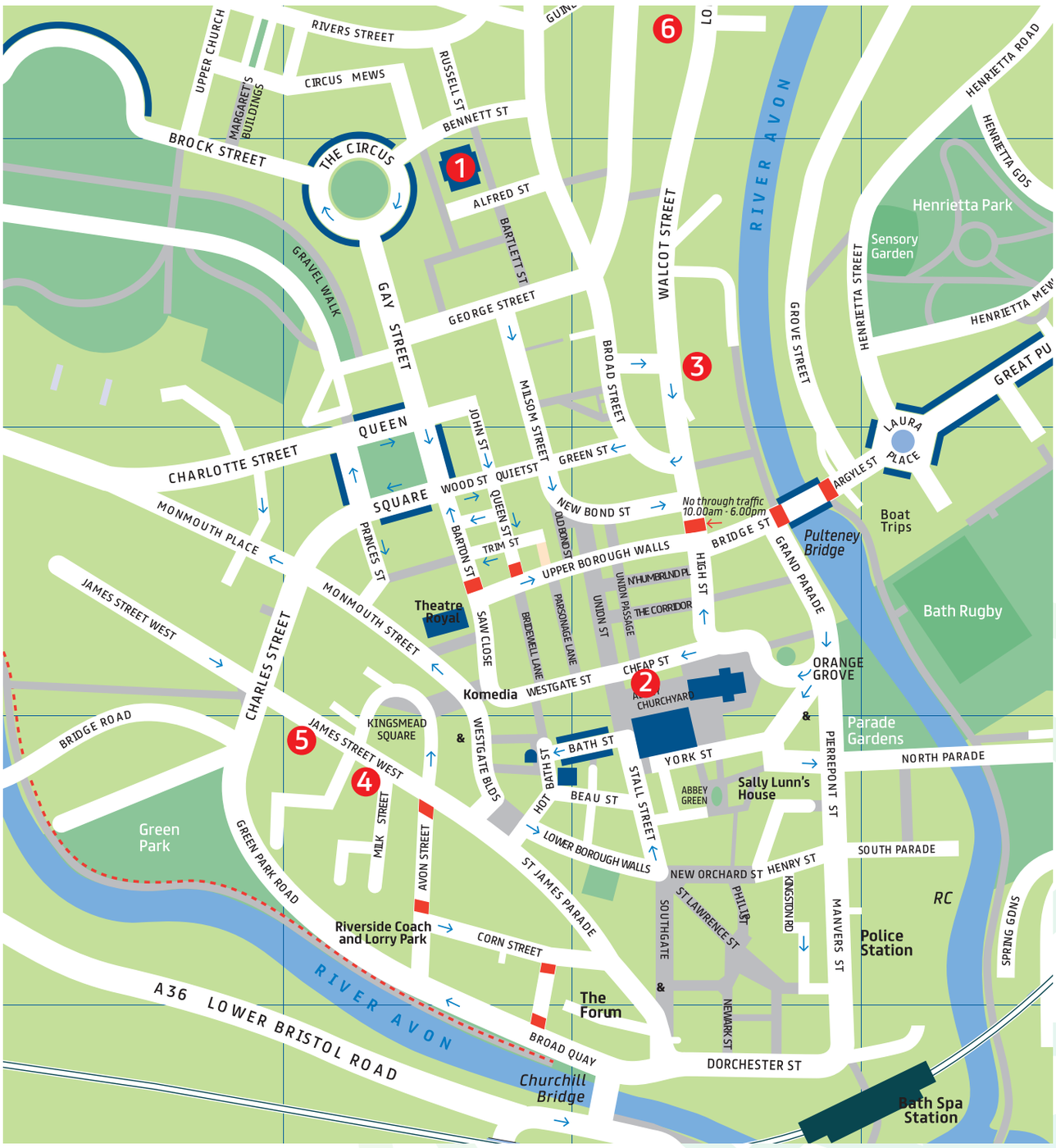
Location map



1 Assembly Rooms (main conference venue)
2 Pump Rooms (Reception venue)
3 Bath Hilton Hotel
4 Bath Premier Inn Hotel
5 Bath Brewhouse – pub of the night (Monday)
6 The Bell – pub of the night (Tuesday)

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INRC 2016 Awardees

FOUNDERS' LECTURE – LAKSHMI DEVI

Dr Lakshmi Devi is a Professor in the Departments of Pharmacology and Systems Therapeutics, Psychiatry and Neuroscience. She is also the Dean for Academic Development and Enrichment and the Director of the Interdisciplinary Training in Drug Abuse Research Program at Icahn School of Medicine at Mount Sinai. As a researcher she has made an impressive contribution to the field of opioids. She has been widely recognized for her research exploring the novel pharmacology of receptor dimerization with a particular focus on receptor heterodimers. An important part of her research is directed to investigation of fundamental mechanisms underlying opioid and cannabinoid receptor activation by combining molecular biological, biochemical, cell-biological, pharmacological and behavioral techniques.

Dr Devi received her PhD at the University of Windsor in Ontario, Canada and her postdoctoral training with Avram Goldstein at the Addiction Research Foundation, Palo Alto, California and at the Vollum Institute, Portland, Oregon with Ed Herbert. She continued her research career as Assistant and Associate Professor at the faculty of Pharmacology at the New York University before joining Mount Sinai School of Medicine as a Professor of Pharmacology and Biological Sciences. Dr Devi has authored around 200 scientific research articles and reviews, most of them published in high ranked international journals including PNAS, Nature and Science. She has mentored more than 70 trainees including medical students, graduate students, postdoctoral research associates, and visiting scientists.

The scientific achievements of Dr Lakshmi Devi have been recognized by many grants, patents and several honors and awards. The latter include the National Institute of Drug Abuse Senior Scientist and Mentorship Award, the Dean's Award for Excellence in Basic Research, and the NIH MERIT award. She has also been elected as a Fellow of the American Association for the Advancement of Science. Dr Devi is widely praised for her role in organizing symposia in national and international conferences. Since her first meeting in Garmisch Partenkirchen in 1983, she has been a frequent attendant at the INRC meetings and together with her students and collaborators provided outstanding contributions to our organization. She served as a member of the executive committee of INRC (1996-1998) and as the President of the INRC (2006-2010). It is fair to say that Dr Lakshmi Devi belongs to the core of successful researchers that has been essential for the development and maintenance of INRC as an organization promoting high-quality science in the opioid field.

YOUNG INVESTIGATORS AWARD – AMYNAH PRADHAN

Dr Amynah Pradhan is currently an Assistant Professor in the Department of Psychiatry at the University of Illinois at Chicago. She attended McGill University in Montreal, Canada, for her undergraduate and graduate studies. She received her PhD in 2005 from the Department of Pharmacology and Experimental Therapeutics; with a thesis entitled "Dissociation between behavioral and biochemical measures of mu and delta opioid receptors in rat central nervous system". She subsequently joined AstraZeneca R&D Montreal for a 1 year postdoctoral fellowship, in which she studied the role of SNSR1 protein in pain perception. In 2006, she joined Dr Brigitte Kieffer's laboratory at the IGBMC in Illkirch, France. There she developed projects to understand the role of ligand directed signaling at the delta opioid receptor. She established that in vivo, delta opioid receptor trafficking critically controlled behavioral outcomes; and that chronic treatment with high- and low-internalizing delta agonists produced different types of tolerance. She subsequently joined the laboratory of Christopher Evans at UCLA, Los Angeles, USA. In this fellowship, she studied the ligand-specific recruitment of arrestins to the delta opioid receptor. She also developed an interest in migraine, and her work has led to the development of delta opioid receptor agonists for the treatment of this disorder. In 2013, she joined the Department of Psychiatry at the University of Illinois at Chicago in a tenue-track faculty position.

During her postdoctoral fellowships with Brigitte Kieffer and Chris Evans, Amynah Pradhan showed great promise as a young scientist, and she was highly recommended for her constructive attitude to scientific questions and her outstanding ability to move projects forward. These achievements gave her a strong and solid basis for her appointment to her present faculty position. Her current research is still focused on the neurobiology of opioid receptors. Her studies aim to elucidate the behavioral consequences of agonist-specific signaling and trafficking of delta opioid receptors. In addition, her lab is also interested in understanding the differing roles of opioid receptors in migraine and post-traumatic headache. Dr Pradhan has published around 30 full papers in high-ranked international journals. She has active grant support from NIH-NIDA, NIAAA, and the US Department of Defense. She has received several honors and awards, including The National Headache Foundation Lectureship Award, International Headache Society Trainee's Excellence Award, and The INRC-NIDA International Travel Award. She has been attending INRC meetings since 2003, where she has contributed excellent oral and poster presentations on her opioid research; and has actively participated with her creative attitude in many discussions.

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Monday 11 July 2016

08.00–08.30 Registration

PLENARY

08.30–09.30 PL001
The A B C of GPCR Structure Based Drug Design
Fiona Marshall
Heptares Therapeutics, Welwyn Garden City, UK

OPIOID RECEPTOR STRUCTURE AND FUNCTION

09.30–09.55 I001
Dynamic Modelling of Opioid Receptors, Dimers and Allostery
Marta Filizola
Icahn School of Medicine at Mount Sinai, New York, NY, USA

09.55–10.20 I002
Structural insights into opioid receptor activation
Aashish Manglik
Stanford University, Stanford, CA, USA

10.20–10.35 HT001
Measuring Efficacy of Orthosteric and Allosteric Ligands at the Mu-Opioid Receptor Using a Conformational Biosensor
K Livingston[1], J Mahoney[1], A Manglik[3], B Kobilka[3], R Sunahara[2], J Traynor[1]
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California- San Diego, San Diego, CA, USA, [3]Stanford University, Palo Alto, CA, USA

10.35–10.55 Coffee

10.55–11.20 I003
Molecular control of δ-opioid receptor functionality by sodium.
Patrick Giguère
University of Ottawa, Ottawa, Ontario, Canada

11.20–11.45 I004
Ligand Bias at Opioid Receptors
Laura Bohn
The Scripps Research Institute, Juptier, FL, USA

11.45–12.00 HT002
Functional Selectivity and Analgesic Effects of LOR17, a Novel Kappa Opioid Receptor (KOR) Selective Agonist
A Bedini[1], L Di Cesare Mannelli[2], R De Marco[3], L Gentilucci[3], C Ghelardini[2], S Spampinato[1]
[1]Department of Pharmacy and Biotechnology (FaBiT) - Universty of Bologna, Bologna, Italy, [2]Department of Neuroscience, Psychology, Drug and Children Health (NEUROFARBA) – University of Florence, Florence, Italy, [3]Department of Chemistry "G. Ciamician" – University of Bologna, Bologna, Italy

12.00–12.15 HT003
Investigating the structural determinants of μ-opioid receptor ligand bias by mutagenesis
J Daniel Hothersall, Rubben Torella, Sian Humphreys, Gordon McMurray, Sarah Nickols
Pfizer NPRU UK, Cambridge, UK

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12.15–12.30 Discussion

12.30–13.30 Lunch

NEW ROLES FOR OPIOIDS

OREXIGEN

13.30–13.55 I005
Opioidergic basis of human social interaction
Lauri Nummenmaa[1,2,3]
[1]Turku PET Centre, Turku, Finland, [2]Department of Psychology, University of Turku, Turku, Finland, [3]Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland

13.55–14.20 I006
Novel Opioid Ligands: Anxiety, Depression and Addiction
SM Husbands[1], AM Almatroudi[1], T Hillhouse[2], J Hallahan[2], CP Bailey[1], JR Traynor[2], SJ Bailey[1]
[1]University of Bath, Bath, UK, [2]University of Michigan, Ann Arbor, USA

14.20–14.35 HT004
The G-protein biased kappa opioid receptor agonist 6'-GNTI blocks hippocampal paroxysmal discharges without inducing aversion
Christoph Schwarzer[1], Johannes Burtscher[1], James P MacKay[2], William F Colmers[2], Luca Zangrandi[1]
[1]Dept. Pharmacology, Medical University of Innsbruck, Innsbruck, Austria, [2]Dept. Pharmacology, University of Alberta, Edmonton, Canada

14.35–14.55 Coffee

14.55–15.20 I007
Mu opioid receptors, social behavior and autism
J Le Merrer, JAJ Becker
Physiologie de la Reproduction et des Comportements, INRA UMR0085, CNRS UMR7247, Université Rabelais, Nouzilly, France

15.20–15.45 I008
Current status of kappa-based therapies
Selena Schattauer, Benjamin Land, Jamie Kuhar, Shao-En Ong, Charles Chavkin
University of Washington, Seattle, WA, USA

15.45–16.00 HT005
Nociceptin/orphanin FQ inhibits contextual fear memory reconsolidation in mice
L Mouledous[1,2], K Rekik[1,2], R Faria Da Silva[1,2], B Frances[1,2]
[1]Research Center on Animal Cognition, Toulouse, France, [2]CNRS/Université Paul Sabatier, Toulouse, France

16.00–16.15 HT006
A Role for the Mu Opioid Receptor in the Antidepressant-like Effects of Buprenorphine
Shivon Robinson, Rebecca Erickson, Caroline Browne, Irwin Lucki
University of Pennsylvania, Philadelphia, PA, USA

16.15–16.30 Discussion

16.30–17.00 **Datablitz Session**

17.00 **POSTER SESSION A**

Takeda

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Tuesday 12 July 2016

08.15–09.15 **PLENARY**

Imaging endogenous and exogenous opiods in the human brain
Irene Tracey
Oxford, UK

OPIOIDS, CRAVING AND ADDICTION

09.15–09.40 I009

An Evaluation of Opioid Substitution Treatment (OST) in prison on risk of mortality in period immediately after prison: does leaving prison on OST reduce the risk of death?
Garry Stillwell[1], Hayley E Jones[2*], Alisha Cooper[3], Nino Maddalena[3] Jenny Shaw[3], Michael Farrell[5], Chris Metcalfe[2] John Marsden[1^], Matthew Hickman[2^]*
Garry Stillwell[2], Hayley Jones[1], Alisha Cooper[3], Nino Maddalena[3], Jenny Shaw[4], Michael Farrell[5], Chris Metcalfe[1], John Marsden[2], Matt Hickman[1]
[1]University of Brtistol, Bristol, UK, [2]National Addiction Centre,Kings College, London, UK, [3]Public Health England, London, UK, [4]University of Manchester, Manchester, UK, [5]UNSW, Sydney, Australia

09.40–10.05 I010

Understanding Opioid Addiction-Depression Comorbidity: The Oxytocin Story
A Bailey
St. George's University of London, London, UK

10.05–10.20 HT007

Pulling the brakes on midbrain dopamine cells: inhibiting substance P prevents opiate reward
AJ Sandweiss, MI McIntosh, A Moutal, AK Giri, VJ Hruby, R Khanna, TM Largent-Milnes, TW Vanderah
University of Arizona, Tucson, AZ, USA

10.20–10.40 Coffee

10.40–11.05 I011

Projection target dependent effects of orexin and dynorphin in the ventral tegmental area
Corey Baimel[1,2], Stephanie Borgland[1]
[1]University of Calgary, Calgary, Canada, [2]University of British Columbia, Vancouver, Canada

11.05–11.30 **Opioid receptors and drug-seeking behaviour**
Rafael Maldonado
Barcelona, Spain

11.30–11.45 HT008

Does Cannabis Use Modify The Relationship Between Opioid Use And Non-fatal Overdose?
Pauline Voon[1,2], M-J Milloy[1,3], Kanna Hayashi[1,3], Sabina Dobrer[1], Evan Wood[1,3], Thomas Kerr[1,3]
[1]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada, [2]School of Population and Public Health, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada, [3]Department of Medicine, University of British Columbia, Vancouver, BC, Canada

11.45–12.00 HT009

Regulation of Morphine Reward by a Novel Neuropeptide Receptor System, BigLEN-GPR171
EN Bobeck[1], D Pena[2], I Gomes[1], AK Fakira[1], LA Devi[1]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]University of Sao Paulo, Sao Paulo, Brazil

12.00–12.15 Discussion

12.15–13.15 Lunch

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OPIOIDS: FROM ITCH TO CANCER

13.15–13.40 I013

Kappa, itch and the immune response

Maria Schneeweiss[1], Ann-Christin Lüdiger[1], Natia Chartolani[1], Michael Soeberdt[2], Ulrich Knie[2], Thomas A. Luger[1], Christoph Abels[2], Karin Loser[1]

[1]University of Münster, Department of Dermatology, Münster, Germany, [2]Dr August Wolff GmbH & Co. KG Arzneimittel, Bielefeld, Germany

13.40–14.05 I014

Opioid-induced immunosuppression

HL Rittner, A Brack

University Hospitals of Wuerzburg, Wuerzburg, Germany

14.05–14.20 HT010

Sewarine, an Indole Alkaloid from Rhazya stricta, and Its Interaction with the κ-Opioid Receptor: A Pharmacological and Molecular Modeling Study

Aquilino Lantero[1], Michael Mairegger[1], Stefan Salcher[2,3], Muhammad F. Asim[1], Elena Guerrieri[1], Helmut Schmidhammer[1], Petra Obexer[2,3], Mariana Spetea[1]

[1]Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences, University of Innsbruck, Innsbruck, Austria, [2]Department of Pediatrics II, Medical University of Innsbruck, Innsbruck, Austria, [3]Tyrolean Cancer Research Institute, Innsbruck, Austria

14.40–15.05 I015

Opioids, the immune system and cancer

Jason Boland

Hull York Medical School, Hull, UK

15.05–15.30 I016

Role of Peripheral Opioid Receptors In Tumor Angiogenesis And Metastasis

Yoshinori Kato, Minoru Narita

Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan

15.30–15.45 HT011

The role of cancer cell secretion in opioid-induced tumor angiogenesis

DA Fux, A Schoos, S Tripolt

VetMedUniversity, Vienna, Austria

15.45–16.00 HT012

Development of a Combination Vaccine for Heroin and HIV-1

R Jalah[1,2], OB Torres[1,2], JFG Antoline[3,4], KK Peachman[1,2], Z Beck[1,2], M Rao[1], AE Jacobson[3,4], NL Michael[1], KC Rice[3,4], CR Alving[1], GR Matyas[1]

[1]U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA, [2]U.S. Military HIV Research Program, Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA, [3]Drug Design and Synthesis Section, Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA, [4]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

16.00–16.15 Discussion

16.15 **Datablitz Session**

16.45 **POSTER SESSION B**

TOCRIS a bio-techne brand

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Wednesday 13 July 2016

FOUNDERS LECTURE

08.15–09.15 PL003

Exploring the Mysteries of the Endogenous Opioid System

Lakshmi Devi
Icahn Medical School at Mount Sinai, New York, USA

OPIOID RECEPTOR REGULATION AND CROSSTALK

09.15–09.40 I017

Allostery at opioid receptors

JR Traynor
University of Michigan, Ann Arbor, USA

09.40–10.05 I018

Peripherally restricted opioid combination therapy synergizes in multiple pain states

George Wilcox, Daniel Bruce, Cristina Peterson, Kelley Kitto, Carolyn Fairbanks
University of Minnesota, Minneapolis, MN, USA

10.05–10.30 I019

Agonist-induced Mechanisms of Mu Opioid Receptor Regulation

Janet Lowe[1], Helen Sanderson[2], Alexandra Cooke[2], Mehrnoosh Ostovar[3], Elena Tsisanova[2], Sarah Withey[2], Charles Chavkin[4], Stephen Husbands[3], Eamonn Kelly[2], Graeme Henderson[2], Chris Bailey[3]
[1]Oregon Health & Science University, Portland, OR, USA, [2]University of Bristol, Bristol, UK, [3]University of Bath, Bath, UK, [4]University of Washington, Seattle, WA, USA

10.30–10.45 HT014

Phosphoproteomic survey of Kappa Opioid Receptor *in vivo* Functional Selectivity

Jeffrey Liu[1], Luca Zangrandi[2], Chongguan Chen[3], Sean Humphrey[1], Lee-Yuan Liu-Chen[3], Christoph Schwarzer[2], Matthias Mann[1]
[1]Max Planck Institute of Biochemistry, Martinsried, Germany, [2]Department of Pharmacology, Medical University of Innsbruck, Innsbruck, Austria, [3]Center for Substance Abuse Research and Department of Pharmacology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

10.45–11.05 Coffee

11.05–11.30 I020

Phosphorylation barcodes for opioid receptors

Stefan Schulz
Institute of Pharmacology and Toxicology, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany

11.30–11.55 I021

Opioid receptor desensitization

Macdonald Christie
University of Sydney, NSW, Australia

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11.55–12.10	HT015 **RGS protein interactions contribute to functional selectivity of agonists acting at G-protein coupled receptors (GPCRs)** *SL Ingram[1], JD Lowe[1], M Li[1], KL Suchland[1], JR Traynor[2]* *[1]Oregon Health & Science University, Portland, OR, USA, [2]University of Michigan, Ann Arbor, MI, USA*
12.10–12.25	HT016 **Inhibition of c-Src reduces morphine analgesic tolerance** *Fiona Bull[1], Daniel Baptista-Hon[1], Wendy Walwyn[2], Tim Hales[1]* *[1]University of Dundee, Dundee, Scotland, UK, [2]University of California, Los Angeles, Los Angeles, CA, USA*
12.25–12.40	Discussion
12.40–13.40	Lunch
13.40	Free afternoon or grant-writing and careers workshop

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Thursday 14 July 2016

PLENARY LECTURE

08.15–09.15 PL004

Neural substrates of addiction

TW Robbins
University of Cambridge, Cambridge, UK

NEURONAL PLASTICITY: PAIN AND ADDICTION

09.15–09.40 I022

Opioids, spinal cord and pain

Tony Dickenson
University College, London, UK

09.40–10.05 I023

Pain resilience and vulnerability and the brain's opioid system

Anthony Jones[1], Christopher Brown[2]
[1]University of Manchester, Manchester, UK, [2]University of Liverpool, Liverpool, UK

10.05–10.20 HT017

A single dose of morphine induces epigenetic changes in dopamine neurons of the VTA associated with potentiation of glutamate mediated excitation and depression of GABA inhibition

BM Cox[1], ME Authement[1], H Kassis[1], LD Langlois[1], S Gouty[1], M Dacher[1,2], RD Shepard[1], FS Nugent[1]
[1]Department of Pharmacology, Uniformed Services University, Bethesda, MD, USA, [2]iEES-Université Pierre et Marie Curie/Paris 6, Versailles, France

10.20–10.40 Coffee

10.40–11.05 I024

Pain alters opioid intake and associated motivated behavior

Nicolas Massaly, Adrianne Poe, Lucia Hipolito, Ream Al-Hasani, Michael Bruchas, Jose Moron-Concepcion
Washington University, St Louis, MO, USA

11.05–11.30 I025

Endogenous Opioids and Placebo Neurobiology: Tapping into Resiliency?

JK Zubieta
University of Utah, Salt Lake City, Utah, USA

11.30–11.45 HT018

Prediction Formulas for Individual Opioid Analgesic Requirements Based on Genetic Polymorphism Analyses

Daisuke Nishizawa[1], Kaori Yoshida[1,2], Takashi Ichinomiya[3], Tatsuya Ichinohe[2], Masakazu Hayashida[4], Ken-ichi Fukuda[5], Kazutaka Ikeda[1]
[1]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Tokyo Dental College, Tokyo, Japan, [3]Gifu University Graduate School of Medicine, Gifu, Japan, [4]Juntendo University School of Medicine, Tokyo, Japan, [5]Tokyo Dental College, Tokyo, Japan

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11.45–12.00 HT019
Intermittent Versus Sustained Morphine Treatment Regimens on Molecular and Behavioral Markers of Withdrawal
Chris Evans[1], Anna Taylor[1], Kevin Lee[1], Samuel Bridges[1], Catherine Cahill[2]
[1]UCLA, California, USA, [2]UCI, California, USA

12.00–12.15 Discussion

12.15–13.15 Lunch

YOUNG INVESTIGATOR SYMPOSIUM: PAIN AND PERCEPTION

13.15–14.00 I026
Novel signaling and therapeutic indications for the delta opioid receptor
Amynah Pradhan
University of lllinois at Chicago, Chicago, IL, USA

14.00–14.25 I027
What can fMRI tell us about the role of opioids in cognitive and emotional modulation of pain?
Tim Salomons
University of Reading, Reading, UK

14.25–14.40 HT020
Nitroglycerin-induced migraine-like pain and trigeminal neuronal hyperactivity is enhanced by reduced CB1 receptor activity
Chihiro Nozaki, Astrid Markert, Andreas Zimmer
Institute of Molecular Psychiatry, University of Bonn, Bonn, NRW, Germany

14.40–15.00 Coffee

15.00–15.25 I028
The Brain in Pain Studies: Central sensitisation as a pharmacological target for chronic pain
Julius Bourke
Barts and the London SMD, QMUL, London, UK

15.25–15.40 HT021
Intra- and Inter-Regional co-Regulation of Opioid Genes: Broken Symmetry in Spinal Circuits
T Yakovleva[1], V Galatenko[2], O Kononenko[1], M Andersson[1], H Watanabe[1], I Mityakina[2], A Tonevitsky[2], DL Adkins[3], G Bakalkin[1]
[1]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [2]Moscow State University, Moscow, Russia, [3]Colleges of Medicine & Health Professions, Medical University of South Carolina, Charleston, South Carolina, USA

15.40–15.55 HT022
Circuit dynamics of in vivo dynorphin release in the nucleus accumbens shell
Ream Al-Hasani[1], Jenny Wong[2], Jordan McCall[1], Omar Mabrouk[2], Gavin Schmitz[1], Kirsten Porter-Stransky[3], Julio Bernardi[1], Brandon Aragona[3], Robert Kennedy[2], Michael Bruchas[1]
[1]Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri, USA, 2Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA, 3Department of Psychology, University of Michigan, Ann Arbor, Michigan, USA

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15.55–16.15	Discussion
16.15–17.00	INRC Business Meeting
17.00	Conference Close
19.00	Conference Dinner

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INRC ABSTRACTS

PLENARY TALK ABSTRACTS

PL001
The A B C of GPCR Structure Based Drug Design
Fiona Marshall
Heptares Therapeutics, Welwyn Garden City, UK

G protein-coupled receptors remain one of the most important target classes for drug discovery particularly in the area of CNS disorders. In the last 20 years GPCR drugs have been identified and progressed to market which target over 40 different GPCRs. X-ray structures of GPCRs across all the major classes, A, B, C and F are now available and for the first time structure based drug design can be applied across the entire GPCRome (1).

In this talk I will describe the methods used in structure based drug design and discuss a number of case studies for Class A, B and C GPCRs. Orexin 1 antagonists have potential in the treatment of craving associated with drug addiction - structures of the orexin 1 and orexin 2 receptors have been solved and are being used to optimise selectivity between OX1 and OX2. In Class B (2) we have solved X-ray structures of CRF1 and the glucagon receptor (3) which have revealed unexpected allosteric binding sites. Finally in Class C I will describe the importance of getting multiple co-structures of different ligands and the challenges of developing allosteric modulator drugs (4,5).

1. Congreve *et al.* (2014) *Prog Med Chem.* 53:1-63.
2. Bortolato *et al.* (2014). *Br J Pharmacol.* 171 3132-45.
3. Jazayeri *et al.* (2016). *Nature* doi:10.1038/nature17414
4. Hollenstein *et al.* (2013). *Nature* 499 :438-43.
5. Bennett *et al.* (2015) *Curr Opin Pharmacol*. 20:1-7

PL003
Exploring the Mysteries of the Endogenous Opioid System
Lakshmi Devi
Icahn Medical School at Mount Sinai, New York, USA

Several decades ago when I became interested in the opioid system, a large number of endogenous opioid peptides had been discovered and multiple receptor subtypes had been characterized. It was not known whether each peptide had a unique function or if the multitude of peptides had redundant functions. Similarly, it was not known if each peptide was generated by a specific enzyme or if a small number of peptidases generated all of the peptides. It was generally assumed that the different receptor subtypes were encoded by distinct genes, as found for other G protein-coupled receptors (dopamine, serotonin, etc). The discovery that mu, delta, and kappa receptors are each encoded by a single gene raised questions regarding the molecular basis for the distinct receptor pharmacology. It was generally assumed that opioid receptors function as monomers and once activated, initiate a specific signal transduction cascade that terminates once the receptor is endocytosed. Many of these myths and mysteries have been addressed. For example, now we know that all neuropeptides (including opioid peptides) are generated by a small set of processing enzymes, opioid receptors associate to form dimers which leads to novel pharmacology, each opioid peptide efficiently activates more than one opioid receptor type and some can activate more than one signal transduction pathway (functional selectivity) and activated receptors continue to signal after endocytosis. This presentation will describe my scientific journey in the field, with interesting excursions and diversions exploring the mysteries of the endogenous opioid system.

Supported by NIH grants, DA008863 and NS026880.

PL004
Neural substrates of addiction
TW Robbins
University of Cambridge, Cambridge, UK

Much evidence now supports the view that drug addiction results in part from aberrant learning mediated by dopamine-dependent processes of the limbic-striatal interface, including the amygdala and nucleus accumbens. In particular, drug addiction may involve a 'switch' between instrumental behaviour controlled by its outcome and habitual responding mediated by stimulus-response associations, corresponding hypothetically to a devolution of behavioural control from (i) the prefrontal cortex to the striatum, and (ii) from the ventral to the dorsal striatum (Everitt and Robbins 2016). I will consider the experimental evidence relevant to these two predictions from studies of both experimental animals and humans with substance use disorders, especially for stimulant drugs, but including consideration of other substance use disorders. For human drug misuse disorders it is difficult to unravel causal neurobehavioural factors contributing to addiction from potential neurotoxic effects of the drugs themselves. I will describe strategies for determining the aetiological role of these factors, based on evidence from (i) experimental animals, using several techniques and (ii) human drug abusers, based on functional neuroimaging methods, in both longitudinal and endophenotype designs. Finally, I will consider implications for therapeutic approaches to drug addiction.

Reference: Everitt BJ and Robbins TW (2016) Drug Addiction: Updating actions to habits to compulsions ten years on. *Annual Review of Psychology* 67, 23-50

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INVITED SPEAKER ABSTRACTS

I001
Dynamic Modelling of Opioid Receptors, Dimers and Allostery
Marta Filizola
Icahn School of Medicine at Mount Sinai, New York, NY, USA

High-resolution crystal structures of opioid receptors provide critical information and new directions for understanding the molecular mechanisms underlying the diverse physiological functions of these receptors. However, current crystal structures do not provide information about allosteric ligand binding, physiological dimers, and/or possible structural modulation of opioid receptors by G protein-biased agonists. This has sparked great interest in obtaining atomistic information about alternative (allosteric) binding sites, ligand-specific (biased) conformations, and likely oligomeric complexes for use as new exciting avenues for drug discovery. I will provide an overview of atomic-level mechanistic insights we have derived from enhanced computational strategies we have implemented and tested over the years. The level of molecular detail we have obtained has laid the foundation for novel experimental studies aimed at furthering our understanding of the physiological functions of opioid receptors, and at developing new therapeutic strategies. I will share a few examples of our findings and their implications.

I002
Structural insights into opioid receptor activation
Aashish Manglik
Stanford University, Stanford, CA, USA

Despite two centuries of medicinal chemistry since the isolation of morphine from the opium poppy, the ideal opioid analgesic devoid of key liabilities like addiction and respiratory depression remains elusive. The recent determination of X-ray crystal structures of the μOR, as well as the other three key opioid receptor subtypes, has provided considerable insight into the molecular recognition of opioidergic drugs. I will describe our recent efforts to understand the structural and biophysical basis of opioid receptor activation using X-ray crystallography as well as NMR and fluorescence spectroscopy. Using these insights, we have recently embarked on structure-based discovery campaigns to identify novel opioid analgesics. I will discuss the identification and optimization of a selective, Gi biased μOR agonist that elicits analgesia without inducing respiratory depression or a conditioned place preference response and will outline our current efforts to elucidate the structural basis for the remarkable efficacy of such compounds.

I003
Molecular control of δ-opioid receptor functionality by sodium.
Patrick Giguère
University of Ottawa, Ottawa, Ontario, Canada

Opiates are widely used pharmaceuticals for the treatment of pain and addictive disorders. However, there are numerous difficulties associated with opioid therapy (overdose, tolerance, addiction, respiratory depression and constipation). Endogenous opioids act through the activation of the three classical opioid receptors (μ, κ and δ-OR) and the related nociceptin/orphanin FQ peptide receptor (NOP), all belonging to the class A (rhodopsin-like) family of G protein-coupled receptors (GPCRs). The δ-OR is well described for its role in pain perception and management. Interestingly, in clinical models, it also showed great potential as an anti-depressor, for the treatment of symptoms associated with spasmodic movements in Parkinson's disease and for migraine. However, the clinical use of δ-OR agonists is limited due to the generation of side effects such as epileptic-like seizures. Recently, we reported the discovery of a sodium-dependent allosteric regulation site in the δ-OR. This work highlights the presence of a cluster of sodium and water molecules housed in a cavity thought to be present only in the inactive conformation of the receptor. While the sodium pocket might be present in most class A GPCRs, the shape of this allosteric cavity seems to have important structural variation making this site a potential target for the design of new allosteric modulators that will be selective for the δ-OR. Our works seek to address the druggability as well as the mechanism underlying the sodium-mediated transmission of the signal outcome using pharmacological, chemical and structural approaches. Supported by the CIHR grant 142219 and Canada Research Chairs program.

I004
Ligand Bias at Opioid Receptors
Laura Bohn
The Scripps Research Institute, Juptier, FL, USA

It is becoming increasingly appreciated that G protein-coupled receptor (GPCR) signaling in vivo is not unilaterally determined by the ability of a ligand to promote coupling of the receptor to a particular G protein. Rather, GPCR signaling is determined by the context within which it is expressed as well as by the nature of the ligand that binds to it. In this regard, it is therefore anticipated that a receptor that resides in the synaptic cleft would have different potential interacting partners than one that resides on a dendritic spine. Furthermore, when multiple effectors are present and able to engage with the receptor, it may also be possible to skew the potential for effector GPCR interactions toward certain effectors and away from others. In vitro, ligands have been identified that have preferential functional affinity for certain signaling pathways over others (G protein vs. barrestin interactions for example). Increasing evidence suggests, in the some cases, that the activation of certain signaling components correlate with beneficial drug effects in vivo while signaling to other effectors promote adverse events. These concepts, falling under the concept of "functional selectivity," point to new means to bias receptor signaling towards improving therapeutic efficacies while eliminating side effects. In this presentation, I will present our work on opioid receptor biased agonism and how we are using these compounds to understand what biased signaling is meaningful and how such biases translate to the in vivo setting. Funding: NIH/NIDA: R01DA038964, R01DA033073, R01DA031927.

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I005
Opioidergic basis of human social interaction
Lauri Nummenmaa[1,2,3]
[1]Turku PET Centre, Turku, Finland, [2]Department of Psychology, University of Turku, Turku, Finland, [3]Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland

Human social bonds endure despite infrequent contact and physical distance between individuals. Because blockade of opioid receptors stimulates grooming and social behaviour in non-human primates it has been hypothesized that modulation of endogenous opioid-system activity during prosocial behaviour could support maintenance and establishment of long-term relationships in humans. Until recently this hypothesis has, however, lacked direct neurophysiological support. Here I review our recent work on the opioidergic basis of human sociability using in vivo positron emission tomography (PET) with mu opioid receptor (MOR) specific ligand [^{11}C]carfentanil combined with functional magnetic resonance imaging (fMRI). These data reveal that 1) prosocial behavior such as social touching (grooming) and group laughter engages the endogenous MOR system, 2) striatal MOR availability predicts empathetic responses (as measured by BOLD-fMRI) when seeing others in pain, and 3) frontocortical MOR availability predicts individual differences in prosociality. Altogether these results suggest that modulation of the MOR system activity by social touching and laughter might provide a neurochemical mechanism reinforcing and maintaining social bonds. Similarly, MOR system activity might promote helping behavior when seeing others in distress. Individual differences in MOR system functioning could have a profound impact on the social relationships individuals establish, thus potentially having a strong impact on psychological well-being.

I006
Novel Opioid Ligands: Anxiety, Depression and Addiction
SM Husbands[1], AM Almatroudi[1], T Hillhouse[2], J Hallahan[2], CP Bailey[1], JR Traynor[2], SJ Bailey[1]
[1]University of Bath, Bath, UK, [2]University of Michigan, Ann Arbor, USA

Buprenorphine is reported to show antidepressant activity in treatment resistant patients with clinical improvements seen within 1 week of treatment. Growing evidence suggests that antagonism of the kappa opioid receptor is responsible (1,2), at least in part, for this clinical effect with debate as to the significance of mu opioid receptor activation. In addition, kappa receptor antagonism has been promulgated to explain the activity of buprenorphine/naltrexone combinations in preventing relapse to drug taking, though here there is evidence that activity at more than just kappa receptors is important (3). To allow further investigation and the potential development of new therapeutics there is a strong rationale for developing single compounds with profiles very similar to buprenorphine but with substantially reduced efficacy at mu opioid receptors. This has been achieved in a series of orvinol analogues in which the C20-methyl group has been moved to the C7-beta position (4). The lead compound, BU10119, shows antidepressant activity in the forced swim and novelty induced hypophagia tests in mice and separately has been shown to inhibit cocaine-primed reinstatement of cocaine conditioned place preference, activities also displayed by buprenorphine/naltrexone combinations.

Acknowledgement: Funding from NIDA, grant # DA07315 (SMH),

(1) Falcon E *et al.* (2016). Neuropsychopharmacology: 1-8
(2) Almatroudi A *et al.* (2015). J Psychopharmacol 29: 812-821
(3) Cordery SF *et al.* (2014). Addiction Biology 19: 575-586
(4) Cueva JP *et al.* (2015). J Med Chem 58: 4242-4249

I007
Mu opioid receptors, social behavior and autism
J Le Merrer, JAJ Becker
Physiologie de la Reproduction et des Comportements, INRA UMR0085, CNRS UMR7247, Université Rabelais, Nouzilly, France

Within the opiate system, the μ opioid receptor (MOR) have been shown to mediate the rewarding properties of natural reinforcers such as food, sex or physical exercise as well as artificial rewards such as drugs of abuse. Social interactions can also be rewarding (1), and converging experimental evidence point to MOR as a key brain substrate for social hedonic abilities, social acceptance and rejection, maternal/affiliative behavior and social communication. Among such evidences, the study of mice lacking the *Oprm1* gene, encoding MOR, has revealed severe deficits in social behavior and communication skills, leading to propose this line as a monogenic model of autism (2). Supporting this hypothesis, we have shown that adult *Oprm1-/-* animals recapitulate core and multiple comorbid behavioral symptoms of autism, and display anatomical, neurochemical and genetic landmarks of the disease (3). We have also revealed that chronic administration of VU0155041, a positive allosteric modulator of the glutamate receptors mGluR4, alleviates autistic deficits in *Oprm1*$^{-/-}$ mice. To further explore the potential role of MOR in the etiology of the autistic syndrome, we are now assessing MOR-dependent responses in other mouse models of the pathology. Identifying MOR as a common molecular mechanism underlying behavioral deficits in autism would open novel promising avenues for the pharmacotherapeutic treatment of this disease.

(1) Trezza *et al.* (2010) *Trends Pharmacol Sci* 31:463-469.
(2) Oddi *et al.* (2013) *Behav Brain Res* 40:1113-1122.
(3) Becker *et al.* (2014) *Neuropsychopharm* 39:2049-2060.

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I008
Current status of kappa-based therapies
Selena Schattauer, Benjamin Land, Jamie Kuhar, Shao-En Ong, Charles Chavkin
University of Washington, Seattle, WA, USA

Kappa opioid receptor antagonists have been shown to have therapeutic efficacy in the treatment of depression, anxiety and drug addiction in numerous preclinical studies, which will be briefly summarized. Recent human trials are starting to confirm that promise, and these studies will be briefly reviewed. The mechanisms of dynorphin action as a mediator of the stress response have become clearer, and these studies will be summarized. The original selective kappa opioid antagonists have unexpectedly long durations of action, but the underlying mechanisms have been somewhat controversial. Progress in the development of theapeutically effective kappa receptor antagonists requires that we understand whether the duration of action is solely controlled by pharmacokinetic clearance as proposed by some researchers or whether selective kappa antagonists can act by a cJun- N-terminal kinase mechanism to functionally inactivate kappa opioid receptor signaling. Evidence supporting the latter hypothesis will be described, and details of a novel JNK-dependent receptor inactivation mechanism will be presented.

The authors have no conflicts of interest to disclose. Support for this research from the National Institute on Drug Abuse P01 DA035764 is gratefully acknowledged.

I009
An Evaluation of Opioid Substitution Treatment (OST) in prison on risk of mortality in period immediately after prison: does leaving prison on OST reduce the risk of death?
Garry Stillwell2, Hayley Jones1, Alisha Cooper3, Nino Maddalena3, Jenny Shaw4, Michael Farrell5, Chris Metcalfe1, John Marsden2, Matt Hickman1
[1]University of Brtistol, Bristol, UK, [2]National Addiction Centre, Kings College, London, UK, [3]Public Health England, London, UK, [4]University of Manchester, Manchester, UK, [5]UNSW, Sydney, Australia

Background: UK and international evidence has shown that the risk of death in the first month leaving prison can be 4-12 times higher compared to later periods. We test whether leaving prison on OST compared to leaving drug free can reduce the risk of death in the period immediately after prison release for opioid dependent prisoners.

Methods: Prospective cohort of opioid dependent adult prisoners ≥18 recruited from 39 prisons in England from September 2010 to August 2013, released and followed up from 123 prisons until September 2015.

Results: We recruited 22,000 incarcerations, 6859 (31%) were excluded because of missing data on date of release, OST at release, or link to NHS, leaving 15,141 prison releases from 12,260 individuals. 8645 (57%) of cases were exposed to OST intervention on release, and 6286 (42%) of people released from prison entered treatment programmes in the community. People released on OST were more likely to enter treatment programmes in the community compared to the unexposed population (Odds Ratio 2.48 95%CI 2.3-2.65). The mortality hazard ratio in the first four weeks of leaving prison on OST vs leaving drug free was 0.24 (95%CI 0.09-0.64) and after adjustment also for community OST the hazard ratio was 0.27 (95%CI 0.11-0.71).

Conclusions: Opioid dependent people leaving prison on opioid substitution treatment (OST) had a mortality risk approximately 75% lower than if they had left prison drug free – removing the excess risk of death PWID experience leaving prison compared to rest of time in the community.

I010
Understanding Opioid Addiction-Depression Comorbidity: The Oxytocin Story
A Bailey
St. George's University of London, London, UK

There is 50-60% comorbidity between addiction and depression which is accompanied by more severe symptoms, higher service utilization and higher relapse rates. Therefore, understanding the neurobiological mechanisms underlying depression-addiction comorbidity will have important therapeutic implications in improving mental health care.

In our laboratory, we recently identified the oxytocin system as a crucial player involved in the comorbidity between opioid addiction and depressive disorders (1). We first developed a translationally relmevant mouse model of opioid abstinence which exhibited marked anxiety and depressive phenotype as well as social deficits. We demonstrated that this emotional impairment was accompanied by marked plasma and hypothalamic oxytocin deficits and a concomitant upregulation of oxytocin receptors. We showed that the oxytocin analogue carbetocin completely attenuated the negative emotional consequences of opioid abstinence and prevented both stress- and opioid priming- induced reinstatement of opioid seeking behaviour. Recent work from our laboratory have revealed several mechanisms underlining the aforementioned properties of carbetocin including modulation of striatal noradrenergic and dopaminergic systems and by means of regulating amygdala activity possibly via a m- opioid receptor mediated mechanism (2).

These studies highlight the oxytocin system as an important target for the treatment of co-existing substance use with affective disorders and thereby prevention of relapse. We are currently carrying out a pilot trial to assess efficacy of intranasal oxytocin administration in post-detoxification opioid dependent individuals in an attempt to translate our preclinical findings.

(1) Zanos P *et al.* (2014) *Neuropsychopharmacology* 39(4):855-65
(2) Georgiou P *et al.* (2015) *European Journal of Neuropsychopharmacology* 25(12):2459-64

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I011

Projection target dependent effects of orexin and dynorphin in the ventral tegmental area

Corey Baimel[1,2], Stephanie Borgland[1]

[1]University of Calgary, Calgary, Canada, [2]University of British Columbia, Vancouver, Canada

Dopamine neurons in the ventral tegmental area (VTA) are critically involved in the expression of motivated behaviour. The activity of dopamine neurons is regulated by intrinsic conductances and by synaptic inputs, both of which are subject to neuromodulatory influences. This talk will demonstrate how the lateral hypothalamic peptide, orexin (also known as hypocretin) alters the synaptic regulation and the activity of VTA dopamine neurons. We demonstrate that orexin signalling in the VTA gates morphine-induced synaptic plasticity in the VTA. Inhibition of orexin receptor signalling in the VTA blocks morphine-induced increases and decreases in the strength of excitatory and inhibitory synaptic transmission, respectively. Orexin neurons coexpress the inhibitory peptide dynorphin and the two are likely coreleased. We found that orexin and dynorphin modulate the activity of dopamine neurons in a projection-target specific manner. Orexin preferentially increased the output of dopamine neurons that project to the lateral shell of the nucleus accumbens, while dynorphin was more effective at inhibiting the activity of dopamine neurons that project to the basolateral amygdala. We propose that through corelease of orexin and dynorphin, these lateral hypothalamic projections coordinate the activity of VTA dopamine neurons to drive motivated reward seeking behaviour.

I013

Kappa, itch and the immune response

Maria Schneeweiss[1], Ann-Christin Lüdiger[1], Natia Chartolani[1], Michael Soeberdt[2], Ulrich Knie[2], Thomas A. Luger[1], Christoph Abels[2], Karin Loser[1]

[1]University of Münster, Department of Dermatology, Münster, Germany, [2]Dr August Wolff GmbH & Co. KG Arzneimittel, Bielefeld, Germany

Opioids can induce analgesia without adverse central effects by binding to peripheral opioid receptors and particularly kappa-opioid receptor agonists (KORA) exhibit anti-inflammatory properties. Here, we investigated the newly developed KORA WOL071-007, belonging to the structural class of decahydroquinoxalines, in inflammatory skin diseases. WOL071-007 down-regulated pro-inflammatory cytokines in mouse and human immune and significantly reduced ongoing skin inflammation in mouse models of psoriasis and atopic dermatitis (AD) as shown by a decreased clinical score or the down-regulated infiltration of immune cells into lesional skin. In humans, AD and psoriasis are associated with severe itch. Hence, we investigated whether WOL071-007 modulated itch. Notably, the scratching frequency and the itch-associated cytokine IL-31 were decreased in WOL071-007-treated atopic and psoriatic mice compared to controls. Of note, the anti-inflammatory and anti-pruritic effect of WOL071-007 was mediated by binding to KOR since it was almost absent in KOR-deficient animals. For clinical use in AD or psoriasis, WOL071-007 would largely benefit from the possibility of topical application. Therefore, we developed a cream formulation and demonstrated that WOL071-007 was equally efficient when applied topically. To investigate the anti-inflammtory potential of WOL071-007 in humans we performed a Phase-IB proof-of-concept-study in AD patients and detected a reduction in local SCORAD (scoring atopic dermatitis) index in WOL071-007-treated patients compared to placebo-treated controls. Together, our data demonstrate that WOL071-007 is able to ameliorate ongoing skin inflammation and itch in mouse models of psoriasis and AD as well as in AD patients, thus suggesting WOL071-007 as a potential candidate for further clinical development.

I014

Opioid-induced immunosuppression

HL Rittner, A Brack

University Hospitals of Wuerzburg, Wuerzburg, Germany

Opioid receptors are not only expressed in the peripheral and central nervous system but also on immune cells. There, they have several functions: opioids promote immunosuppression in cell culture experiments but also in different animal models. Morphine or fentanyl administration increases lethality in sepsis due to altered endotoxin tolerance, augmented bacterial translocation in the gut via an impaired barrier function and increased interleukin-17A. Mortality in mice with streptococcus-induced pneumonia rises with morphine treatment and wound closure is delayed due to a lag in immune cell recruitment and bacterial clearance. The convincing data from the animal studies, however, are so far in contrast to the paucity of studies in humans. Opioid use correlates with infectious complications in patients with burn injuries but does not increase the rate of sepsis in neonates. Long-term use of opioids increases the risk of pneumonia in elderly patients and incidence of hospitalization due to serious infection among patients with rheumatoid arthritis.

Secondly opioid receptors on immune cells mediate peripheral analgesia. Leukocytes synthesize endogenous opioid peptides, which can be released in the tissue in inflammatory or neuropathic pain. Stimulation of opioid receptors but also toll-like, chemokine and formyl peptide receptors triggers release of opioid peptides and contributes to peripheral analgesic effects. Selective blockade of peripheral opioid receptors impairs opioid analgesia in postoperative patients.

In summary, the neuroimmune interaction of opioids with leukocytes leads to immunosuppression and analgesia, which need further basic and clinical exploration in the light of an increased prescription of opioids.

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I015
Opioids, the immune system and cancer
Jason Boland
Hull York Medical School, Hull, UKK

Opioids are important in the management of cancer pain. By acting on endogenous opioid receptors, prescribed opioids affect many organ-systems. This leads to a range of side effects, including suppression of some aspects of the immune response. As anti-cancer immunity is important in the defence against cancer, the use of opioids for pain management in patients with cancer is potentially concerning. However, the evidence for the effects of opioids on the immune system principally comes from pre-clinical studies. Data from these and clinical studies, exploring the immune effects of opioids in patients with cancer will be presented. As pain might also be immune suppressive, there is a triangulation with the beneficial effect of opioids on pain potentially being offset the immune suppressive effects of opioids. Based on this data, the clinical implications will be discussed.

I016
Role of Peripheral Opioid Receptors In Tumor Angiogenesis And Metastasis
Yoshinori Kato, Minoru Narita
Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan

The relationship between peripheral μ-/κ-opioid receptors and cancer progression has received a lot of attention in recent years. As peripheral μ-opioid receptors paly a supportive role in cancer progression, we found that a μ-opioid antagonist methylnaltrexone enhanced the efficacy of anticancer agents (1). The mechanism underlying enhancement of the anticancer activity by methylnaltrexone is considered to be blocking of opioid growth factor signaling. In contrast, peripheral κ-opioid receptors have a suppressive role in cancer progression and, therefore, we proposed that a κ-opioid agonist nalfurafine had a potential for anticancer agents through the inhibition of tumor angiogenesis (2). Inhibition of angiogenesis, however, correlates to hypoxia in tumors, implying that the use of κ-opioid agonists may increase a chance of metastasis. We are currently investigating the extent to which κ-opioid agonists affect cancer metastasis using the in vivo optical imaging system that permits noninvasive monitoring of cancer cells in live animals. Considering its pharmacological mechanism, μ-opioid antagonists may not negatively affect cancer metastasis. Overall, our findings indicate that peripheral μ-/κ-opioid receptors are involved in different types of cancers, and managing peripheral opioid receptors by μ-antagonists or κ-agonists will provide a great benefit to cancer patients. Furthermore, it is of significance to appropriately choose target peripheral opioid receptors in order to make use of opioid agonists/antagonists for cancer therapy.

(1) Suzuki M *et al.* (2015). *PLoS One* 10: e0123407.
(2) Yamamizu K *et al.* (2013). *Sci Rep* 3: 3213.

I017
Allostery at opioid receptors
JR Traynor
University of Michigan, Ann Arbor, USA

Positive allosteric modulators have been recently described for the μ-opioid receptor and provide a new avenue to explore for the development of drugs for moderate to severe pain. Allosteric modulators bind to a site on the receptor distinct from the orthosteric site for conventional opioid drugs and endogenous opioid peptides. Positive allosteric modulators increase the potency and/or efficacy of opioid agonists, both opioid analgesic drugs and endogenous opioid peptides and thus have the potential benefits of reducing or even eliminating the need for morphine-like drugs to obtain efficient analgesia. However, we know little about the mechanisms and outcomes of allosteric modulation of opioid receptors. In this presentation I will discuss the discovery of positive allosteric modulators for the μ- (and δ-) opioid receptors, their receptor selectivity, structure-activity relationships, dependence on the ligand occupying the orthosteric site (so-called "probe dependence"), and emerging ideas about how these allosteric modulators function at the receptor level. I will also provide proof-of-principle for the effectiveness of allosteric modulators as antinociceptive agents in mouse models to reduce the level of opioid drug required and/or enhance endogenous opioid peptide efficacy. Overall, the findings provide a basis for the further investigation of positive allosteric modulators of the opioid receptors as potential therapeutic agents and as pharmacological tools to examine endogenous opioid tone.

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I018
Peripherally restricted opioid combination therapy synergizes in multiple pain states
George Wilcox, Daniel Bruce, Cristina Peterson, Kelley Kitto, Carolyn Fairbanks
University of Minnesota, Minneapolis, MN, USA

Background: Most adverse effects of opioids are mediated by CNS mu-opioid receptors (MORs), but some opioid analgesia is mediated at the peripheral terminals of primary sensory afferents. Targeting peripheral opioid receptors should provide effective pain control without respiratory depression or addiction liability. We showed last year that delta-opioid receptor (DOR)-MOR spinal synergy is dependent on PKCε, suggesting that it occurs in the central terminals of nociceptors and might generalize to their peripheral terminals. We tested the MOR agonist loperamide (Lo, excluded from the CNS) and the DOR agonist oxymorphindole (OMI).

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Methods: We evaluated the analgesic or anti-hyperalgesic effect of Lo, OMI, or their 1:1 combination (i.t., i.pl., s.c. or topical) in naive or CFA-pretreated ICR-CD1 mice using the Hargreaves method.

Results: When combined in a 1:1 dose ratio —locally, topically or systemically — OMI-Lo produced analgesia (naïve) at 4-10-fold lower doses or anti-hyperalgesia (inflamed) at 50-100-fold lower doses than either agent alone. This synergy was blocked by naloxone methiodide, reinforcing the peripheral localization of the effect. The synergistic interaction was also inhibited by tertiapin-Q, which blocks G protein-coupled, inwardly rectifying potassium channels (GIRKs).

Conclusions: MOR agonists significantly synergized with DOR agonists at peripheral sites of action, providing strong evidence in support of peripherally restricted combination opioid therapy. The systemic and transdermal efficacy of the combination, along with loperamide's extremely low abuse liability, suggests that this combination therapy might be therapeutically useful to control inflammatory pain in the clinic without manifesting any of the CNS-mediated side effects of traditional opioid analgesics.

I019

Agonist-induced Mechanisms of Mu Opioid Receptor Regulation

Janet Lowe[1], Helen Sanderson[2], Alexandra Cooke[2], Mehrnoosh Ostovar[3], Elena Tsisanova[2], Sarah Withey[2], Charles Chavkin[4], Stephen Husbands[3], Eamonn Kelly[2], Graeme Henderson[2], Chris Bailey[3]

[1]Oregon Health & Science University, Portland, OR, USA, [2]University of Bristol, Bristol, UK, [3]University of Bath, Bath, UK, [4]University of Washington, Seattle, WA, USA

There is ongoing debate about the role of G protein receptor kinases (GRKs) in agonist-induced desensitization of the mu-opioid receptor (MOPr) in brain neurons (see Williams *et al.* (2013) Pharmacol Rev 15: 223-254). We have used a novel, membrane permeable, small molecule inhibitor of GRK2 and GRK3, Takeda Compound 101 (Cmpd101), to study the involvement of GRK2/3 in acute agonist-induced MOPr desensitization. We observed that Cmpd101 inhibits the desensitization of the G protein activated potassium current (GIRK) evoked by receptor- saturating concentrations of Met Enkephalin, DAMGO, endomorphin-2 and morphine in rat and mouse locus coeruleus (LC) neurons. In LC neurons from GRK3 knockout mice Met Enkaphalin-induced desensitization was unaffected implying a role for GRK2 in MOPr desensitization. Quantitative analysis of the loss of MOPr function following acute agonist exposure revealed that Cmpd101 partially reversed MOPr desensitization. Inhibition of ERK, PKC or JNK did not inhibit the Cmpd101-insensitive component of desensitization. In HEK 293 cells Cmpd101 produced almost complete inhibition of DAMGO-induced MOPr phosphorylation at Ser375, arrestin translocation and MOPr internalization. Our data demonstrate a role for GRK2 (and potentially also GRK3) in agonist-induced MOPr desensitization in the LC, but leave open the possibility that another, as yet unidentified, mechanism of desensitization also exists. Support from the MRC [MR/J013269/1], BBSRC[BB/J003506/1], Wellcome Trust Value in People award and USPHS-NIDA grant RO1DA030074. None of the authors has a conflict of interest related to this research.

I020

Phosphorylation barcodes for opioid receptors

Stefan Schulz

Institute of Pharmacology and Toxicology, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany

Phosphorylation of G protein-coupled receptors (GPCRs,) by GPCR kinases (GRKs) plays an essential role in the regulation of receptor function by promoting interactions of the receptors with interacting proteins. For the mu-opioid receptor (MOR), agonist-induced phosphorylation occurs at a conserved 10-residue sequence, ^{370}TREHPSTANT^{379}, in the carboxyl-terminal cytoplasmic tail. Morphine induces a selective phosphorylation of serine^{375} (S375) in the middle of this sequence that is predominantly catalyzed by GRK5. By contrast, high-efficacy opioids not only induce phosphorylation of S375 but also drive higher-order phosphorylation on the flanking residues threonine370 (T370), threonine^{376} (T376), and threonine^{379} (T379) in a hierarchical phosphorylation cascade that specifically requires GRK2/3 isoforms. In vivo GRK3 facilitates MOR desensitization, whereas GRK5 seems to be required for opioid dependence. These findings suggest that agonist-selective recruitment of distinct GRKs can influence different opioid-related behaviors. To further explore the physiological consequences of MOR phosphorylation, we have now generated and characterized a series of different phosphorylation-deficient mice.

I021

Opioid receptor desensitization

Macdonald Christie

University of Sydney, NSW, Australia

Agonist induced desensitization of μ-opioid receptor (MOPr) is widely considered to be an initial step in the development of opioid tolerance. Recruitment of GRK-β-arrestin is known to produce MOPr desensitization but other mechanisms, e.g. protein kinase C (PKC), have also been identified. It is established that some agonists which strongly recruit β-arrestins to MOPr (e.g. met-enkephalin) show little PKC-dependent desensitization and vice versa. We have mutated S/T phosphorylation sites in the MOPr C-terminal to examine the mechanisms of desensitization in cell lines and, to some extent, in native neurons using activation of GIRK channels with whole cell and perforated patch clamp recording. Phosphorylation of sites around and including S375 is required for β-arrestin recruitment and endocytosis but desensitization by met-enkephalin persists in most mutants regardless. Desensitization by met-enkephalin is only abolished by mutation of all C-terminal S/T phosphorylation sites (11S/T-A). In contrast, desensitization by morphine (which weakly recruits β-arrestins) was observed in all mutants, including 11S/T-A, and was blocked by PKC inhibitors. Surprisingly, mutation of sites that reduce β-arrestin recruitment (except 11S/T-A) increased the PKC-dependence of met-enkephalin-induced desensitization. This suggests that reduced β-arrestin recruitment can increase PKC-mediated MOPr regulation. Supporting this,

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engagement of PKC-dependent mechanisms was also increased for G-protein biased agonists that recruit β-arrestin at wild type MOPr even more weakly than morphine. Whether PKC-mediated desensitization is determined solely by G-protein bias or other agonist properties is still unclear. The importance bias towards PKC-regulation for opioid safety and tolerance also remains uncertain.

I022
Opioids, spinal cord and pain
Tony Dickenson
University College, London, UK

Opioids work at spinal levels by pre- and post-synaptic mechanisms and the spinal application of morphine rapidly lead to the human epidural route in patients.

Messages to the brain from the spinal cord activates sensory and affective areas. Brainstem pathways then descend to facilitate spinal mechanisms of pain showing the key interplay between sensory and psychological events in pain processing. Opioids reduce descending facilitations by CNS actions. All of these mechanisms alter as pain shifts from acute to chronic.

Opioids can be useful in chronic pain but there are issues with side-effects from the opioid load and paradoxical hyperalgesia as the inhibited spinal neuronal systems compensate. An advance has been tapentadol which is a mu opioid with noradrenaline reuptake inhibition, with key spinal actions. The latter action targets and enhances descending inhibitions and so opioid side-effects are reduced.

Another approach has been to combine opioids with other drugs such as pregabalin (PGB). In opioid induced hyperalgesia, enhancing noradrenaline levels and use of PGB reduces the abnormal pain signalling.
Opioid receptors sit on the facilitatory neurones in the brainstem. Using the binding of dermoprhin to the GPCR mu opioid receptor as a Trojan horse, the neurotoxin, saporin can be used to selectively ablate these neurones and chronic pains fail to maintain. Finally, opioid activation of descending inhbitions shows plasticity in persistent pains.
Thus a number of drugs interacting with opioids with final actions at spinal levels alter aberrant transmitter or channel function and have efficacy in different pain states.

I023
Pain resilience and vulnerability and the brain's opioid system
Anthony Jones[1], Christopher Brown[2]
[1]University of Manchester, Manchester, UK, [2]University of Liverpool, Liverpool, UK

Although the distribution of opioid peptides and receptors is well described their physiological function in the brain is still not entirely clear. Human functional brain imaging with Positron Emission Tomography PET) allows in vivo quantification of available opioid receptor binding sites. Changes in opioid receptor binding may reflect alterations of receptor density or occupation of binding sites by endogenous opioid peptides or a combination of both.

The focus will be on human studies of chronic pain. Because neuropathic pain can be relatively resistent to synthetic opiates it has been suggested that the endogenous opioid system was not involved. Early studies using [^{11}C]diprenorophine and PET in patients with trigemminal neuralgia showed changes in OR binding consistent with increased occupation of OR in the brain during pain compared to after surgical pain relief. Similar findings were found in patients with rheumatoid arthritis in and out of inflammatory pain . PET studies have shown reduced OR binding within the pain matrix in patients with post-stroke central deafferantation pain in patients without cortical damage. This study has been well replicated suggesting a potential mechanisms for this types of pain.

More recently we have shown in patients with arthritis that there is increased OR binding in relation to the severity of recent pain within the striatum confirming findings in animals suggesting a compensatory upregulation of OR binding in the brain in response to recent pain. We also showed a correlation with pain threshold suggesting OR upregulation reduces pain perception. This suggests that pain itself may lead to adaptive upregulation of OR binding in humans.

I024
Pain alters opioid intake and associated motivated behavior
Nicolas Massaly, Adrianne Poe, Lucia Hipolito, Ream Al-Hasani, Michael Bruchas, Jose Moron-Concepcion
Washington University, St Louis, MO, USA

Pain patients with a prior history of drug use are sensitized to opioid exposure such that they are more prone to opioid abuse, dose escalation and/or relapse. However, the effect of pain on opioid intake patterns in animal models of opioid abuse has not been investigated. In our studies, we examined the regulation of μ- and k- receptor systems 48 hours after the induction of inflammatory pain using the complete Freund's adjuvant (CFA) rat model of inflammation. In addition, we conducted behavioral studies to examine the role of these opioid systems in the effects of pain on motivated behavior. We used a combination of electrophysiology and in vivo microdialysis approaches to assess pain-induced changes in μ - and k- receptors. In addition, we assessed the role of k-receptors in opioid intake, and associated motivated behavior, in the presence of pain using the intravenous drug self-administration procedure in rats. Our data indicate that the presence of pain impacts the effects of opioids and natural reinforcers in the mesolimbic reward pathway by 1) decreasing μ-receptor regulatory function and 2) enhancing k-receptor repressive effects on dopamine release in the NAc. We are currently investigating the involvement of opioid systems in the regulation of rewarding properties of positive stimuli during painful conditions, as well as the role of adaptations in these systems in the development of addiction.

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I025
Endogenous Opioids and Placebo Neurobiology: Tapping into Resiliency?
JK Zubieta
University of Utah, Salt Lake City, Utah, USA

Endogenous opioid and non-opioid mechanisms [e.g. dopamine (DA), endocannabinoids (eCB)] have been implicated in the formation of placebo analgesic effects. The information so far acquired points to neurobiological systems that when activated by positive expectations and maintained through conditioning and reward learning are capable of inducing physiological changes that lead to the experience of analgesia and improvements in emotional state. Functional and molecular neuroimaging techniques have significantly contributed to our understanding of the neurobiological systems involved in the formation of placebo effects [1, 2]. This line of research has described neural and neurotransmitter networks implicated in placebo responses across pathological states and provided the technical tools to examine inter-individual differences in the function of placebo responsive mechanisms. As a consequence, the formation of biological placebo effects is now being linked to the concept of resiliency mechanisms, partially determined by genetic factors and learning mechanisms. The delineation of these processes within and across diseases would point to biological targets that have not been contemplated in traditional drug development. This line of work is of particular importance in the study of pathologies that present high levels of placebo response, such as Major Depression, persistent pain conditions, or even their comorbidities.

(1) Peciña *et al.* (2015). Association Between Placebo-Activated Neural Systems and Antidepressant Responses: Neurochemistry of Placebo Effects in Major Depression. *JAMA Psychiatry*, 72(11), 1087-1094.
(2) Scott *et al.* (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Arch Gen Psychiatry*, 65(2), 220-231.

I026
Novel signaling and therapeutic indications for the delta opioid receptor
Amynah Pradhan
University of lllinois at Chicago, Chicago, IL, USA

The delta opioid receptor regulates a number of biological processes; including pain, anxiety, and depression. Agonists to the delta opioid receptor are being developed for clinical use; and activation of this receptor produces fewer adverse events compared to currently available mu opioid receptor-based therapies. Like many G protein coupled receptors, delta opioid receptors display ligand directed signaling. We have previously demonstrated that not all delta agonists induce receptor internalization, and that this difference in receptor trafficking can have profound effects on acute and chronic tolerance. We have also recently found that delta agonists differentially recruit arrestin isoforms. Binding of high-internalizing agonist preferentially recruits arrestin 2, while low-internalizing agonists promote receptor interactions with arrestin 3. This work has revealed a novel role for arrestin 3 as a facilitator of receptor resensitization, and protective from tolerance mechanisms. A further area of interest has been to determine novel therapeutic roles for the delta opioid receptor. We have recently shown that delta agonists are highly effective in multiple models of migraine. We are currently characterizing the role of central and peripheral delta opioid receptors in the regulation of migraine pathophysiology. Taken together, this work serves to encourage the development of novel therapies targeting the delta opioid receptor.

I027
What can fMRI tell us about the role of opioids in cognitive and emotional modulation of pain?
Tim Salomons
University of Reading, Reading, UK

Functional magnetic resonance imaging allows us a window into neural processes associated with critical human functions. One such function is the ability to cope with pain. Pain is a sensory and emotional experience associated with actual or potential tissue damage. One critical way to cope with pain is by altering the cognitive and emotional response to nociceptive input. This talk will focus on the neural processes underlying cognitive and emotional coping. Given the role of endogenous opioids in pain modulation and emotional processes, understanding the role of the opioid system in these processes is a critical goal. The talk will review evidence, but also limitations on inferences drawn from fMRI.

I028
The Brain in Pain Studies: Central sensitisation as a pharmacological target for chronic pain
Julius Bourke
Barts and the London SMD, QMUL, London, UK

A challenge in the development of treatments for chronic pain is the incomplete understanding of mechanisms contributing to the transition from acute to chronic pain and to chronic pain disorders in which an underlying pathology is not identifiable, such as functional somatic syndromes (FSS).

FSS such as chronic fatigue syndrome and fibromyalgia are common and poorly understood causes of chronic pain. Chronic pain and FSS are accompanied by affective and cognitive disturbances that add to group heterogeneity and the development of a model that encompasses these dimensions represents a further challenge. Neural adaptations in reward-related circuits and the neurophysiological basis for 'central sensitisation' have been proposed as more appropriate models but without translation into patient benefit.

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During this session I will present established data suggesting that opioid neurotransmission may be upregulated in functional somatic syndromes with a corresponding adaptation in mu-opioid binding potential. I will also present novel data that suggests this adaptation may occur at mu-opioid receptors, implicating these receptors as pharmacological targets for antagonism.

The therapeutic potential of opioid antagonists in the management of chronic pain represents a paradigmatic shift in our understanding and treatment of this disabling and costly problem. I will also outline current theories of central sensitisation as a model of chronic pain and how tests for this might be employed to stratify patient subgroups to more appropriate treatment options whilst also improving group homogeneity in future research.

HOT TOPIC ABSTRACTS

HT001

Measuring Efficacy of Orthosteric and Allosteric Ligands at the Mu-Opioid Receptor Using a Conformational Biosensor

K Livingston[1], J Mahoney[1], A Manglik[3], B Kobilka[3], R Sunahara[2], J Traynor[1]

[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California- San Diego, San Diego, CA, USA, [3]Stanford University, Palo Alto, CA, USA

The intrinsic efficacy of a ligand reflects the ability of that ligand to stabilize a signaling-competent state of the receptor and is one of the major predictors of its physiological effect. Difficulties arise when measuring intrinsic efficacy as values can change depending upon factors including cell type, signaling output measured, and the kinetics of the assay. Here, we present a cell-free, signal transduction-independent method for the determination of intrinsic efficacy of both orthosteric and allosteric ligands acting at the mu opioid receptor (MOPr). Utilizing purified MOPr reconstituted into high density lipoprotein particles we have studied binding of the MOPrconformation-sensitive biosensor nanobody39 (Nb39) by interferometry to monitor formation of active-state MOPr by ligands in real-time. Differences in orthosteric ligand efficacy were seen to correlate with different kinetics of Nb39 association and dissociation. In addition, we found that positive allosteric modulators (PAMs) of MOPr are capable of stabilizing active-state MOPr alone and that the kinetics of Nb39 binding correlate to thein vitro efficacy of the PAMs to enhance agonist affinity. The technique can readily be applied to other G protein-coupled receptors to analyze the efficacy of both orthosteric and allosteric ligands.

Funded by DA039997.

HT002

Functional Selectivity and Analgesic Effects of LOR17, a Novel Kappa Opioid Receptor (KOR) Selective Agonist

A Bedini[1], L Di Cesare Mannelli[2], R De Marco[3], L Gentilucci[3], C Ghelardini[2], S Spampinato[1]

[1]Department of Pharmacy and Biotechnology (FaBiT) - University of Bologna, Bologna, Italy, [2]Department of Neuroscience, Psychology, Drug and Children Health (NEUROFARBA) – University of Florence, Florence, Italy, [3]Department of Chemistry "G. Ciamician" – University of Bologna, Bologna, Italy

Background: Kappa opioid receptor (KOR) agonists determine antinociception by G protein signaling; conversely, they trigger relevant side effects through GRK3-, arrestin 3-dependent activation of p38MAPK. Thus, studies supporting the hypothesis that functionally selective KOR agonists might represent novel analgesics with reduced side effects are in progress.We aimed to investigate functional selectivity and analgesic effects of LOR17, a novel KOR selective and potent agonist that we recently identified.

Results: LOR17 displayed nanomolar affinity and selectivity for KOR in binding assays performed in HEK-hKOR cells, inhibited adenylyl cyclase and activated early (5-15min), G protein-dependent component of ERK1/2 phosphorylation in HEK-hKOR and U87-MG astrocytoma cells, as assessed by EIA and western blot; furthermore, LOR17 did not trigger in the same cells late (60min), arrestin-dependent component of ERK1/2 or p38MAPK phosphorylation. On the contrary, U50,488 activated all the above mentioned signaling pathways. Moreover, U50,488 induced a p38MAPK-dependent increase in cell proliferation and IL-1beta mRNA levels in U87-MG cells, as assessed by [3H]-thymidine incorporation and real-time PCR, whereas LOR17 was not effective. Experiments on normal human astrocytes are ongoing and will be presented at the conference.

Interestingly, LOR17 and U50,488 determined KOR-mediated antinociception in mice in warm-water tail-withdrawal test (LOR17-ED50=10.07±0.36mg/kg; U50,488-ED50=9.93±0.37mg/kg) and in acetic acid-induced visceral pain (LOR17-ED50=5.74±0.46mg/kg; U50,488-ED50=8.24±0.59mg/kg), but only the former was anti-hyperalgesic in a mouse model of oxaliplatin-induced neuropathy (LOR17-ED50=6.63±0.23mg/kg).

Conclusion: LOR17 is a novel KOR agonist displaying functional selectivity towards G protein signaling and analgesic effects in different pain models; oxaliplatin-induced neuropathy was resistant to U50,488.

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HT003

Investigating the structural determinants of μ-opioid receptor ligand bias by mutagenesis

J Daniel Hothersall, Rubben Torella, Sian Humphreys, Gordon McMurray, Sarah Nickols

Pfizer NPRU UK, Cambridge, UK

Ligand bias describes observations that some GPCR ligands can preferentially activate certain receptor pathways over others. There is growing enthusiasm for optimising ligand bias in drug discovery for anticipated pharmacotherapeutic benefit; for example μ-opioid receptor G protein-biased agonists as improved analgesic. Despite this, our ability to rationally develop novel biased ligands is hindered by an incomplete understanding of precisely how ligand binding site interactions influence differential receptor activation.

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To investigate which residues within the μ-opioid binding site play a role in generating bias, we introduced point mutations rationally informed by structural biology data. Mutant receptor pharmacology was compared to the wildtype for a panel of ligands at the G protein and β-arrestin2 branches of signalling, and bias factors calculated as ΔΔlog(Emax/EC50).

We identified W320A as a mutant with a particularly interesting effect on opiate bias. This mutation caused DAMGO to switch from moderate cAMP-bias to β-arrestin2 bias. Whilst DAMGO gained relative activity at W320A, there was a dramatic loss in endomorphin-1 responses, especially in the β-arrestin2 assay. These changes in function were uncoupled from effects on relative binding affinity, indicating that the mutation did more than simply perturb ligand binding.

Thus, chemically altering the MOR binding pocket by mutagenesis changes the balance of pathway activation by certain ligands. This highlights a role for the binding site residue W320 in qualitatively regulating how a ligand activates the receptor to cause ligand bias.

HT004

The G-protein biased kappa opioid receptor agonist 6'-GNTI blocks hippocampal paroxysmal discharges without inducing aversion

Christoph Schwarzer[1], Johannes Burtscher[1], James P MacKay[2], William F Colmers[2], Luca Zangrandi[1]

[1]Dept. Pharmacology, Medical University of Innsbruck, Innsbruck, Austria, [2]Dept. Pharmacology, University of Alberta, Edmonton, Canada

With a prevalence of 1–2%, epilepsies belong to the most frequent neurological diseases worldwide. Although antiepileptic drugs are available since several decades, medication is ineffective over 30 %. Antiepileptic effects of kappa opioid receptor (k receptor) agonists were suggested since the early 1980s. However, clinical use of their potential was hampered by dysphoric side effects. In recent years, G-protein biased k receptor agonists were developed, which displayed little activation of the b-arrestin pathway in-vitro, suggesting reduced aversive effects.

We investigated the effects of the full k receptor agonist U-50488H and the G-protein biased partial k receptor agonist 6'-GNTI in models of acute seizures and drug resistant temporal lobe epilepsy and in the conditioned place avoidance test. Moreover, we performed slice electrophysiology to understand the functional mechanisms of 6'-GNTI.

Like previously shown for U-50488H, 6'-GNTI markedly increased the threshold for pentylenetetrazole induced seizures. All treated mice displayed reduced paroxysmal activity in response to 6'-GNTI (10 - 30 nmoles) or U-50488H (20 mg / kg) treatment in the mouse model of unilateral intra-hippocampal injection of kainic acid. Moreover, 6'-GNTI did not induce conditioned place avoidance, a measure of aversive effects, while U-50488H did. Single cell recordings on hippocampal pyramidal cells revealed enhanced GABAergic signaling as potential mechanisms causing the reduction of paroxysmal activity.

Our data provide the proof of the principle that anticonvulsant / anti-seizure and aversive effects of k receptor activation can be pharmacologically separated *in-vivo*.

HT005

Nociceptin/orphanin FQ inhibits contextual fear memory reconsolidation in mice

L Mouledous[1,2], K Rekik[1,2], R Faria Da Silva[1,2], B Frances[1,2]

[1]Research Center on Animal Cognition, Toulouse, France, [2]CNRS/Université Paul Sabatier, Toulouse, France

Several neuropeptidergic systems act as modulators of cognitive performances. Among them, nociceptin, an opioid-like peptide also known as orphanin FQ, has recently gained some attention. Its receptor named NOP is expressed in brain regions involved in emotion, memory and stress response such as the amygdala, the hippocampus and the hypothalamus. The peptide has inhibitory effects on the acquisition and/or consolidation of various types of spatial and emotional memory in rodent models. Recently, nociceptin was also proposed to be linked to the pathogenesis of Post-Traumatic Stress Disorder in humans. Overall the nociceptin system could represent an interesting pharmacological target for interfering with so-called "pathological memories" associated with stress, emotion and trauma. However, until now the effect of the nociceptin system on late phases of memory, such as retrieval and reconsolidation, has never been explored. Thus, in the present study, we investigated the consequences of systemic injection of a NOP agonist or icv injection of the nociceptin peptide itself on the retrieval and the reconsolidation of contextual fear memory in mice. We demonstrate that the activation of the nociceptin system does not affect recall but interferes with the reconsolidation process. We are currently investigating the site of action of the peptide and the mechanism by which it prevents efficient memory reconsolidation.

HT006

A Role for the Mu Opioid Receptor in the Antidepressant-like Effects of Buprenorphine

Shivon Robinson, Rebecca Erickson, Caroline Browne, Irwin Lucki

University of Pennsylvania, Philadelphia, PA, USA

Buprenorphine (BPN) has been shown to produce behavioral responses in rodents that are similar to those of antidepressant and anxiolytic drugs. The novelty-induced hypophagia (NIH) test, a behavioral assay previously shown to be responsive to chronic antidepressants, is also sensitive to BPN treatment. The roles of mu (MORs) and kappa (KORs) opioid receptors were examined in mediating conditioned approach behavior in the NIH test. The effects of BPN (0.25 mg/kg, i.p.) were evaluated in the NIH test 24 h post-administration in mice with genetic deletion of MOR ($Oprm1^{-/-}$) or KORs ($Oprk1^{-/-}$), or after pharmacological blockade with opioid receptor antagonists. Behavioral responses to BPN in the NIH test tested 24 h post-administration were blocked in $Oprm1^{-/-}$ mice, but not $Oprk1^{-/-}$ mice. To further elucidate the opioid receptor mechanisms underlying behavioral response in the NIH paradigm, other opioid receptor compounds were tested. The selective MOR antagonist cyprodime (10 mg/kg) and the nonselective antagonist naltrexone (1 mg/kg) significantly reduced approach latency in the NIH test when tested 1 h after treatment. In contrast, morphine and the KOR antagonist nor-BNI (both at 10 mg/kg) were both ineffective in the NIH test. Moreover, antinociceptive studies confirmed

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MOR antagonist effects of cyprodime and naltrexone and revealed MOR antagonist properties of BPN persisting at 24 h post-administration. Altogether, these data support the idea that reduced signaling at MORs is a key component of BPN's antidepressant-like effects in the NIH paradigm.

HT007

Pulling the brakes on midbrain dopamine cells: inhibiting substance P prevents opiate reward

AJ Sandweiss, MI McIntosh, A Moutal, AK Giri, VJ Hruby, R Khanna, TM Largent-Milnes, TW Vanderah

University of Arizona, Tucson, AZ, USA

Chronic pain affects approximately 100 million Americans. Opioids are the mainstay therapy for the treatment of chronic pain. While physicians and patients alike are apprehensive about using opioids due to their side effects including respiratory depression and addiction, 259 million opioid prescriptions were written in 2012. Although opioids are the best available analgesics, they increase both positive and negative reinforcement, ultimately leading to addiction. The pro-nociceptive neurotransmitter Substance P (SP) and its corresponding receptor (NK1R) are found in the ventral tegmental area associated with dopamine neurons. Studies have shown that Substance P can potentiate positive reinforcement of opiates and may play a role in opioid reward. Here using in vivo microdialysis, we show that systemic morphine significantly increases SP release in the VTA, an effect mediated by ventral midbrain GABAergic neurons. Substance P given in the VTA results in a significant increase in dopamine in the nucleus accumbens (NAc). Using CRISPR-Cas9 knockdown of NK1R in the VTA we prevent the induction of opiate reward as tested using a conditioned place preference paradigm (CPP). Finally, we developed a novel opioid agonist/NK1R antagonist bifunctional compound, TY032, which inhibits acute and chronic pain in male rats. Importantly, TY032 microinjection into the VTA did not increase extracellular dopamine release in the NAc. These data indicate dual targeting of the dopamine reward circuitry and pain pathways with multifunctional opioid-NK1R compounds may be an effective strategy in developing future analgesics that lack the potential for abuse.

HT008

Does Cannabis Use Modify The Relationship Between Opioid Use And Non-fatal Overdose?

Pauline Voon[1,2], M-J Milloy[1,3], Kanna Hayashi[1,3], Sabina Dobrer[1], Evan Wood[1,3], Thomas Kerr[1,3]

[1]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada, [2]School of Population and Public Health, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada, [3]Department of Medicine, University of British Columbia, Vancouver, BC, Canada

Background: Opioid overdose is a major public health concern. A recent population-level analysis found a 25% lower mean annual opioid overdose mortality rate in U.S. states with medical cannabis laws compared to those without. However, there is a lack of individual-level data evaluating the effect of cannabis on opioid overdose patterns.

Methods: Data from June to November 2014 were derived from two prospective cohorts of illicit drug users in Vancouver, Canada. The sample was restricted to individuals reporting at least one opioid use (non-medical prescription opioid or heroin) in the six months prior to interview. The relationship between daily opioid use and non-fatal opioid overdose (OOD), stratified by marijuana use, was examined using bivariable and multivariable logistic regression (adjusting for age, gender, ethnicity, homelessness, and recent incarceration).

Results: Among the 536 participants eligible for this analysis, 38 reported OOD during the study period. Daily opioid use was significantly associated with OOD among the total sample (AOR: 2.11, 95%CI: 1.02-4.35) and among those who did not use cannabis daily (AOR: 2.46, 95%CI: 1.07-5.67) or at all (AOR: 5.48, 95%CI: 1.52-19.72). However, daily opioid use was not significantly associated with OOD among individuals reporting any cannabis use (AOR: 0.86, 95%CI: 0.29-2.56) or daily cannabis use (AOR: 0.90, 95%CI: 0.12-6.77).

Conclusions: Consistent with recent ecological and experimental research, these findings suggest that cannabis may have a protective effect in the context of OOD. Further research on this potentially modifying effect may inform future policies and clinical practices related to medicinal marijuana.

HT009

Regulation of Morphine Reward by a Novel Neuropeptide Receptor System, BigLEN-GPR171

EN Bobeck[1], D Pena[2], I Gomes[1], AK Fakira[1], LA Devi[1]

[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]University of Sao Paulo, Sao Paulo, Brazil

ProSAAS derived peptides, such as BigLEN, are abundantly expressed throughout the brain. We recently identified Big-LEN as the endogenous peptide for the orphan receptor GPR171. Immunohistochemical analysis shows that both Big-LEN and GPR171 are highly expressed in areas of the brain involved in opioid function, such as nucleus accumbens (NAc), medial prefrontal cortex (mPFC), and basolateral amygdala (BLA). Following conditioned place preference to morphine there is an increase in GPR171 mRNA expression in NAc and BLA. Whereas we see no change in GPR171 expression under these conditions in the PFC, there is enhanced signaling as determined by BigLEN-mediated decreases in cAMP production. Next we examined the role of GPR171 in the BLA using lentiviral-mediated local knockdown in C57bl/6 mice and find that this leads to a reduction in conditioned place preference to morphine. We have recently identified MS21570 as a selective antagonist of GPR171 by in silico screening of a library of small molecules using a homology model of GPR171. Pretreatment with MS21570 (5 mg/kg, i.p. in 6% DMSO) prior to 4 morphine (10 mg/kg, s.c.) pairings, tends to reduce conditioned place preference to morphine. Together, these data support the idea that the BigLEN-GPR171 system plays an important role in the regulation of reward-related behaviors.

Supported by NIH grants, DA019521 and NS026880 (to LAD) and NIDA postdoctoral training grant DA007135 (to ENB).

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HT010

Sewarine, an Indole Alkaloid from Rhazya stricta, and Its Interaction with the κ-Opioid Receptor: A Pharmacological and Molecular Modeling Study

Aquilino Lantero[1], Michael Mairegger[1], Stefan Salcher[2,3], Muhammad F. Asim[1], Elena Guerrieri[1], Helmut Schmidhammer[1], Petra Obexer[2,3], Mariana Spetea[1]

[1]Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences, University of Innsbruck, Innsbruck, Austria, [2]Department of Pediatrics II, Medical University of Innsbruck, Innsbruck, Austria, [3]Tyrolean Cancer Research Institute, Innsbruck, Austria

Natural products are an excellent source of promising lead compounds for the generation of new therapeutic drugs. Rhazya stricta is an alkaloid-rich herb that is used in traditional oriental medicine to treat several human diseases, including tumors. Applying a pharmacophore-based virtual screening strategy, we have recently discovered a new κ-opioid receptor (KOR) ligand, sewarine, a natural alkaloid from *R. stricta*. Here, we present a pharmacological and molecular modeling study on sewarine examining (i) the interaction and signaling at the rodent and human KOR, and (ii) the antiproliferative and anticancer effects *in vitro*. Sewarine shows high KOR selectivity and similar binding affinity to the guinea-pig and human KOR. While sewarine displays antagonism at the rodent KOR, it is a partial agonist at the human KOR. It effectively inhibits poliferation and induces apoptosis in lymphoblastic leukemia, neuroblastoma and breast cancer cells. The apoptotic effect of sewarine is mediated via activation of caspase pathways and by modulating pro-apoptotic and anti-apoptotic proteins, and it involves the KOR, based on the antagonism by nor-binaltorphimine. Docking of sewarine to the structure of the human KOR revealed that the salt bridge between the protonable nitrogen in sewarine and Asp138 was maintained, and hydrophobic contacts were formed with Val108, a residue responsible for KOR selectivity. Our results established the significant anticancer activity of sewarine *in vitro*, and thus provided valuable information on the role of KOR in apoptosis, as well as the first evidence and a rational for the anticancer effects of alkaloid extracts of *R. stricta*.

HT011

The role of cancer cell secretion in opioid-induced tumor angiogenesis

DA Fux, A Schoos, S Tripolt

VetMedUniversity, Vienna, Austria

Opioids have been revealed to modulate tumor angiogenesis, albeit by yet obscure signaling mechanisms. Tumor angiogenesis includes migration of endothelial (END) cells, which is centrally driven by the focal adhesion kinase (FAK). Thus we started to analyze the effect of opioids on FAK regulation in murine END cells. However, incubation of END cells with the μ-agonist [D-Ala2, N-MePhe4, Glyol]-enkephalin (DAMGO), the delta-agonist [D-Ala2, D-Leu5]-Enkephalin (DADLE) and kappa-agonist U50,488H (1μM each) failed to induce FAK activation. To rather meet tumor-like conditions, we next used END cells that were cultured in conditioned medium (CM) obtained from breast cancer cells (MDA-MB-231, 4T1). Under this condition, DADLE triggered a naloxone-sensitive FAK activation and END cell migration; the other opioids failed. We found by a cytokine array that the fibroblast growth factor-2 (FGF-2) is released from the tumor cells. Thus, END cells were tested next for DADLE-mediated FAK regulation after FGF-2 (10 ng/ml) pre-treatment. Again DADLE incubation resulted in FAK activation and cell migration suggesting that tumor cell-released FGF-2 renders END cells susceptible to DADLE. This notion is further supported by the finding that FGF-2 treatment enhanced DOR expression in END cells via the NF-kappaB transcriptional pathway. Inhibition of NF-kappaB activation by Resveratrol (10μM) or the FGF-2 receptor inhibitor BGJ398 (1 μM) abolished DADLE-promoted FAK activation and migration of FGF-2 conditioned END cells. Our findings let us thus suggest that FGF-2 secretion from breast cancer cells promotes expression of DORs in END cells, which subsequently allows opioid-induced cell migration via FAK activation.

HT012

Development of a Combination Vaccine for Heroin and HIV-1

R Jalah[1,2], OB Torres[1,2], JFG Antoline[3,4], KK Peachman[1,2], Z Bec[k1,2], M Rao[1], AE Jacobson[3,4], NL Michael[1], KC Rice[3,4], CR Alving[1], GR Matyas[1]

[1]U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA, [2]U.S. Military HIV Research Program, Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA, [3]Drug Design and Synthesis Section, Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA, [4]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

Background: There is a high prevalence of HIV-1 amongst injection drug users. We aimed to develop a combination heroin-HIV vaccine that could block the effects of heroin and also prevent HIV-1 transmission. The HIV portion of the vaccine utilized a cyclic V2 (cV2) peptide identified as a correlate of prevention of HIV acquisition in the RV144 phase III HIV vaccine clinical trial.

Methods: Mice were immunized with the heroin hapten MorHap conjugated to tetanus toxoid and mixed with Army Liposome Formulation (ALF) containing palmitoylated cV2 peptide. Sera were assayed for antibodies to MorHap and HIV Env antigens by ELISA and their ability to block the binding of cV2 to human α4β7 integrin. Mice were challenged at week 10 with 1 mg/kg subcutaneous heroin and efficacy was assessed by antinociception assays.

Results: The heroin-HIV combination vaccine induced high serum levels of IgG to MorHap (1.3 mg/ml) and protected mice from heroin challenge with a % Maximal Potential Effect of 15% in a tail-flick assay that was similar to the heroin-only vaccine group. Antibody endpoint titers to HIV Env cV2, gp70-V1V2, and gp120 were from 200,000-600,000, similar to the group receiving ALF-cV2 vaccine alone. The combination vaccine group sera inhibited the binding of cV2 to α4β7 integrin by 65%.

Conclusions: The heroin-HIV vaccine induced protection against heroin challenge and very high titer cV2 antibodies that blocked α4β7 integrin receptor binding, a proposed mechanism of efficacy of the V2 antibodies in RV144. The findings suggest that an effective dual heroin-HIV vaccine is feasible.

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HT014

Phosphoproteomic survey of Kappa Opioid Receptor in vivo Functional Selectivity

Jeffrey Liu[1], Luca Zangrandi[2], Chongguan Chen[3], Sean Humphrey[1], Lee-Yuan Liu-Chen[3], Christoph Schwarzer[2], Matthias Mann[1]

[1]Max Planck Institute of Biochemistry, Martinsried, Germany, [2]Department of Pharmacology, Medical University of Innsbruck, Innsbruck, Austria, [3]Center for Substance Abuse Research and Department of Pharmacology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

Kappa Opioid Receptor (KOR) modulates mood, pain and stress in the human. Agonist-mediated signaling through KOR is initiated by activation of either G-proteins and/or beta-arrestins, from which different downstream responses were produced, an effect known as "functional selectivity". It was proposed that KOR agonists, which initiate preferentially G-protein signaling, do not elicit dysphoric effects typically associated with prototypic KOR agonists, while preserve analgesic effectsq. To date, little is known about KOR-mediated signaling pathways beyond the initial steps and some downstream effectors (e.g. MAP kinases), especially in the brain. Despite its promising potential to yield the first true "non-addictive" opioid drug, no known systems biology study was conducted on KOR-mediated signaling pathways. Here, we applied the cutting-edge high-throughput mass spectrometry-based phosphoproteomics to investigate KOR ligand functional selectivity in a neuroblastoma-derived cell line (Neuro2A) and in mouse brain, using a collection of kappa agonists, such as the non-biased agonist U50,488H, and the G-protein biased 6'GNTI. From this study, we 1) showed that phosphoproteomics can be used as a novel tool to assess functional selectivity of ligands *in vivo*; 2) compared and contrasted KOR functional selectivity in the cell line model and *in vivo* and 3) in various brain regions, including cortex, striatum, hippocampus, and medulla oblongata; 4) dissected downstream signaling pathways initiated by different functionally selective ligands.

HT015

RGS protein interactions contribute to functional selectivity of agonists acting at G-protein coupled receptors (GPCRs)

SL Ingram[1], JD Lowe[1], M Li[1], KL Suchland[1], JR Traynor[2]

[1]Oregon Health & Science University, Portland, OR, USA, [2]University of Michigan, Ann Arbor, MI, USA

RGS proteins are GTPase accelerating proteins (GAPs) that accelerate the hydrolysis of Galpha bound GTP and promote the formation of inactive Galpha GDP at GPCRs. Using a transgenic knock-in mouse that expresses Galpha proteins that are insensitive to the GAP activity of RGS proteins, we show that GPCR coupling to effectors in the periaqueductal gray (PAG) is differentially affected in the knock-in mice compared to wildtype mice. We compared mu opioid receptor (MOR) and GABAB receptor agonist inhibition of presynaptic GABA release and activation of postsynaptic GIRK channels in the PAG. MOR agonists met-enkephalin and morphine induced greater inhibition of presynaptic GABA release in the knock-in mice but significantly reduced GIRK currents. Interestingly, the differences noted in presynaptic inhibition were larger at EC50 concentrations of the agonists, while differences in GIRK current activation were more evident at maximal concentrations. Consistent with these results, a maximal concentration of DAMGO (5 μM) produced comparable inhibition of GABA release in the knock-in and wildtype mice but significantly reduced GIRK currents in the knock-in mice. Similarly, a maximal concentration of the GABAB agonist baclofen (20 μM) produced comparable inhibition of GABA release in the knock-in and wildtype mice but GIRK currents were significantly reduced in the knock-in mice. These results suggest that RGS proteins act to limit GPCR signaling in presynaptic terminals but are critical for GPCR-mediated by signaling to GIRK channels.
Supported by NIH R01DA035316.

HT016

Inhibition of c-Src reduces morphine analgesic tolerance

Fiona Bull[1], Daniel Baptista-Hon[1], Wendy Walwyn[2], Tim Hales[1]

[1]University of Dundee, Dundee, Scotland, UK, [2]University of California, Los Angeles, Los Angeles, CA, USA

A limitation of opioid analgesia is tolerance, leading to the requirement for increasing doses for the treatment of persistent pain. Morphine tolerance is attenuated in mice that lack β-arrestin2 (βarr2-/-), a scaffolding protein that participates in mu opioid receptor (MOR) endocytosis and signalling through several kinases including c-Src. We examined whether c-Src participates in morphine analgesic tolerance and/or reinforcement. Tail withdrawal from noxious heat and conditioned place preference (CPP) were used to assess morphine analgesia and reinforcement, respectively.

Morphine dose-dependently prolonged the latency for tail withdrawal from 48oC water in wild type (WT) C57BL/6 mice, which was not seen in homozygous MOR knockout (MOR-/-) mice. As expected, higher doses of morphine were required for analgesia in MOR+/- mice. Morphine (10 mg/Kg) analgesic tolerance developed faster and to a greater extent in MOR+/- mice than in WT mice. Intraperitoneal (ip) injection of, dasatinib (5 mg/Kg, daily, 30 mins prior to morphine), a c-Src inhibitor used to treat leukaemia, reduced the development of morphine tolerance in WT and MOR+/- mice. The selective c-Src inhibitor, PP2 (5 mg/Kg ip), also reduced morphine tolerance in MOR+/- mice, while the inactive analogue, PP3, did not. Dasatinib reversed tolerance when administered on day 4, 30 mins prior to morphine (10 mg/Kg). Neither PP2 nor dasatinib altered tail withdrawal latencies in the absence of morphine.

Furthermore, dasatinib neither affected the locomotor response to morphine nor CPP. These data suggest that c-Src signalling can be targeted to produce sustained MOR-mediated analgesia without affecting the psychomotor effects of opioids.

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HT017

A single dose of morphine induces epigenetic changes in dopamine neurons of the VTA associated with potentiation of glutamate mediated excitation and depression of GABA inhibition

BM Cox[1], ME Authement[1], H Kassis[1], LD Langlois[1], S Gouty[1], M Dacher[1,2], RD Shepard[1], FS Nugent[1]

[1]Department of Pharmacology, Uniformed Services University, Bethesda, MD, USA, [2]iEES-Université Pierre et Marie Curie/Paris 6, Versailles, France

Previous studies have shown that a single dose of morphine increases the excitability of dopamine (DA) neurons in the ventral tegmental area (VTA) and induces plasticity at GABAergic synapses on DA neurons, initiating processes that are consolidated after repeated dosing. We now show that a single dose of morphine (10 mg/kg) in rats induces epigenetic changes in the VTA 24 hrs later. Specifically, the expression of HDAC2 measured by immunohistochemistry in VTA tyrosine hydroxylase-positive neurons was increased markedly 24 hrs after morphine treatment, while the level of acetylated H3K9 histone decreased. This morphine treatment also triggered plasticity at glutamatergic excitatory synapses (with an increase in GluA2-lacking AMPA receptors and increases in the AMPA/NMDA ratio and the amplitude of mEPSCs, indicating glutamatergic long-term potentiation). Plasticity was also observed at GABAergic inhibitory synapses (which showed induction of both pre-and post-synaptic long-term depression) on DA neurons in VTA slices at 24 hrs after morphine treatment. These changes were reversed by incubation of VTA slices from morphine treated rats with the Class I HDAC inhibitor, CI-994. Our results suggest that a single dose of morphine initiates via epigenetic mechanisms a cascade of events resulting in long-term changes in plasticity at both glutamaterigic and GABAergic synapses regulating the VTA DA neurons.

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HT018

Prediction Formulas for Individual Opioid Analgesic Requirements Based on Genetic Polymorphism Analyses

Daisuke Nishizawa[1], Kaori Yoshida[1,2], Takashi Ichinomiya[3], Tatsuya Ichinohe[2], Masakazu Hayashida[4], Ken-ichi Fukuda[5], Kazutaka Ikeda[1]

[1]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Tokyo Dental College, Tokyo, Japan, [3]Gifu University Graduate School of Medicine, Gifu, Japan, [4]Juntendo University School of Medicine, Tokyo, Japan, [5]Tokyo Dental College, Tokyo, Japan

Introduction: The analgesic efficacy of opioids is well known to vary widely among individuals. We sought to construct prediction formulas for individual opioid analgesic requirements based on genetic polymorphisms and clinical data from patients who underwent cosmetic orthognathic surgery and validate the utility of the prediction formulas in patients who underwent major open abdominal surgery.

Methods: We constructed the prediction formulas by multiple linear regression analyses using data from subjects who underwent cosmetic orthognathic surgery. The dependent variable was 24-h postoperative or perioperative fentanyl use, and the independent variables were age, gender, height, weight, pain perception latencies (PPL), and genotype data of five single-nucleotide polymorphisms (SNPs). To examine the utility of the formulas, we performed simple linear regression analyses using subjects who underwent major open abdominal surgery.

Results: Multiple linear regression analyses showed that the four SNPs, PPL, and weight were retained as independent predictors of 24-h postoperative fentanyl use ($R^2 = 0.145$, $P = 5.66 \times 10^{-10}$) and the two SNPs and weight were retained as independent predictors of perioperative fentanyl use ($R^2 = 0.185$, $P = 1.99 \times 10^{-15}$). Simple linear regression analyses showed that the predicted values were retained as an independent predictor of actual 24-h postoperative analgesic use ($R^2 = 0.033$, $P = 0.030$) and perioperative analgesic use ($R^2 = 0.100$, $P = 1.09 \times 10^{-4}$), respectively.

Conclusions: We constructed prediction formulas, and found the possible utility of these formulas in another type of surgery (1).

Reference: (1) Yoshida K *et al.* (2015). PLoS ONE 10: e0116885.

HT019

Intermittent Versus Sustained Morphine Treatment Regimens on Molecular and Behavioral Markers of Withdrawal

Chris Evans[1], Anna Taylor[1], Kevin Lee[1], Samuel Bridges[1], Catherine Cahill[2]

[1]UCLA, California, USA, [2]UCI, California, USA

Opioid dependence has been linked to profound changes in brain circuitry that lead to a negative hedonic state. We have previously shown that chronic, intermittent morphine treatment leads to microglial activation in the Ventral Tegmental Area (VTA), and blocking microglial activation in this region restores cocaine and opioid reward behaviors in opioid dependent animals. We hypothesized that the repeated withdrawal associated with the intermittent morphine treatment exacerbates neuroinflammation and negative affective states associated with dependence. To test this hypothesis, male C57Bl/6 mice were treated with either subcutaneous morphine pellet (25mg) or an intermittent ascending morphine regimen (10-40mg/kg, i.p.) for four days. A tail flick assay confirmed that morphine injection and pellet develop equivalent tolerance to morphine, suggesting each paradigm administers comparable amounts of drug. Twelve hours after the last intermittent morphine injection, molecular and behavioral assays of neuroinflammation and dependence were assessed and compared between groups. Microglia activation was measured by IBA1 immunostaining in the VTA. Reward behavior was assessed in a balanced two-chamber conditioned place preference (CPP) apparatus, using cocaine (10mg/kg) as the reward stimulus. We have previously shown that intermittent morphine injection impairs cocaine preference using this paradigm. Here, we demonstrate that intermittent morphine injection, but not morphine pellet, significantly increases microglial cell body size in the VTA and impairs cocaine place preference. This result is suggestive that the cellular and behavioral adaptations in reward circuitry following chronic morphine exposure are in part attributable to the repeated periods of withdrawal, rather than direct effects of the drug itself.

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HT020

Nitroglycerin-induced migraine-like pain and trigeminal neuronal hyperactivity is enhanced by reduced CB1 receptor activity

Chihiro Nozaki, Astrid Markert, Andreas Zimmer

Institute of Molecular Psychiatry, University of Bonn, Bonn, NRW, Germany

Migraine is a chronic neurological disorders characterized by recurrent episodes of headache which affects more than 10% of the population worldwide. Clinical evidence suggests that a dysregulated endocannabinoid signaling may contribute to the pathophysiology of migraine. Thus, patients suffering from migraine showed alterations in the activity of the arachidonoylethanolamide (AEA) degrading enzyme fatty acid amide hydrolase (FAAH) and a specific AEA membrane transporter, alongside with changes in AEA levels. However, the precise role of the endocannabinoid system on migraine still remains unclear. We have therefore examined the role of endocannabinoid activity using an animal model. Acute migraine has been induced by systemic injection of NO donor nitroglycerin in mice lacking the cannabinoid receptors CB1 and CB2, or the endocannabinoid degrading enzymes FAAH and monoacylglycerol lipase (MAGL). We found that nitroglycerin-induced mechanical allodynia and neuronal activation of the trigeminal nucleus were completely abolished in FAAH-deficient mice. In contrast, CB1 knockout showed enhanced pain and trigeminal activity. We also confirmed that two structurally different FAAH inhibitors, URB597 and PF3945, blocked nitroglycerin-induced hyperalgesia and the activation of trigeminal neurons in a dose-dependent manner. The analgesic effects of FAAH were completely disrupted with the CB1 antagonist rimonabant or knockout of CB1 receptors, suggesting that CB1 receptor activity is crucial for NTG-induced migraine-like pain and trigeminal activity. These results identify FAAH and CB1 signaling as a target for migraine pharmacotherapy.

HT021

Intra- and Inter-Regional co-Regulation of Opioid Genes: Broken Symmetry in Spinal Circuits

T Yakovleva[1], V Galatenko[2], O Kononenko[1], M Andersson[1], H Watanabe[1], I Mityakina[2], A Tonevitsky[2], DL Adkins[3], G Bakalkin[1]

[1]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [2]Moscow State University, Moscow, Russia, [3]Colleges of Medicine & Health Professions, Medical University of South Carolina, Charleston, South Carolina, USA

Regulation of formation and rewiring of neural circuits by neuropeptides may require coordinated production of these signaling molecules and their receptors. Here, we demonstrated that expression patterns of the opioid genes, the largest neuropeptide family highly correlate within and across functionally and anatomically different areas. The opioid peptide genes compared to their receptor genes are transcribed at much greater absolute levels suggesting formation of "neuropeptide cloud" covering the receptor-expressed circuits. Surprisingly both the expression levels and proportion of opioid receptors are strongly lateralized in the spinal cord; inter-regional co-expression patterns are side-specific; and intra-regional co-expression profiles are differently affected by the left- and right-side unilateral body injury. We propose that the opioid genes are regulated as interconnected components of the same molecular system distributed between distinct anatomical regions. The striking feature of this system is its asymmetric co-expression patterns suggesting the side-specific regulation of selective neural circuits by opioid neurohormones.

HT022

Circuit dynamics of in vivo dynorphin release in the nucleus accumbens shell

Ream Al-Hasani[1], Jenny Wong[2], Jordan McCall[1], Omar Mabrouk[2], Gavin Schmitz[1], Kirsten Porter-Stransky[3], Julio Bernardi[1], Brandon Aragona[3], Robert Kennedy[2], Michael Bruchas[1]

[1]Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri, USA, [2]Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA, [3]Department of Psychology, University of Michigan, Ann Arbor, Michigan, USA

We recently used an optogenetic approach to demonstrate that stimulation of dynorphinergic cells in the ventral nucleus accumbens shell (vNAcSh) elicits robust aversive behavior and photostimulation of dorsal NAcSh dynorphin (dNAcSh) cells induces a place preference and is positively reinforcing. Both of which appear to be dependent on kappa opioid receptor (KOR) activation. To follow these recently published findings, we are investigating how KOR is able to mediate these opposing behaviors in two distinct regions of the NAcSh. We are using an opto-microdialysis approach which combines optogenetics with microdialysis for use in awake, freely moving mice. This system allows quantification of neuropeptide release while directly modulating cell-type specific neuronal firing in the NAcSh. We have identified that the amount of dynorphin and met-enkephalin released during optogenetic stimulation is equal in the dNAc and vNAc. Interestingly, release of leu-enkephalin and dopamine is only detectable following photostimulation in the dNAc release. To further understand the circuitry driving the opposing unique behaviors and distinct neuropeptide release profiles, we are mapping the projections to and from discrete regions with the dyn-reporter mouse (dyn-Cre^{tdTomato}) and using tracing approaches (Rabies, canine adenovirus and cholera-toxin B). Thus far we have identified projections from the lateral septum, dorsal and ventral tegmental area (VTA) in addition to a unique GABAergic projection from the VTA to the vNACSh that drives a preference behavior. Together these experiments will help us understand how these distinct populations of dynorphin neurons in the NAcSh are engaged, altered, and recruited in stress and reward-related behaviors.

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MONDAY 11 JULY POSTER SESSION A ABSTRACTS

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M1.01

MOR Agonist/DOR Antagonist Peptidomimetics that Display Reduced Development of Tolerance and Dependence

J Anand, N Griggs, K Kochan, J Traynor, E Jutkiewicz, H Mosberg

University of Michigan, Michigan, USA

Mu-opioid receptor (MOR) agonists are currently used for pain management, however, their use is limited by the development of tolerance and dependence. The co-administration of a MOR agonist with a delta opioid receptor (DOR) antagonist produces antinociception with reduced side effects, but administering drug cocktails is potentially complicated by the diverse pharmacokinetics. We have developed a small molecule peptidomimetic scaffold that displays MOR agonist/DOR antagonist profile in vitro and produces antinociception in the mouse 50°C warm water tail withdrawal (WWTW) assay in male C57BL6/N mice after peripheral administration. Three of these peptidomimetics, AAH8, AMB46, and AMB47, were evaluated for the development of tolerance to antinociceptive effects and physical dependence after five days of twice daily treatment with escalating doses of drug; morphine was chosen as a comparator as it has a similar duration of action in the mouse WWTW assay. Chronic administration of morphine and AMB46 produce significant rightward shifts in the antinociceptive dose response curves, while dose effect curves of AAH8 and AMB47 are unaltered following chronic administration. Naltrexone precipitates fewer signs of withdrawal (e.g. jumps) in mice treated chronically with AAH8 or AMB47 than those treated with morphine or AMB46. Balanced affinity for MOR and DOR appears to predict reduced development of tolerance and dependence, while compounds that have higher affinity for MOR over DOR produce greater tolerance. In conclusion, these peptidomimetics may be better alternatives to traditional opioid analgesics for chronic pain management, producing antinociception without development of tolerance and dependence.

M1.02

Conformationally restricted κ-opioid receptor agonists: Synthesis and pharmacological evaluation of diastereoisomeric and enantiomeric decahydroquinoxalines

A Wegert[1], P Molenveld[1], R Bouzanne des Mazery[1], G Sterk[1], R Storcken[1], R Autar[1], B van Oss[1], R van der Haas[1], R Fröhlich[2], D Schepmann[2], B Wünsch[2], M Soeberdt[3]

[1]Mercachem, Nijmegen, The Netherlands, [2]Westfälische Wilhelms-Universität Münster, Münster, Germany, [3]Dr August Wolff GmbH & Co. KG Arzneimittel, Bielefeld, Germany

Opioid receptor agonists activating especially μ receptors are clinically used for their analgesic efficacy but are associated with adverse effects. Compared with μ agonists, κ agonists show a different side effect profile with minimal respiratory depression, negligible inhibition of gastrointestinal motility and reduced physical dependence.[1] Recently, racemic trans/trans decahydroquinoxaline 4,[2] a conformationally restricted analog of the well-known κ-opioid agonists U-50,488 and GR-89,696, was reported as a potent and selective κ-opioid receptor agonist. The objective of our study was the preparation of all diastereoisomers and enantiomers of decahydroquinoxaline 4, and identification of the stereoisomer with the highest κ-opioid receptor affinity and agonistic activity.

References

1 (i) Stein, C.; Machelska, H. *Pharm. Rev.* 2011, 63, 860; (ii) Trescot, A. M.; Datta, S.; Lee, M.; Hansen, H. *Pain Physician* 2008, Opioid Special Issue 11, S133.

2 (i) Bourgeois, C.; Werfel, E.; Galla, F.; Lehmkuhl, K.; Torres-Gómez, H.; Schepmann, D.; Kögel, B.; Christoph, T.; Straßburger, W.; Englberger, W.; Soeberdt, M.; Hüwel, S.; Galla, H.-J.; Wünsch, B. J. *Med. Chem.* 2014, 57, 6845; (ii) Bourgeois, C.; Werfel, E.; Schepmann, D.; Wünsch, B. *Bioorg. Med. Chem.* 2014, 22, 3316

M1.03

Design and Synthesis of Opioid-Fluorophore Conjugates for Bioimaging

R Lam[1], B Kellam[2], B Graham[1], PJ Scammells[1]

[1]Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia, [2]School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, Nottinghamshire, UK

Bioimaging using fluorescent probes has become an important tool in the study of G protein-coupled receptors, allowing for real-time tracking of receptor translocation and recycling. Fluorescent probes have been previously used to characterize other receptor systems, such as the adrenergic and purinergic systems. Small molecule fluorescent probes for the opioid receptors have been previously described, and we wish to continue the development of these probes using clinically relevant ligands and alternative fluorescent systems.

We have synthesized fluorescent opiate agonists that may be used to study the opioid receptors. Morphine was used as the initial targeting ligand, and was synthetically modified to allow for conjugation to the desired fluorophore. Two linking methods were selected and full morphine-fluorophore conjugates synthesized, consisting of the targeting ligand morphine, an appropriately sized linker, and Cy5 as the fluorophore.

In addition to this, we have created small molecule targeted nanoparticles, also to be used as bioimaging agents. Nanoparticles have gained interest as an alternative to organic fluorophores, as single particles may be visualized, giving higher resolution data. For this work, gold nanorods were selected. We have created protocols that allow for functionalization of these nanoparticles using a discrete

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monomeric biocompatible surface coating in addition to a morphine congener. Preliminary data suggests successful coating of these particles, and their maintained ability to activate the μ-opioid receptor.

This work will assist in elucidating the underlying mechanisms behind opioid tolerance and dependence, allowing for the design of the next generation of analgesics lacking these side effects.

M1.04

14-O-Methylmorphine: A Novel Selective Mu-Opioid Receptor Agonist With High Efficacy And Affinity

F Zádor[1], E Lackó[2], K Király[2], M Balogh[2], A Váradi[3], E Szűcs[1], ZS Zádori[2], S Hosztafi[3], P Riba[2], S Fürst[2], S Benyhe[1], M Al-Khrasani[2]

[1]Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Csongrád, Hungary, [2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary, [3]Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary

Previous studies have shown that the introduction of 14-O-methyl (14-O-Me) group in morphine-6-O-sulfate (M6SU) results in significant improvement of binding properties for mu opioid receptor (MOR) and analgesic potency [1]. In the present work we determine the pharmacological properties of the new compound, 14-O-methylmorphine (14-O-MeM) in in vitro and in vivo assays.

The potency and efficacy of 14-O-MeM on opioid receptors were examined in [35S]GTPgammaS binding, isolated mouse vas deferens (MVD) and rat vas deferens (RVD) assays as previously described [1]. The affinity of 14-O-MeM for opioid receptors was assessed by radioligand binding assay [1]. Finally, we also determined the analgesic effect of the test compound in rat tail flick tests (RTF) as described earlier [1].

14-O-MeM together with 14-O-MeM6SU showed much higher efficacy and potency than morphine or M6SU in MVD, RVD or [35S] GTPgammaS binding. In addition, 14-O-MeM similar to 14-O-MeM6SU and in contrast to morphine or M6SU displayed higher affinity for MOR. However, the selectivity for MOR (delta/mu) dramatically decreased and improved for 14-O-MeM6SU and 14-O-MeM, respectively. In RTF test 14-O-MeM produced stronger analgesia than morphine or M6SU.
Our present data indicates that 14-O-MeM has a high efficacy and affinity for MOR and also reflects that small but proper modification of the morphine molecule results in a novel analgesic agent of potential clinical value.

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References:
[1] Lacko E *et al.* (2012). *Curr Med Chem* 19: 4699-707.

M1.05

Polyglycerol-morphine conjugates for the treatment of inflammatory pain

Sara González-Rodríguez[1], Shilpi Gupta[2], Xuejiao Zhang[2], Karolina Walker[2], Antonio Rodriguez-Gaztelumendi[1], Halina Machelska[1], Rainer Haag[2], Christoph Stein[1]

[1]Freie Universität - Charité - Anesthesiology, Berlin, Germany, [2]Freie Universität - Chemistry, Berlin, Germany

Inflammation, accompanied by tissue acidosis, is an essential component of painful syndromes, including arthritis, cancer and surgery. For treatment, we target opioid receptors in injured tissue to block the excitation of peripheral sensory neurons. Thereby, the main source of pain generation is eliminated without central or intestinal side effects. We are developing conjugates that selectively release opioids in injured acidic tissue and prevent blood-brain barrier permeation due to high molecular weight and hydrophilicity. Here, we examine nanoparticulate pH-sensitive morphine polyglycerol (PG-M) conjugates in comparison to conventional morphine hydrochloride (M-HCl). We hypothesized that morphine is released from PG-M selectively at pH values below 7 in a rat model of unilateral hindpaw inflammation. Following intravenous (i.v.) or intraplantar (i.pl.) injection of PG-M, an analgesic effect (as measured by elevated paw pressure thresholds; PPT) was only detected in inflamed paws and was completely abolished by i.pl. injection of the quaternary opioid antagonist naloxone methiodide (NLXM). The highest dose of i.pl. M-HCl also increased PPT in contralateral noninflamed paws indicating central effects. I.v. M-HCl increased PPT dose-dependently in both hindpaws and was only partially reversible by i.pl. NLXM, suggesting the involvement of central opioid receptors. M-HCl produced sedation and constipation at higher doses, while such effects were not detected after PG-M administration. Microdialysis experiments revealed the presence of free morphine in both hindpaws following i.v. M-HCl, but only in inflamed paws after i.v. PG-M. Thus, PG-M may serve as prototype of a peripherally restricted opioid analgesic designed to avoid central and intestinal side effects.

M1.06

Development of Novel Compounds for 6TM- and 7TM- μ-opioid receptor isoforms

A Samoshkin[1,2], M Convertino[3], A Muralidharan[1,4], P Gris[1], R Klares III[1], J.S. Mogil[1,4], W Maixner[5], N.V. Dokholyan3, L Diatchenko[1,2]

[1]Alan Edwards Centre for Research on Pain, McGill University, Montreal, QC, Canada, [2]Department of Anesthesia, McGill University, Montreal, QC, Canada, [3]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [4]Department of Psychology, McGill University, Montreal, QC, Canada, [5]Center for Translational Pain Medicine, Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA

The μ-opioid receptor (MOR) is the primary target for opioid analgesics. Higher expression of truncated 6-transmembrane (TM) isoform of MOR has been associated with high pain sensitivity and poor response to morphine in humans (1), and is involved in opioid-

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induced hyperalgesia in mice (2). Therefore, we aim to develop effective 6TM- and 7TM-MOR selective drugs for pain management to ensure high analgesic efficacy and low risk of treatment-limiting adverse effects. We generated the structural models of MORs by applying minor changes to the crystallographic coordinates of the 7TM-MOR chimera, as available from the Protein Data Bank (PDB ID: 4DKL; 3). Models were used for virtual screening to identify lead compounds with high activity and selectivity towards the MOR-isoforms. Three-dimensional coordinates of molecules from the publicly available virtual library ZINC (4) were built and refined using LigPrep module in Maestro (Schroedinger) and docking calculations were performed with MedusaDock (5). From ~11 million ZINC-entries, we ultimately retrieved 30 compounds exhibiting putative high affinity for MOR-isoforms. In vitro screening of computationally retrieved MOR compounds by measuring NO production and cAMP accumulation revealed four potentially active compounds on 6TM or 7TM-MOR transfected cells. Pre-selected compounds were further evaluated in vivo in the mouse thermal nociception test (tail-flick assay).

(1) Shabalina *et al.* (2009). *Hum Mol Genet* 18:1037-51.
(2) Samoshkin *et al.* (2015) *Sci Rep* 5:18198.
(3) Manglik *et al.* (2012) *Nature* 485:321-6.
(4) Irwin *et al.* (2012) *J Chem Inf Model* 52:1757-68.
(5) Ding *et al.* (2010) *J Chem Inf Model* 50:1623-32.

M1.07

Developing a Better Opioid Analgesic: Mixed Efficacy Mu Opioid Receptor (MOPr) Agonist/Delta Opioid Receptor (DOPr) Antagonists to Combat Opioid Tolerance and Dependence

N Griggs, J Anand, A Harland, H Mosberg, J Traynor
University of Michigan, Ann Arbor, MI, USA

Drug discovery and development of opioid ligands has largely favored highly selective agonists/antagonists for a single opioid receptor (OPr); opioid analgesics, such as morphine, primarily activate mu opioid receptors (MOPr). However, evidence suggests that modulation of other opioid receptors may be beneficial in MOPr-mediated analgesia. In particular, simultaneous activation of MOPr with inhibition of the delta opioid receptor (DOPr) has been reported to reduce the development of morphine tolerance and dependence. Thus one strategy is to design single compounds that target both mu and delta opioid receptors (activate MOPr and inhibit DOPr). Here we report the synthesis of small molecule peptidomimetics, which maintain key elements of opioid peptides vital for activity but have features to provide for bioavailability, blood brain barrier permeability and longer duration of action. Compounds were characterized by radioligand binding to provide affinity (Ki) values and for potency and relative efficacy (EC50, % stimulation) using the degree of binding of $GTP_{\gamma}35S$ to G proteins in membranes from cells expressing MOPr or DOPr. Compounds were evaluated for antinociceptive activity using the mouse warm water tail withdrawal assay, and development of tolerance and dependence was measured after a 5-day escalating drug treatment. Lead compounds displayed maximal antinociceptive activity with a long duration of action and also with a marked reduction in tolerance and dependence. This research provides a strong foundation for further exploration of mixed efficacy MOPr-DOPr compounds as potential analgesics for the treatment of moderate to severe pain. Funded by DA-03910 and NIGMS-GM007767.

M1.08

***In vitro* pharmacological characterization and comparison of NOP receptor non peptide agonists**

F Ferrari[1], D Malfacini[1], C Trapella[1], R Guerrini[1], N Zaveri[2], G Calò[1]
1University of Ferrara, Ferrara, Italy, 2Astraea Therapeutics, California, USA

Nociceptin/orphanin FQ (N/OFQ) modules several biological functions via the activation of the N/OFQ peptide (NOP) receptor. The aim of this study was the in vitro pharmacological characterization of the non peptide NOP agonists MCOPPB, Ro 65-6570, Ro 2q, SCH-221510, AT-202 and AT-403. The compounds were assayed in calcium mobilization studies performed in cells stably co-expressing NOP or classical opioid receptors and chimeric G proteins, in BRET studies for investigating NOP/G protein and NOP/beta arrestin2 interaction, and in the electrically stimulated mouse vas deferens. In the calcium assay, all compounds mimicked the stimulatory effect of N/OFQ with the following rank order of potency MCOPPB>AT-403>Ro 65-6570=Ro 2q>SCH-221510>AT-202, and moderate to high NOP selectivity over opioid receptors. The NOP antagonist SB-612111 displayed similar potency against N/OFQ and the non peptide agonists. In the BRET assay, the compounds mimicked the stimulatory effects of N/OFQ in both interactions showing the same rank order of potency as in the calcium mobilization assay and a moderate bias toward G protein with the exception of AT-403 that displayed no bias. In the mouse vas deferens all compounds mimicked the effect of N/OFQ showing a very slow kinetic of action. In tissues taken from NOP(-/-) animals, N/OFQ was inactive while non peptide agonists were still active even if with lower potency. The present results suggest that MCOPPB and AT-403 are the best pharmacological tools to be used in future in vitro and in vivo studies aimed to investigate the therapeutic potential of selective NOP agonists.

M1.09

PPL-101 And PPL-103 high affinity kappa partial agonists are potent analgesics with low abuse potential but without dysphoria

Lawrence Toll[1], Andrea Cippitelli[1], Nicholas Toll[1], Taline Khroyan[2], John Lawson[3]
[1]Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA, [2]SRI International, Menlo Park, CA, USA, [3]Phoenix PharmaLabs, Logan, UT, USA

PPL-101 and PPL-103 are opiate derivatives that bind with high affinity to mu, kappa and delta receptors. The a-methyl constituent produces two diastereomers and constrains the cyclopropylmethyl moiety into an R or S orientation, with the R orientation having higher affinity for opiate receptors. [35S]GTPgS binding studies have indicated that these compounds have partial agonist activity at

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kappa receptors, with lower efficacy at delta and very low efficacy (10-20%) at mu receptors. Both compounds are potent analgesic with an ED50 in tail flick 3-10 times more potent than morphine. Despite kappa agonist activity, PPL-101 and PPL-103 induce no dysphoria and in fact, display a strong trend towards CPP in mice. In contrast, in rats neither compound was self-administered. PPL-101 also blocked morphine self-administration at 3 mg/kg. To determine whether PPL-101 was not self-administered because kappa agonist activity blocked mu reward or because mu efficacy was too low to mediate the reward, self-administration studies were carried out in the presence of JDTic to block kappa receptor activity. A single dose of JDTic (10 mg/kg) neither influenced morphine self-administration nor induced self-administration of PPL-101, suggesting the mu efficacy is too low to induce reward, and probably the kappa agonist activity is too low to be aversive. Previous studies demonstrated that PPL-101 and PPL-103 substitute for morphine and thereby blocked withdrawal symptoms in addicted monkeys. These results demonstrate that these kappa opiates have unusual profiles that could be ideal as an analgesic with low abuse potential or potentially as a drug abuse medication.

M1.10

In vitro and ex vivo investigations of opioid-ORL-1 hybrid peptide ligands

AI Erdei[1], E Szucs[1], A Borbély[2], A Magyar[2], M Al-Khrasani[3], S Benyhe[1]

[1]Biological Research Centre, Hungarian Academy of Science, Institute of Biochemistry, Szeged, Hungary, [2]MTA-ELTE, Research Group of Peptide Chemistry, Budapest, Hungary, [3]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Opioid receptor subtypes (MOR, DOR and KOR) and nociceptin receptor (ORL-1) are G protein-coupled receptors (GPCRs). In animal pain models, activation of opioid receptors results in analgesia, whereas the activation of nociceptors produces analgesia or hyperalgesia.

In addition, opioid receptors have been reported to constitute dimeric/oligomeric complexes. These findings encourage us to design ligands targeting these homo- or heterodimers.

The present study aimed to synthesize nociceptin-opioid hybrid ligands and assess their pharmacological properties compared to that of highly selective MOR, DOR, KOR or ORL-1 ligands in biochemical and isolated mouse vas deferens (MVD), hosting opioid receptor subtypes and ORL-1.

The novel hybrid peptides, H-YGGFGGGRYYRIK-NH2 (BA55), H-YGGFRYYRIK-NH2 (BA62) and Ac-RYYRIKGGGYGGFL-COOH (BA61), contain YGGF residues and the sequence of Ac-RYYRIK-NH2, which displayed nociceptin antagonist effect in [35S]GTP$_\gamma$S assays (Berger *et al.* 1999). In [35S]GTP$_\gamma$S assay, BA55 and BA62 showed equipotent agonist potency (IC50), 368 nM and 369 nM, respectively.

In binding experiments, BA55 and BA62 displayed a higher affinity for the KOR, than MOR, DOR or ORL-1. BA61 showed affinity for DOR (1300 nM) and agonist activity in G-protein activation assay. In MVD bioassay, the electrically evoked muscle contractions were inhibited by BA61 in a concentration-dependent manner. This inhibition was DOR mediated, and justified by DOR selective antagonist, naltrindole. Taken together, these novel hybrid peptides bind to and activate multiple opioid receptors, however, their selectivity profiles need to be studied further.

M1.11

Studies With Peptide Bivalent Mu/NOP Ligands In A Mu/NOP Double Expression System

Mark Bird[1], Remo Guerrini[2], David Rowbotham[1], Girolamo Calo[2], David Lambert[1]

1University of Leicester, Leicestershire, UK, 2University of Ferrara, Ferrara, Italy

Targeting multiple opioid receptors produces analgesia with reduced side-effect profile[1]. We have used a Mu/NOP double expression system to explore the bivalent peptides [dermorphin]-[N/OFQ](DeNo) and [dermorphin]-[UFP-101](DeUFP).

We have used Human Embryo Kidney (HEK) cells expressing Mu (HEKhMu) or NOP (HEKhNOP) receptors, or a Mu/NOP co-expression system (HEKhMu/NOP) and measured functional potency/efficacy using GTP$_\gamma$[35S] binding assays [2]. All compounds were dissolved in water (1pM-10μM). Data are mean ±SEM.

In GTP$_\gamma$[^{35}S] assays, Dermorphin (pEC_{50}:$8.13_{(0.06)}$; E_{max}:$1.43_{(0.03)}$) DeNo (pEC_{50}:$8.25_{(0.11)}$; E_{max}:$1.53_{(0.03)}$) and DeUFP (pEC_{50}:$8.26_{(0.11)}$; E_{max}:$1.44_{(0.06)}$) showed activity at Mu, while N/OFQ and UFP101 did not. At HEK_{hNOP}, N/OFQ (pEC_{50}:$8.69_{(0.13)}$; E_{max}:$1.47_{(0.04)}$) and DeNo (pEC_{50}:$9.24_{(0.19)}$; E_{max}:$1.47_{(0.05)}$) are full agonists. The remaining ligands showed no activity at NOP. At $HEK_{hMu/NOP}$, Dermorphin (pEC_{50}:$7.60_{(0.07)}$; Emax:$1.31_{(0.04)}$) and DeNo (pEC_{50}:$7.63(^{0.22})$; Emax:$1.26_{(0.05)}$) lost potency in the co-expression system, while N/OFQ (pEC_{50}:$9.23_{(0.16)}$; Emax:$1.25_{(0.05)}$), and DeUFP (pEC_{50}:$8.67_{(0.15)}$; Emax:$1.34_{(0.04)}$) were unchanged.

We demonstrate reduced potency at Mu for dermorphin and DeNo when NOP is co-expressed, while N/OFQ potency remains unchanged. Interestingly, DeUFP has a similar potency to that seen in a Mu single expression system, implying the binding of a NOP antagonist [3]in a Mu/NOP expression system leads to changes that do not reduce Mu signaling. These results suggest that differential functional pairings of Mu/NOP ligands may widen clinical utility.

1. Bird, M.F. and D.G. Lambert. *Curr Opin Support Palliat Care*, 2015. 9(2): p. 98-102.
2. Dietis, N., *et al. British Journal of Anaesthesia*, 2012. 108(2): p. 262-270.
3. Calo, G., *et al. CNS Drug Reviews*, 2005. 11(2): p. 97-112.

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M1.12

Improved Analgesics: BU08028 a Novel, Bifunctional NOP/MOP Ligand

G Cami-Kobeci[1], M-Ch Ko[2], L Toll[3], SM Husbands[1]

[1]Department of Pharmacy and Pharmacology, University of Bath, Bath/BANES, UK, [2]Wake Forest University, Winston-Salem/North Carolina, USA, [3]Torrey Pines Institute for Molecular Studies, Port St. Lucie/Florida, USA, [4]Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

When a NOP (nociceptin receptor) agonist is co-administered with a mu opioid (MOP) receptor agonist, the combination produces a synergistic effect, resulting in enhanced analgesia (1), suggesting the possibility of strong analgesia with only low efficacy partial agonism at both receptors. Whilst buprenorphine has been reported to display both MOP and NOP partial agonism in rodents, in primates it lacks NOP receptor involvement (2) and so an improved analgesic might have a buprenorphine-like profile but with enhanced NOP activity.

We have discovered BU08028, and other compounds, having binding affinity at MOP receptors similar to that of buprenorphine and, as desired, higher affinity and considerably higher efficacy than buprenorphine at NOP receptors (3). In measures of hyperalgesia, both in rodents (rats) and non-human primates (rhesus monkey) BU08028 was a potent, long-acting anti-hyperalgesic with both MOP and NOP components to its activity. More importantly, BU08028 at antinociceptive doses did not induce itch scratching, did not compromise physiological functions including respiration and cardiovascular activities and did not produce reinforcing effects. Therefore BU08028 represents a potential analgesic agent, with low side effect profile.

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(1) Ko, M. Ch, Husbands, S. M.; *ACS Symposium Series* 1131, 2013. (2) Cremeans, C. *et al. J. Pharmacol*. Exp. Ther., 2012, 343, 72-81. (3) a) Cami-Kobeci, *et al.*; *J. Med. Chem.*, 2011, 54, 6531-6537. b) Khroyan, T. V. *et al.J. Pharmacol*. Exp. Ther., 2011, 336, 952-961.

M1.13

New Biphenylmethylenepiperidine Derivatives As Dual Opioid-NPFF Ligands: In Vitro And In Vivo Evaluation

M Mottinelli[1], CR McCurdy[1], VB Journigan[1], C Mesangeau[1], SJ Cutler[1], SO Eans[2], JM Medina[2], JP McLaughlin[2]

[1]University of Mississippi, Oxford, Mississippi, USA, [2]University of Florida, Gainsville, Florida, USA

Introduction: NPFF receptor antagonists may prevent opioid-induced hyperalgesia (OIH) (1) and tolerance (2). Hence, compounds with both opioid agonist and NPFF antagonist features may induce analgesia with reduced tolerance.
Methods: To obtain selective high affinity small molecules at both the opioid and the NPFF receptors we chose to synthesise a series of biphenylmethylenepiperidines. Compounds were evaluated for affinities using a radioligand competition binding assay and cAMP or $GTP_{\gamma}S$ based functional assays. One selected compound was studied in vivo to determine its effects on analgesia and tolerance development using the mouse warm-water tail-withdrawal test following intracerebroventricular (icv) administration.

Results: We synthesised and evaluated a series of compounds for receptor binding and functional activity. We were then able to draw structure-activity relationship (SAR) conclusions leading to compounds with affinities for opioid and NPFF receptors, in the nM and μM range, respectively. Selected compound produced potent analgesia by icv administration that lasted at least 50min with an ED50 (95% CI) of 6.9(4.7-9.5) nmol and was antagonised by the MOP antagonist β-FNA. Moreover, although repeat treatment with morphine shifted the ED50 value 9.6-fold rightward, demonstrating significant acute antinociceptive tolerance, the 1.6-fold rightward shift produced by the test compound was not significantly different.

Conclusions: Our SAR investigations yielded compounds with affinities at the opioid and NPFF receptors in the nM and μM range, respectively. In addition, selected compound demonstrated in vivo full antinociception and absence of acute antinociceptive tolerance. Hence, our results support the idea of dual opioid-NPFF ligands as promising analgesics with fewer liabilities.

M1.14

Consumption of Milk Caseins Beyond the Normal Age of Weaning Increases Depressive-Like Behaviour and Induces Brain Region Specific Alterations to Opioid Receptors: Implications of the Gut-Brain Axis

A Osman, A Bailey, J Swann, I Kitchen, G Walton

University of Surrey, Guildford, UK

Nutrition during the postnatal period can influence brain development and play an important role in the development of neuropsychiatric disorders such as depression. In this study we show that in rats, exposure to milk caseins beyond the normal age of weaning results in depressive-like behaviour, which is accompanied by changes in the gut bacteria and host-metabolism. Depressive like behaviour was studied using the forced swim test (FST) on postnatal day (PND) 25 in rats provided with, no milk (controls), casein free (CF) or casein rich (CR) milk from PND 21. CR treated animals displayed a depressive phenotype as indicated by increased immobility times in the FST. Autoradiographic binding of μ opioid receptors (MOPr) showed a significant down-regulation of MOPr in a number of brain regions including the amygdala in CR animals compared to CF, and in the deep layer of somatosensory cortex of CR compared to CF and controls. Furthermore, autoradiographic binding of δ opioid receptors (DOPr) showed a significant decrease in DOPr density in the deep layer of the somatosensory cortex of CF animals vs. CR and controls. Metabonomic analysis of urine from CR and CF groups revealed 6 differently expressed metabolites some of which were gut microbial metabolites. Fluorescence *in situ* hybridization showed CR animals harbor significantly more *Clostridium histolyticum* bacterial groups in the caecum and colon compared to CF animals. These results show first evidence that a casein rich diet in early postnatal life influences development of the opioid system and subsequent behaviour via a potential gut-brain axis mediated mechanism.

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M1.15

Delta Opioid Receptor as a Target for Migraine - CGRP Co-expression and Inhibition of Medication Overuse Headache

AF Tipton, L Segura, AA Pradhan

University of Illinois at Chicago, Chicago, IL, USA

Migraine is an extraordinarily common brain disorder for which therapeutic options continue to be limited. We have previously demonstrated that in preclinical animal models, delta opioid receptor agonists may be promising targets for the treatment of migraine. Delta agonists effectively inhibit cortical spreading depression, as well as nitroglycerin-induced hyperalgesia and negative affect. A better understanding of how delta opioid receptor modulates migraine mechanisms would encourage future development of this target. The neuropeptide, calcitonin gene related peptide (CGRP) plays a pivotal role in the induction and maintenance of migraine, primarily through the peripheral afferents projecting from the trigeminal ganglia. The aim of this study was to characterize the expression of delta opioid receptor in trigeminal ganglia, and on CGRP-expressing neurons specifically. To visualize the delta opioid receptor, we used knockin mice in which the endogenous receptor was replaced by a fluorescent tagged delta opioid receptor (DOR-eGFP). We observed a significant population of trigeminal ganglia which co-expressed CGRP with DOR-eGFP. This data suggests that delta agonists may produce their anti-migraine effects by directly modulating CGRP-expressing ganglia. As a further goal of this study we also tested delta agonists in a model of sumatriptan-induced medication overuse headache (MOH). In this case, C57BL6 mice were treated chronically with sumatriptan for 11 days, which produced severe mechanical hypersensitivity. The delta agonist, SNC80, inhibited this hyperalgesia, and suggests that delta agonist could be an effective strategy for managing MOH. Together, this work provides further evidence that delta opioid receptors are promising targets for migraine treatment.

M1.16

Opioid and cannabinoid signalling systems are impaired in different brain regions of a rat model of schizophrenia

Edina Szücs[1], Csaba Tömböly[1], Szabolcs Dvorácskó[1], Alexandra Büki[2], Gabriella Kékesi[2], Gyöngyi Horváth[2], Sándor Benyhe[1]

[1]Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary, 2Department of Physiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

Clinical reports suggest that many patients with schizophrenia are less sensitive to pain than other individuals. Numerous studies have implicated both the endocannabinoid and opioid system in the pathophysiology of schizophrenia. Animal models do not interpret schizophrenia completely, but they can model a number of symptoms of the disease, including decreased pain sensitivities and increased pain thresholds. We investigated changes in the signalling properties of the mu-opioid (MOP) receptor and cannabinoid receptors in different brain regions, some of them are involved in the pain transmission, i.e. thalamus, olfactory bulb, prefrontal cortex, hippocampus and cerebellum. Our goal was to compare the transmembrane signalling mediated by MOP and cannabinoid receptors in control rats and in a recently developed rat model of schizophrenia. Regulatory G-protein activation via MOP receptors were measured in [^{35}S]GTPgS binding assays in the presence of a highly selective MOP receptor peptide agonist, DAMGO. It was found that the MOP receptor mediated activation of G-proteins was substantially lower in membranes prepared from the 'schizophrenic' model rats than in control animals. The potency of DAMGO to activate MOP receptor was also decreased in all brain regions studied. Cannabinoid signalling was triggered by the non-selective agonist ligand WIN-55,212. The highest stimulation was found in the cerebellar membranes, moreover an extremely significant decrease in G-protein activation was observed in the cerebellum of the model animals. Taken together in our rat model of schizophrenia, MOP receptor and cannabinoid receptor mediated G-proteins have a reduced stimulatory activity compared to membrane preparations taken from control animals.

M1.17

The effects of the synthetic cannabinoid AB-FUBINACA on body temperature, nociceptive threshold, locomotor parameters and anxiety in mice

Chaim G. Pick[1], Miaad Bader[1], Vardit Rubovitch[1], Yiffat Katz[2], Etia Cohen[2], Ehud Wolf[2], Shaul Schreiber[3]

[1]Department of Anatomy and Anthropology, Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel, [2]Analytical Laboratory, Division of Identification and Forensic Science, Israel Police National HQ, Jerusalem, Israel, [3]Department of Psychiatry, Tel-Aviv Sourasky Medical Center & Tel-Aviv University Sackler Faculty of Medicine, Tel Aviv, Israel

Synthetic cannabinoids are designer drugs which attempt to mimic the effects of natural cannabinoids. Abuse of synthetic cannabinoids began in the early 2000s. Increase in recreational use of these compounds has been observed despite the efforts to control their availability. New, unregulated compounds appear once older compounds become controlled by law authorities around the world. It has been previously reported that synthetic cannabinoids are not just other forms of Δ9-tetrahydrocannabinol (Δ9-THC); the active component of cannabis. These compounds have chemical structures unrelated to Δ9-THC, different metabolism, and often greater toxicity. Synthetic cannabinoids are often abused by heroin addicts and methadone maintenance treatment (MMT) patients. AB-FUBINACA, a synthetic cannabinoid, was originally developed as an analgesic medication, but was never pursued for human use. This study investigated the effects of AB-FUBINACA on body temperature, nociceptive threshold, locomotor activity, muscle strength, gate balance and anxiety levels using a battery of behavioral and motor tests among adult ICR mice. AB-FUBINACA lowered the body temperature exclusively at a high dose of 4 mg/kg, decreased locomotor activity, exploratory behavior, muscle strength and gate balance in a range of doses. However, AB-FUBINACA did not affect the nociceptive threshold of the mice. Further studies are needed to assess the effects of AB-FUBINACA on cognition and depression or psychosis like behavior to characterize the overall effects of this synthetic cannabinoid. In addition, future study will investigate the effect of AB-FUBINACA on MMT mice model to assess the influence of this compound on MMT patients.

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M1.18

Transcriptional regulation of endogenous opioid system genes in obesity

Claudio D'Addario[1,3], Mariangela Pucci[1], Maria Vittoria Micioni Di Bonaventura[2], Carlo Cifani[2]

[1]University of Teramo, Teramo, Italy, [2]University of Camerino, Camerino, Italy, [3]Karolinska Institutet, Stockholm, Sweden

Obesity, one of the major global health issues is strongly influenced by our genetic background. Many data provide a role for endogenous opioid genes in regulation of feeding, energy balance and obesity, and, among the different mechanisms leading to inter-individual differences in obesity, epigenetic regulation of gene expression recently emerged as a potentially important contributor [1]. We used the Diet-induced obesity (DIO) rat model to analyze the epigenetic regulation of endogenous opioid system genes between outbred Sprague-Dawley rats placed on a high-fat diet becoming obese (DIO), compared their diet resistant (DR) counterparts, as well as to low-fat chow diet [2]. Gene expression analysis revealed in the hypothalamus (HY) of DIO rats a significant increase of PNOC mRNA when compared to DR and chow-fed group. In the nucleus accumbens (NA) of DIO rats a significant decrease in PNOC counteracted by an increase in PDYN mRNA was observed. Consistently, DNA methylation at PDYN gene promoter resulted to be reduced in DIO rats in the NA. No changes in DNA methylation status at PNOC promoter were observed in both HY and NA. We thus show selective changes in opioid peptides gene expression in DIO rats key brain regions in homeostatic and hedonic eating, confirming a major role for PNOC in body weight gain in the HY. Moreover, in the NA, PDYN epigenetic regulation provides new insight for possible interventions, either through nutrition or specific drugs, to modify obesity risk.

1. Campion *et al.*, (2009) *Obes.Rev.* 10,383-392.
2. Sclafani, (1976) *Physiol.Behav.* 17,461-471.

M1.19

In Vitro and In Vivo Immunosuppressive Effects of the δ-Opioid Receptor Antagonist HS-378 and Mechanisms Thereof

Mariana Spetea[1,2], Jian Li[2], Aquilino Lantero[1], Tashfeen Ahmad[2], Tore Saxne[3], Claire Gaveriaux-Ruff[4], Harald Mittendorfer[1], Johann Hofmann[5], Mahmood Ahmed[2], Andris Kreicbergs[2], Helmut Schmidhammer[1]

1Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria, 2Department of Molecular Medicine and Surgery, Section of Orthopaedics, Karolinska Institutet, Stockholm, Sweden, 3Department of Rheumatology, Lund University Hospital, Lund, Sweden, 4Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université de Strasbourg, Illkirch, France, 5Section of Medical Biochemistry, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

The *in vitro* and *in vivo* immunosuppressive actions of non-peptidic δ-opioid receptor antagonists were long recognized, although the underlying mechanisms remained so far unknown. We have investigated the anti-inflammatory and anti-arthritic effects of the δ opioid antagonist HS-378 (17-cyclopropylmethyl-4,5α-epoxy-14β-ethoxy-5β-methylindolo[2',3':6,7]morphinan-3-ol) in a rat model of adjuvant arthritis. We have initiated the analysis of the mechanistic basis of immunosuppressive and anti-inflammatory effects of HS-378, and herein we report on the molecular target responsible for these specific actions. Intraperitoneal administration of HS-378 (0.1-8 mg/kg/day) for 21 days significantly reduced the degree of chronic inflammation and tissue damage associated with arthritis. *In vitro*, HS-378 displayed marked immunosuppressive effects by inhibiting rat, mouse and human lymphocyte proliferation. Similar to naltrindole, HS-378 inhibited the mixed lymphocyte reaction assayed with cells from wild-type or triple μ/δ/κ opioid receptors knockout mice, indicating that a non-opioid mechanism contributes to the immunosuppressive activity. *In vitro* production interleukin-2 from lymphocytes was down-regulated by HS-378 in a concentration-dependent manner, associating the inhibitory effects to a interleukin-2-dependent mechanism and a molecular target linked to this cytokine activation pathway. We have established that *in vitro* HS-378 produces a direct inhibition of calcineurin activity and calcineurin-mediated cellular dephosphorylation of the cytoplasmic component of the nuclear factor of activated T cell (NFAT). HS-378 was about three-times more potent as calcineurin inhibitor than naltrindole. In summary, HS-378 has significant therapeutic effects in experimental arthritis and evolves as an inhibitor of the calcineurin/NFAT pathway, therefore providing functional evidence on the mechanistic basis of the immunosuppressive action.

M1.20

A free fatty acid receptor 1, GPR40/FFAR1, deficient mice show the changes of emotional behavior

Shogo Tokuyama[1], Fuka Aizawa[1], Akira Hirasawa[2], Takashi Kurihara[3], Akira Miyata[3], Kazuo Nakamoto[1]

[1]Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan, 2Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan, [3]Department of Pharmacology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

A free fatty acid receptor 1 (GPR40/FFAR1) is one of the G protein coupled receptors which are activated by polyunsaturated fatty acids (PUFA) including arachidonic acid or docosahexaenoic acid. Our previous study has demonstrated that activation of brain GPR40/FFAR1 exerts an antinociceptive effect mediated by the modulation of descending pain control system and also produced antidepressant-like effect in forced swim test. Thus, we propose that PUFA-GPR40/FFAR1 signaling in the central nervous system could have a various physiological function. Recently, the decrease of PUFA in the brain is closely related to the episode and pathological mechanism of psychiatric diseases associate with emotional disorder such as depression and schizophrenia. However, the detailed mechanism remains unknown. In this study, we investigated whether the deficiency of GPR40/FFAR1 signaling have negative effect against emotional behavior. Emotional behavior in wild (C57BL/6J) and GPR40 deficient (KO) male mice was evaluated at 9-10 weeks of age and by elevated plus maze test (EPM), open field test, social interaction test and sucrose preference test. The EPM revealed that the KO mice show reduced anxiety-like behavior. Locomotor activity or social interaction behavior is similar between wild and KO mice. In sucrose preference test, the KO mice showed reduced sucrose preference and intake. Thus, these results suggest that brain GPR40/FFAR1 is associated with the regulation of anxiety behavior and sucrose intake in male mice.

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M1.21

Investigation of 7-benzylidenenaltrexone derivatives as a novel structural antitrichomonal lead compound

Yasuaki Koyama[1], Noriki Kutsumura[1], Ryo Nakajima[1], Yasuyuki Nagumo[1], Tsuyoshi Saitoh[1], Yoshiyuki Miyata[2], Hiroshi Nagase[1]

[1]University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Keio University, Shinjuku-ku, Tokyo, Japan

BNTX (7-benzylidenenaltrexone) is an opioid δ receptor selective antagonist. Recently, we reported that BNTX and its derivatives showed the chloroquine (CQ)-resistance reversing activities for CQ-resistant Plasmodium chabaudi.[1] As a result of the detailed studies, it suggested that both of the antagonistic activity for opioid δ receptor and the α,β-unsaturated ketone group in BNTX are likely to be important to the activity for malaria. Based on this information, we tried to apply the BNTX against Trichomonas vaginalis, which is pathogenic protozoa of sexually transmitted infections (STIs). As a result, BNTX and its derivatives also showed antitrichomonal activity with MIC of 20-80 μM.[2] In addition, the development of a more effective synthetic method for the BNTX derivatives was achieved using the Knoevenagel condensation. The details of improved synthetic method of BNTX and the relationship of opioid δ receptor affinities and antitrichomonal activities derivatives will be presented.

Reference

(1) Miyata, Y.; Fujii, H.; Uenohara, Y.; Kobayashi, S.; Takeuchi, T.; Nagase, H. *Bioorg. Med. Chem. Lett*. 2012, 22, 5174-5176; (b) Miyata, Y.; Fujii, H.; Osa, Y.; Kobayashi, S.; Takeuchi, T.; Nagase, H. *Bioorg. Med. Chem. Lett*. 2011, 21, 4710-4712.

(2) Kutsumura, N.; Nakajima, R.; Koyama, Y.; Miyata, Y.; Saitoh, T.; Yamamoto, N.; Iwata, S.; Fujii, H.; Nagase, H. *Bioorg. Med. Chem. Lett.* 2015, 25, 4890-4892.

M1.22

Mu and delta opioid receptor autoradiographic binding in brains of a mouse model of autism

Fani Pantouli[1], Lucie Pellissier[2], Jerome Becker[2], Julie Le Merrer[2], Alexis Bailey[1]

[1]University of Surrey, Guildford, UK, [2]INRA-0085 CNRS-7247 INSERM, Université de Tours Rabelais, Nouzilly, France

Emerging preclinical and clinical evidence support a crucial role for the endogenous opioid system in the neurobiology of autistic-like traits (1). To further investigate the contribution of the endogenous opioid system in autistic-like disorders, we have challenged this system in a well-established animal model of Fragile X syndrome, which includes autistic symptoms (2), the Fmr1 knock-out (KO) mice. We detected significant reduction in morphine-induced hyperlocomotion in the Fmr1 KO mice compared to their wild type (WT) controls. Moreover, under basal conditions Fmr1 KO mice displayed lower nociception thresholds, whereas morphine-induced analgesia was increased suggesting altered mu opioid receptor (MOR) signalling. The present study aimed at identifying potential alterations in MOR and delta opioid receptors (DOR) binding in the brains of Fmr1 KO mice and assessing transcription levels for a collection of genes of interest. Quantitative receptor autoradiography was performed in order to measure binding of MOR and DOR using [3H] DAMGO and [3H] deltorphin respectively, with and without naloxone, in WT and Fmr1 KO male mice (3). Full autoradiographic mapping of MOR and DOR showed no significant difference regarding the density of either of these receptors between genotypes. Ongoing qRT-PCR experiments, however suggest modified transcriptional levels for several genes in the striatum of Fmr1 KOs, including genes coding for opioid peptides and their receptors.

(1) Becker JA *et al.* (2014). *Neuropsychopharmacology*, 39: 2049-60.
(2) Lozano R *et al.* (2014). *Intract & Rare Diseas Res.*, 3: 134-146.
(3) Kitchen I *et al.* (1997). *Brain Research*, 778: 73-88.

M1.23

Intrinsic properties of central amygdala dynorphin neurons

JG McCall, BA Copits, VK Samineni, RW Gereau

Pain Center, Department of Anesthesiology, Washington University in St. Louis, Saint Louis, MO, USA

The central amygdala (CeA) is a critical anatomical substrate for emotional regulation in response to stress, pain, and drugs of abuse. While much is known the identity of cell-types composing the CeA, much less is understood about the unique properties of these molecularly defined neurons. Here we focus on a subset of neurons in the CeA expressing the neuropeptide dynorphine (Dyn+), the endogenous ligand of the kappa opioid receptor. To genetically identify dynorphinergic (Dyn) neurons, we crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse that expresses Cre recombinase under the same promoter as preprodynorphin (Dyn-Cre). In this model, only dynorphinergic cells express tdTomato, allowing complete visualization of dynorphinergic circuitry throughout the brain. These animals enable targeted whole-cell recordings in amygdala slices. We report the intrinsic electrophysiological properties of these neurons including the input resistance, resting membrane potential, and firing profiles. Furthermore, the morphology of these Dyn+ neurons is defined by filling the cells with Neurobiotin. In addition to its local circuitry, the CeA is also a major output nuclei of the amygdala. To determine the long-range connectivity of Dyn+ CeA neurons, we utilize cell-type selective expression of reporter viruses to identify these molecularly-defined projections throughout the brain. Together these data provide a base knowledge for further cell-type selective manipulation and observation in vivo. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates emotional processing in the context of stress, chronic pain, and addiction will provide valuable insight into potential therapeutic targets for these neurological and neuropsychiatric disorders.

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M1.24

Endogenous Opioids Regulate Moment-to-Moment Neuronal Communication and Excitability

E Bagley[1], B Winters[1], G Gregoriou[1], S Kissiwaa[1], O Wells[1], S Hermes[2], N Burford[3], A Alt[3], S Aicher[2]

[1]University of Sydney, NSW, Australia, [2]Oregon Health & Sciences University, OR, USA, [3]Bristol-Myers Squibb, CT, USA

Fear and emotional learning are modulated by endogenous opioids but the cellular basis for this is unknown. The intercalated cells (ITC) gate amygdala output and thus regulate the fear response. Here we find, using patch-clamp electrophysiology in brain slices from Sprague-Dawley rats (1), that endogenous opioids are released by minimal synaptic stimulation to act via two distinct mechanisms within the main ITC cluster. Endogenously released opioids inhibit glutamate release through the delta-opioid receptor (DOR), an effect potentiated by a DOR positive allosteric modulator, BMS-986187 (1μM). Post-synaptically, the opioids activate a potassium conductance through the mu-opioid receptor (MOR), suggesting for the first time that endogenously released opioids directly regulate neuronal excitability. Ultrastructural localization of endogenous ligands, using immuno-electron microscopy for met-enkephalin (2), support these functional findings. This study demonstrates a new role for endogenously released opioids as neuromodulators engaged by basal synaptic activity to regulate moment-to-moment neuronal communication and excitability. These distinct actions through MOR and DOR may underlie the opposing effect of these receptor systems on anxiety and fear.

1. Bagley *et al.*, (2011). *Nat. Neurosci.* 14:1548-54.
2. Hermes, S. M *et al.*, (2014). *J. Comp. Neurol*. 522, 642-653.

M1.25

Sex differences in voluntary drinking by μ-opioid receptor knockout mice following early stress

Yuki Moriya[1,2], Yoshiyuki Kasahara[2,3], F.Scott Hall[4,5], Georgr R Uhl[5], Kazutaka Ikeda[1], Ichiro Sora[2,6]

[1]Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Department of Biological Psychiatry, Tohoku University Graduate School of Medicine, Sendai, Japan, [3]Department of Disaster Psychiatry, International Research Institute of Disaster Science (IRIDeS), Tohoku University, Sendai, Japan, [4]Molecular Neurobiology Branch, Intramural Research Program, National Institute on Drug Abuse, MD, USA, [5]Department of Pharmacology and Experimental Therapeutics, University of Toledo, Toledo, USA, [6]Department of Psychiatry, Kobe University Graduate School of Medicine, Kobe, Japan

Introduction:Early social experience has been consistently shown increase alcohol consumption, perhaps by influencing stress systems. One factor that has not been adequately examined in previous studies is sex, which is a substantial shortcoming in the field given that there are significant influences of sex on alcohol consumption patterns in humans and in animal models. Genetic factors, such as differences in genes for μ-opioid receptor(MOP)systems, also have a substantial influence on alcohol consumption, but only a limited set of such genetic influences on stress-induced alcohol consumption have been examined. This experiment was based on the hypothesis that the effects of chronic stress on alcohol consumption would be influenced by both sex and the functioning of MOP systems.

Methods:This study assessed the effects of isolation-rearing on later alcohol intake using a two-bottle home-cage paradigm in wild-type(WT) and MOP gene knockout(KO) male and female mice.

Results: Isolation-rearing significantly altered ethanol consumption in both male and female MOP-KO mice. Interestingly, the direction of change was opposite in male and female MOP-KO mice: increased ethanol consumption in male mice, and decreased ethanol consumption in female mice. In socially-rearing male mice, MOP-KO reduced ethanol consumption slightly. While isolation-rearing did not affect ethanol consumption in WT male mice, it produced an increase in ethanol consumption in MOP-KO male mice($p<0.01$). In female mice, socially-rearing MOP-KO mice alone consumed more ethanol than isolation-rearing MOP-KO($p<0.01$), socially-rearing WT mice($p<0.05$), or isolation-rearing WT mice($p<0.05$).

Conclusion: The study shows that disturbances of MOP influence the effects of isolation-rearing on ethanol consumption in a sex-dependent manner.

M1.26

Antidepressant-like effects of BU10119, a novel kappa opioid receptor antagonist, in the novelty-induced hypophagia task in mice

Abdulrahman Almatroudi, Christopher Bailey, Stephen Husbands, Sarah Bailey

Dept Pharmacy & Pharmacology, University of Bath, Bath, UK

Antagonists at kappa-opioid receptors have been proposed as novel antidepressants. The standard high-affinity, selective kappa-antagonists have a long lasting duration of action which potentially limits their use (1). We have previously shown that the combination of buprenorphine (1mg/kg) with naltrexone (1mg/kg) produced a functional short-acting kappa-antagonist, that was non-sedating and non-rewarding, with antidepressant-like effects (2). Here, we present preliminary data on a novel compound, BU10119 (3) that combines the properties of buprenorphine/naltrexone.

Adult male CD-1 mice (8-9 weeks) were used. For novelty-induced hypophagia, mice were individually housed and trained for 3 days to consume condensed milk. On test days, mice were injected intraperitoneally (10 ml/kg) with saline, buprenorphine/naltrexone combination (1 mg/kg), fluoxetine (20 mg/kg) or BU10119 (1mg/kg) one hour prior to testing behaviour. One-way ANOVA, revealed that there was a significant effect of drug treatment on the latency to drink in the novel cage ($F_{(4, 45)}=9.15$, $P<0.001$) but not consumption ($F_{(4, 45)}=1.25$, $P=0.3$).

BU10119 is a relatively short acting kappa-antagonist with little efficacy at the mu-opioid receptor. BU10119 has demonstrated activity in the novelty-induced hypophagia test that is consistent with the behavioural effects of fluoxetine and therefore has an antidepressant-like profile.

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1) Carroll and Carlezon 2013. *J Med Chem* 56: 2178-2195
2) Almatroudi *et al.* 2015. *J. Psychopharmacol*. 29: 812-21
3) Cueva *et al.* 2015. *J Med Chem*, 58: 4242-9

M1.27

Can we protect neurons from opioid-induced cell death?

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

Introduction: Chronic use of opioids can cause cognitive dysfunction that is associated with neuronal cell death and inhibited neurogenesis. Recent studies suggest that growth hormone (GH), an endogenous hormone released from the anterior pituitary gland, alleviates drug-induced cognitive impairment.
Aim: The aim of the current study was to assess the acute neurotoxic effects of methadone and the neuroprotective effects of GH in primary cortical neuronal cultures.

Methods: Tissue from the cortex, an area important for cognitive function, was harvested from embryonic day 17 rats. After digestion and mechanical dissociation, cells were seeded into tissue culture plates. At 7 days *in vitro*, the lethal dose 50% (LD50) of methadone was determined. This dose was later used to assess the neuroprotective effects of GH (1 µM – 0.01 nM) for 24 hours. Cell viability was determined using the colometric MTT-assay and cytotoxicity was assessed using the lactate dehydrogenase (LDH) assay.

Results: The calculated LD50 dose of methadone was 60 µM. Cell viability was significantly increased when cells were co-treated with methadone and 0.01, 0.1 or 1 µM GH. Similarly, cytotoxicity was significantly decreased and restored to control-levels when cells were co-treated with methadone and 1 µM GH.

Conclusion: Acute treatment with methadone induced neurotoxicity in primary cortical neurons, an effect that could be reduced by GH. These data further highlight the potential of GH as a neuroprotectant and cognitive enhancer.

M1.28

Release Of ß-Endorphin From Brainstem POMC Neurons In Mice Is Anxiolytic But Not Rewarding

F E Holmes, S Cerritelli, A E Pickering
University of Bristol, Bristol, UK

β-endorphin is cleaved from pro-opiomelanocortin (POMC) produced in the CNS by neurons in the arcuate nucleus and the nucleus of the solitary tract (NTS). Relatively little is known of the functional role of NTS POMC neurons, however, we have shown that their activation produces opioidergic analgesia and cardiorespiratory depression (1).

To investigate the behavioural consequences of NTS POMC neuron activation, the DREADD system was employed (2). Engineered human M3-muscarinic receptors (hM3Dq) were expressed selectively in NTS POMC neurons by stereotaxic microinjection of a Cre-inducible AAV into POMC-Cre mice, and activated by clozapine-N-oxide. Sedative and anxiolytic effects were assessed in the open field test (OFT); rewarding effects were assessed using the conditioned place preference paradigm (CPP).

In agreement with (4), activation of NTS POMC neurons caused acute suppression of feeding. NTS POMC neuronal activation increased the time spent in the central zone of the OF ($p<0.05$), indicating an anxiolytic effect which was attenuated by naloxone. There was no effect on total distance travelled or mean velocity. In the CPP there was no change in time spent in the environment associated with neuronal activation, suggesting that the β-endorphin released does not produce reward or aversion.

This approach provides a means to test whether the release of β-endorphin within the brainstem may be targeted to dissociate its analgesic action from its reward-related abuse potential.

(1) Cerritelli S *et al.* (2016). *PLoS One* 11: e0153187.
(2) Alexander GM *et al.* (2009). *Neuron* 63: 27-39.
(3) Zhan C *et al.* (2013). *J Neurosci* 33: 3624-3632.

M1.29

In Vivo Pharmacological Characterization of HS665, a Highly Potent and Selective κ Opioid Receptor (KOPR) Agonist

J Tunis[1], P Huang1, H Schmidhammer[2], M Spetea[2], LY Liu-Chen[1]
[1]Center for Substance Abuse Research & Department of Pharmacology, Lewis Katz School of Medicine, Temple University,, Philadelphia, PA, USA, [2]Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences, University of Innsbruck, Innsbruck, Austria

KOPR agonists can potentially be used as analgesics and anti-pruritic agents water diuretics; however, the aversive effects have limited their clinical development. The new KOPR agonist HS665 was designed, synthesized and characterized by Spetea *et al.* (*J Med Chem* 55:10302-10306, 2012). It is a diphenethylamine derivative, with a structure distinctly different from other known KOPR agonists, including the arylacetamide U50,488H. HS665 has high binding affinity defined by a Ki value of 0.49 nM for the KOPR, and 1100- and >20,000-fold selectivity for the KOPR over the MOPR and DOPR, respectively. In this study, we examined if HS665 caused aversive effects within the dose ranges that produces antinociceptive and anti-scratching effects, and compared it with U50,488H. Male adult CD-1 mice were used. In the formalin test, HS665 and U50,488H given s.c. reduced licking of the injected paw in a dose-dependent manner with A50 values of 1.96 mg/kg and 0.58 mg/kg, respectively, indicating potent antinociceptive effects. HS665 and U50,488H

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s.c. dose-dependently inhibited scratching induced by the pruritogen compound 48/80 injected into the nape of the neck, with A50 values of 2.46 mg/kg and 2.07 mg/kg, respectively. These results indicate that HS665 and U50,488H have similar potencies in eliciting antinociceptive and anti-scratching behaviors. HS665 at a dose of 2.5 mg/kg caused conditioned place aversion (CPA) to a similar degree as U50,488H (1, 2.5 and 5 mg/kg). Thus, comparable to U50,488H, HS665 exhibits aversive effects within the dose ranges effective in antinociceptive and anti-scratching effects.

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M1.30

Protein kinase C is involved in KOPR-mediated aversion, but not antinociception or anti-scratching effects

YT Chiu, A Cowan, LY Liu-Chen

Center for Substance Abuse Research & Department of Pharmacology, Philadelphia, PA, USA

Activation of the KOPR produces many effects, including analgesia, antipruritic effects, motor incoordination, sedation and dysphoria. Activation of the KOPR has been shown to inhibit calcium currents through PKC and to stimulate ERK signaling via PKC. In this study, we investigated the role of PKC in the pharmacological effects of KOPR activation in adult CD1 mice. The nonselective PKC inhibitor chelerythrine (CHL) was used in the study because CHL is a cell permeable inhibitor that can cross blood brain barrier into the brain. Mice were treated with CHL (s.c.) for 1 hr and then injected with U50,488 (s.c.). CHL abolished U50,488H-promoted conditioned preference aversion and partially inhibited U50,488H-induced hypolocomotion and motor incoordination. In contrast, U50,488H-induced antinociception in the formalin test and U50,488H-induced anti-scratching effect following compound 48/80 injection were not affected by CHL. These results indicate PKC plays a role in KOPR-mediated sedation, motor incoordination and aversion, but not in KOPR-induced antinociception and anti-scratching. Thus, PKC inhibition reduces the unwanted effects of KOPR activation, yet leaves the desired effects intact.

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M1.31

Interrogating the role of peripheral opioid receptors using an optogenetic approach

H Beaudry[1,2], AR Ase[1,2], I Daou[1,2], A Ribeiro-Da-Silva[1,2], P Séguéla[1,2]

[1]McGill University, Montreal, Québec, Canada, [2]Alan Edwards Centre for Pain Research, Montréal, Québec, Canada

Opioids are the most potent analgesics currently available, but their use is often accompanied by unwanted side effects such as nausea, constipation, respiratory depression and sedation. To overcome this limitation, peripheral administration of opioids represents an interesting alternative. Until recently, DOPR and MOPR were thought to be co-expressed in both peptidergic and non-peptidergic nociceptors in most species. In the mouse however, opioid receptors have been shown to be segregated in primary afferent neurons, with DOPR and MOPR expressed in non-peptidergic (MrgprD-positive) and peptidergic (TRPV1-positive) neurons respectively. In addition to these distinct expression patterns, it has been reported that MOPR activation exclusively relieves thermal pain whereas DOPR activation decreases mechanical pain, which casts a doubt on many past studies.

Optogenetics enables neural control in vivo through expression of light-sensitive channels (e.g. ChR2) that transduce pulses of light into action potentials in genetically-identified neurons. We investigated the role of peripheral MOPR and DOPR by comparing the analgesic effect of deltorphin II and DAMGO on light-induced pain behaviors in Nav1.8-ChR2, TRPV1-ChR2 and MrgprD-ChR2 mice. Unexpectedly, our results show that intradermal delivery of opioids did not affect ChR2-mediated pain behaviors at doses that decreased mechanical hypersensitivity in a neuropathic (SNI) model. In line with in vivo data, bath application of DAMGO did not modify light-induced action potentials in dissociated DRG neurons from Nav1.8-ChR2 mice. These results may reflect a different opioid pharmacology between artificial light-activated vs natural stimuli-activated nociceptors and we are investigating this hypothesis.

Support: CIHR, NSERC, LAEF and QPRN.

M1.32

Low Dose Naltrexone (LDN) - Potential As An Analgesic

Anette Bendiksen, Kirsten Bested

Multidisciplinary Pain Clinic, Friklinikken, Give, Denmark

Introduction: Naltrexone is an opioid antagonist which in few small placebo controlled studies showed to provide pain relief to patients with fibromyalgia using a low oral dose of 2-4.5 mg daily. The question why LDN acts as an analgesic is still unanswered, - is it the antagonistic effect on opioid receptors or maybe an immunological activation of microglia? Can LDN be used for other pain types as well?

Aim: To examine LDN regarding pain relief and side effects among patients with mixed chronic non-malignant pain.
Methods: A retrospective record review of 575 women and 125 men treated orally with LDN 4.5 mg per day. 203 had fibromyalgia, 497 other pain diagnoses.

Results: 31% achieved pain relief of LDN. 44% had no effect and 25% had side effects resulting in withdrawal. The effect was similar for both genders and independent of the type of pain. The mentioned adverse effects were: Nausea 7%, headache 5.29%, dizziness 4.57%, abdominal pain 4%, tiredness 3.71%, sleep problems 2.57%, muscle pain 2.14%, diarrhea 1.57%, fever feeling 1.14%, itching 1%, palpitations 0.57%, shaking 0.57%, sweating 0.43%, anxiety 0.43%, hallucinations 0.43%, aggressiveness 0.29%.

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Conclusion: LDN shows potential in chronic non-malignant pain, not only fibromyalgia. The drug has relatively few side effects in the low dose, and no serious adverse reactions were registered. More studies are wanted to confirm the analgesic effect and clarifying the mechanism of action. We believe that LDN in the future may be a good and safe alternative to both opioids, antidepressants and anticonvulsants.

M1.33

Altered endocannabinoid modulation of GABA release in the adult RVM following persistent inflammation

MH Li, KL Suchland, SL Ingram

Oregon Health & Science University, Portland, USA

Chronic pain is a major health issue that affects 30% of the US population. The treatment of chronic pain has been challenging to date for multiple reasons, including tolerance and/or serious side effects with prolonged use of typical analgesics. Endocannabinoids represent an alternative analgesic strategy but relatively little is known about the endocannabinoid system in chronic pain states.

The rostral ventromedial medulla (RVM) is an important relay of the descending pain modulatory system and a site of endocannabinoid modulation of pain. Using whole-cell patch-clamp recordings from RVM slices, we studied endocannabinoid modulation of GABAergic transmission in the RVM of rats pretreated with complete Freund's adjuvant (CFA) to induce persistent inflammation. Endocannabinoid-mediated inhibition of GABAergic miniature inhibitory postsynaptic currents (mIPSCs) is reduced in RVM slices from CFA-treated compared to naïve rats. The reduction is due to downregulation of CB1 receptor levels and function in the RVM. Although inhibition of GABAergic mIPSCs induced by the non-selective CB1 and CB2 agonist WIN 55,212-2 (5 μM) is similar in both naïve and CFA-treated rats, WIN 55,212-2 effects are only reversed by the CB1 antagonist rimonabant in naïve animals. In CFA-treated rats, the effects of WIN 55,212-2 are blocked with the selective CB2 receptor antagonist SR144528 (3 μM) indicating that CB2 receptor function in the RVM is increased during persistent inflammation. In addition, CB2 receptor-mediated inhibition of mIPSC frequency is blocked by naloxone (10 μM). Our results suggest that exogenous application of selective CB2 receptor agonists may be useful for treatment of persistent inflammatory pain.

M1.35

Novel endomorphin analogs provide potent, longer lasting relief of neuropathic, inflammatory, and postoperative pain relative to morphine

AK Feehan[1], J Morgenweck[4], A Amgott-Kwan[1], X Zhang[2,3], JE Zadina[2,3]

[1]Tulane University Neuroscience Program, New Orleans, LA, USA, [2]Tulane University Department of Medicine, New Orleans, LA, USA, [3]SE Louisiana Veterans HCS, New Orleans, LA, USA, [4]The Jackson Laboratory, Bar Harbor, ME, USA

Activation of the mu-opioid receptor provides the gold standard for pain relief, but a majority of opioids used clinically are based on opium-derived compounds with side effects that have contributed to an epidemic of abuse. We recently characterized mu-opioid receptor selective endomorphin (EM) analogs that provide potent antinociception with reduction or absence of a number of side effects of traditionally prescribed opioids including: abuse liability, respiratory depression, motor impairment, tolerance, and inflammation (1). The current study explores the effectiveness of these EM analogs relative to morphine in three major pain models by both intrathecal and intravenous injection in male, Sprague-Dawley rats. In the spared nerve injury (SNI) model of neuropathic pain, mechanical allodynia and mechanical hyperalgesia were assessed with Von Frey and Randall-Selitto tests, respectively. In the paw incision model of postoperative pain, mechanical allodynia was assessed by Von Frey testing. In the Complete Freund's Adjuvant (CFA) model of inflammatory pain, thermal hyperalgesia was assessed by Hargreaves testing. In all tests, EM analogs, particularly analog 4 (ZH853), had equal or greater potency and duration of action relative to morphine. The data suggest that this EM analog could provide effective therapy for a diverse spectrum of pain conditions with low risk of the adverse side effects caused by currently used opioids such as morphine.

(1) Zadina, JE *et al.* (2016). *Neuropharmacology* (105): 215-227.

M1.36

A role for peripheral pro-inflammatory cytokines in chronic morphine-induced hyperalgesia in mice

S Kishioka[1], S Matsuzaki[1], F Saika[1], M-C Ko[2], N Kiguchi[1,2]

[1]Wakayama Medical University, Wakayama, Japan, [2]Wake Forest University School of Medicine, North Carolina, USA

Long-term opioid use may paradoxically elicit pain, being opioid-induced hyperalgesia. Although the mechanism has been investigated within the brain and spinal cord, there is no report showing peripheral mechanism as far as we know. On the other hand, we clarified that the pro-inflammatory cytokines, including chemokines, in peripheral nerves were involved in chronic pain induced by vincristine or peripheral nerve injury. In this experiments, we evaluated the participation of pro-inflammatory cytokines in peripheral nerves on chronic morphine-induced hyperalgesia in ICR male mice. Morphine (1–100 mg/kg, s.c.) was injected twice a day for 4 days. Mechanical hyperalgesia was evaluated by von Frey test. The mRNA of cytokines was quantified by RT-PCR. For the assessment of spontaneous morphine withdrawal, the serum corticosterone (SCS) increase and body weight loss were used. On the next day after last morphine, pain threshold (g) was decreased in a dose of morphine-dependent manner, being mechanical hyperalgesia. Concomitantly, neither SCS increase nor body weight loss was observed, indicating no spontaneous morphine withdrawal. Interleukin-6 mRNA in the sciatic nerve was significantly increased by chronic morphine (100 mg/kg). The CC chemokine ligand-2 and -3 mRNA in the sciatic nerve was also significantly up-regulated by chronic morphine. In contrast, these mRNA up-regulations were not observed in the spinal cord. The present study suggests that chronic morphine elicits mechanical hyperalgesia, which may be associated with the up-regulation of peripheral pro-inflammatory cytokines derived from macrophages, and the hyperalgesia may not be due to spontaneous morphine withdrawal.

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M1.37

Pain-Induced Upregulation of Endogenous Opioid Tone in the Reward Pathway

Adrianne Wilson-Poe[1], Nicolas Massaly[1], Lucia Hipolito[2], Cathy Cahill[3], Jose Moron-Concepcion[1]

[1]Washington University in Saint Louis, Saint Louis, MO, USA, [2]University of Valencia, Valencia, Spain, [3]University of California, Irvine, Irvine, CA, USA

The opioid epidemic is one of the most significant health problems of the 21st century. Opioids exert their pain relieving and addictive effects primarily through the mu-opioid receptor (MOR). Our previous work has demonstrated that pain induces MOR desensitization in the ventral tegmental area (VTA), part of the brain circuitry that mediates reward, and ultimately addiction. This MOR desensitization contributes to altered motivational states, and a propensity to self-administer very high doses of opioids (in Sprague Dawley rats). However, the mechanism of pain-induced MOR desensitization is unknown. One possible explanation is that persistent inflammatory pain (CFA in the hindpaw) induces an upregulation in the release of endogenous opioid peptides, which play an important evolutionary role in pain modulation. *Elevated opioid tone may continually activate MORs in the VTA, leading to desensitization.* Behavioral pharmacology, ex vivo electrophysiology, and autoradiography were employed to test this hypothesis, with approval from the Washington University IACUC, and in accordance with the Guidelines for the Care and Use of Laboratory Animals. Bilateral intra-VTA MOR antagonism (2ug CTAP) in rodents experiencing persistent pain elicits an opioid withdrawal syndrome. Autoradiography in this region demonstrates significant pain-induced down-regulation of MOR function, as measured by g-protein activation. Our results indicate that pain itself is sufficient to upregulate endogenous opioid tone in the VTA, which is critically involved in motivated behavior, such as drug seeking. These findings provide important mechanistic insight into the pattern and clinical challenge of opioid self-administration in human patients with chronic pain.

M1.38

Increased number of glial and neuronal cells in the periaqueductal gray matter (PAG) of μ-opioid receptor knockout mice

Kazumasu Sasaki[1,2], Ichiro Sora[3], Yoshiyuki Kasahara[4], Kazutaka Ikeda[5], F. Scott Hall[6], George R. Uhl[7,8], Ryuta Kawashima[1]

[1]Department of Functional Brain Imaging, Institute of Development, Ageing and Cancer, Tohoku University, Sendai, Japan, [2]Department of Preclinical Evaluation, Institute of Developing, Ageing and Cancer, Sendai, Japan, [3]Department of Psychiatry Kobe University Graduate School of Medicine, Kobe, Japan, [4]Department of Disaster Psychiatry, International Research Institute of Disaster Science (IRIDeS), Tohoku University, Sendai, Japan, [5]Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [6]Department of Pharmacology and Experimental Therapeutics, The University of Toredo, Toledo, USA, [7]Research, New Mexico VA Healthcare System and BRINM, Albuquerque, USA, [8]NIDA/IRP/NIH, Baltimore, USA

The periaqueductal gray matter (PAG) is an important region for μ-opioid receptor (MOP) regulated physiological functions such as pain modulation. We reported that MOP knock out mice displayed robust increase of regional gray matter volume in PAG, elucidated by analyses of MRI voxel-based morphometry (VBM). Histological analysis failed to identify evidence for neuronal or glial pathological degeneration.

In the current study, we investigate the cell types that contribute to PAG volume difference when MOP receptors are absent. Seven male MOP-KO mice and 7 wild-type mice were studied at 12 weeks of age. Differences in numbers of PAG microglia, astrocytes and neurons in the PAG were quantitated following immunostaining with the respective markers, Iba-1, GFAP, and NeuN. These immunohistochemical analysis was performed at four different PAG regions:dorsomedial, dorsolateral, lateral, ventrolateral. Larger numbers of microglia, astrocyte, and neuron were observed in each of the four different PAG regions in mice without MOP receptors.

These analysis aid understanding of the relationships between individual differences in the PAG, behavioural alterations caused by MOP receptor deletion, and possibly even effects of opiate agonists and antagonists on specific developmental processes.

M1.39

Loss of morphine-induced hyperalgesia in mu opioid receptor knockout mice

Claire Gaveriaux-Ruff[1,2], Laurie-Anne Roeckel[1,2], David Reiss[1], Yannick Goumon[5,2], Hervé Maurin[1,3]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France, [2]Université de Strasbourg, Strasbourg, France, [3]Centre National de la Recherche Scientifique, UMR7104, Illkirch, France, [4]Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France, [5]CNRS UPR3212, Institut des Neurosciences Cellulaires et Intégratives, Strasbourg, France

Opiates are potent analgesics but their clinical use is limited by adverse events including analgesic tolerance and opioid-induced hyperalgesia (OIH). As mu opioid receptor (MOR) has been shown mandatory for most morphine effects, here we examined the involvement of MOR in OIH by comparing chronic morphine effect in MOR knockout (KO) and wild-type (WT) mice.

The repeated morphine administration with 20 mg/kg/d intraperitoneal (ip) injections for 6 days produced analgesic tolerance in both female and male WT animals as measured in the tail immersion assay. Under this tolerance condition, OIH was detected in WT mice but not in MOR KO mice. These behavioural results have also been observed in tests for mechanical and cold pain modalities.

The morphine metabolite morphine-3-glucuronide (M3G) has been previously shown to induce OIH. M3G did produce hyperalgesia in WT but not in MOR KO animals. In the agonist-activated GTPgS binding assay, G protein activation was triggered by DAMGO, morphine and M3G on brain membrane preparations from WT mice, and was absent when using brain membranes from MOR KO mice. Furthermore, M3G-induced G-protein activation was blocked by the selective mu receptor antagonist CTOP ([H-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2].

Altogether, our findings on WT and MOR KO mice indicate that mu receptor is necessary for hyperalgesia induced by both chronic morphine and M3G acute administration.

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M1.40

Assessment of pain-depressed wheel running in male and female rats reveals the limitations of opioids to treat pain

Ram Kandasamy[1], Jonas Calsbeek[2], Michael Morgan[2]

[1]Washington State University, Pullman, WA, USA, [2]Washington State University Vancouver, Vancouver, WA, USA

Opioids are very effective at inhibiting responses to noxious stimuli in laboratory animals, but have limited efficacy and produce many side effects in chronic pain patients. One of the reasons for this disconnect could be that nociception is typically assessed using withdrawal from a noxious stimulus in animals, whereas chronic pain patients suffer from disruption of normal function. We hypothesized that opioid efficacy would be much lower in animal studies if assessed as a restoration of normal function. We tested this hypothesis by measuring restoration of home cage wheel running depressed by inflammatory pain induced by an intraplantar injection of Complete Freund's Adjuvant (CFA) into the right hindpaw. One day after CFA administration, morphine (0.32-3.2 mg/kg, s.c.), buprenorphine (0.032-0.32 mg/kg, s.c.), or saline was administered. CFA administration produced mechanical allodynia and depressed wheel running in both sexes. Moderate doses of morphine (0.32 and 1.0 mg/kg) reversed allodynia and restored running for one hour in both sexes; however, the magnitude of antinociception was greater in male compared to female rats. In contrast, administration of buprenorphine had no effect on inflammation-induced depression of wheel running in male or female rats, although it reversed allodynia in male rats. Administration of buprenorphine and higher doses of morphine depressed wheel running in non-inflamed rats, suggesting that side effects interfere with restoration of function. These data indicate that restoration of pain-depressed function requires maximizing antinociceptive efficacy and minimizing disruptive side effects - the same goal for the treatment of chronic pain in humans.

M1.41

Antiallodynic Treatment with Duloxetine Partially Reverses Neuropathy-Induced Changes in Peripheral Delta Opioid Receptor Distribution

RA Ceredig[1], F Pierre[1], I Yalcin[1], C Gaveriaux-Ruff[2], M Barrot[1], D Massotte[1]

[1]INCI UPR 3212, Strasbourg, France, [2]IGBMC UMR 7104, Illkirch, France

Peripheral delta opioid (DOP) receptors represent novel attractive targets for chronic pain management and are essential for anti-allodynic effect of antidepressants. We therefore addressed the impact of neuropathic pain on DOP receptor distribution in dorsal root ganglia using a knock in mouse expressing a fluorescent version of the DOP receptor (DOPeGFP). In our model, we observed neuronal loss 8 weeks after cuff surgery that affected small size neurons. Also, remaining small peptidergic and non-peptidergic neuronal populations expressing DOPeGFP were decreased. Oral chronic treatment with the antidepressant Duloxetine reversed mechanical allodynia in wild type animals but not in conditional knock-out mice that do not express DOP receptors in Nav1.8 positive neurons (Nav1.8 cKO), establishing that DOP receptor expression in this population is required for treatment effectiveness. Also, we found that chronic neuropathy increased DOPeGFP translocation to the plasma membrane. Interestingly, the antidepressant treatment reversed neuropathy-induced changes DOPeGFP distribution and enhanced surface expression. These data confirm the critical involvement of DOP receptors in the relief of mechanical allodynia by antidepressants in the context of neuropathic pain.

M1.42

Opposing effects of the nociceptin antagonist J113397 and the opioid antagonist naltrexone on mouse models of hyperalgesia

N Senese, J Traynor

University of Michigan, Ann Arbor, USA

Preclinical evidence suggests that both mu opioid receptor (MOPr) and nociceptin receptor (NOPr) signaling increase during chronic pain states. NOPr signaling increases immediately after injury and may contribute to the development of hyperalgesia, while MOPr signaling increases at later time points and may contribute to the alleviation of pain as recovery progresses.

Regulator of G-protein signaling (RGS) proteins are a family of accessory proteins that act to turn off signaling downstream of G-protein coupled receptors including the mu- opioid receptor (MOPr) and the nociception receptor (NOPr). Here we demonstrate that mice with a point mutation in the gene encoding Galphao that renders this G-protein insensitive to the negative regulatory effect of RGS proteins exhibit a tactile hyperalgesic phenotype, in contrast to our earlier work showing a small antinociceptive phenotype in response to thermal stimuli in these animals.

To understand the mechanism underlying the hyperalgesic phenotype in the mutant mice we examine how antagonism of the MOPr and NOPr affects nociceptive behavior using the Von Frey test. We compare these findings to mice with an inflammatory hyperalgesia following intra-plantar carrageenan injection. The results show that NOPr antagonism decreases hyperalgesic behavior in both the genetic and inflammatory models, while MOPr antagonism trends towards increasing hyperalgesia in both models. Neither antagonist changes nociceptive behavior in control mice. These results suggest that signaling through both NOPr and MOPr increases significantly in these hyperalgesic models, and that NOPr antagonists should be explored as anti-hyperalgesic agents. Supported by DA035316 and GM007767.

M1.43

Synergistic antihyperalgesic combination of endogenous protected-enkephalins by an orally active dual enkephalinase inhibitor (DENKI), PL265, with various analgesic drugs acting on different targets, in a murine model of bone cancer-induced pain

BP Roques[1,2], S Gonzalez-Rodriguez[3], H Poras[1], L Menendez[3], A Lastra[3], T Ouimet[1], MC Fournié-Zaluski[1], A Baamonde[3]

[1]Pharmaleads, Paris, France, [2]Université Paris Descartes, Paris, France, [3]Facultad de Medicina, Oviedo, Spain

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The protection of endogenous enkephalins from their degradation by dual enkephalinase inhibitors (DENKIs) has been shown to exert antinociceptive responses in rodents bearing different types of pain. The antinociceptive effect of PL265, a new DENKI, was evaluated in mice intratibially inoculated with B16-F10 melanoma cells. The oral administration of PL265 (12.5-100 mg/kg), decreased thermal hyperalgesia and mechanical allodynia. The antihyperalgesic effect was antagonized by earlier administration of naloxone-methiodide (2 mg/kg) or cyprodime (1 mg/kg) but not naltrindole (0.1 mg/kg) or nor-binaltorphimine (10 mg/kg), thus confirming the involvement of peripheral mu-opioid receptors. Several other antinociceptive drugs, acting through different neuronal pathways, such as gabapentin, a ligand of calcium channel sub-unit $\alpha 2\delta$, a P2X3 receptor antagonist (A-317491), a CB1 receptor agonist (ACEA), two CB2 receptor agonists (AM1241 and JWH-133), an inhibitor of the degradation of endogenous cannabinoids (URB937) and a Nav1.7 blocker (NAV26), yielded by themselves antihyperalgesic effects in these mice. As shown by isobolographic analysis, their combined administration with PL265 indicated drug synergism (with interaction index from 0.3 to 0.7) except for JWH133, a drug whose antihyperalgesic effect is unrelated to the stimulation of opioid receptors. The observed synergistic antihyperalgesic combination of drugs can be advantageous in therapeutics since the decrease of the active dose might reduce the development of side-effects of both components.

M1.44

Opioid Effects on Operant Nociception in Nonhuman Primates

BD Kangas

Harvard Medical School, Boston, USA

The effective management of pain remains an important public health concern. Although morphine-like opioids have long been front-line analgesics for most painful conditions, they have a number of undesirable side effects including abuse liability and sedation that can limit their utility. Slow progress in the development of novel medications for pain management using traditional assays has highlighted the need for new animal models that might more effectively identify clinically useful drugs. Most models (tail flick, hot plate, warm water tail withdrawal) assay simple spinal reflexes or unconditioned behavioral reactions to nociceptive stimuli rather than the disruption of task performance, which may be more clinically relevant. We have recently developed a novel apparatus and procedures to study the disruptive effects of nociceptive stimuli on "voluntary" responses and the restorative effects of analgesics. Monkeys were trained to pull a cylindrical thermode for a palatable food reinforcer. Sessions were conducted in which the temperature of the thermode was increased stepwise until responding stopped, permitting the determination of nociceptive thresholds. Tests with several opioid analgesics revealed dose-related increases in threshold that differed in efficacy consistent with their effects using standard measures of antinociception, whereas d-amphetamine or THC did not. Unlike traditional reflex-based measures, however, the results also permitted the concurrent evaluation of response disruption, providing an index with which to characterize the behavioral selectivity of antinociceptive drugs.

M1.45

Inhibition of brain free fatty acid receptor GPR40/FFAR1 signaling exacerbates pain behavior

Kazuo Nakamoto[1], Fuka Aizawa[1], Takuya Yamashita[2], Akira Hirasawa[3], Takashi Kurihar[a4], Mitsumasa Mankura[5], Atsuro Miyata[4], Yutaka Koyama[6], Fumiyo Kasuya2, Shogo Tokuyama[1]

[1]Department of Clinical Pharmacy School of Pharmaceutical Sciences Kobe Gakuin University, Kobe, Japan, [2]Biochemical Toxicology Laboratory, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan, [3]Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan, [4]Department of Pharmacology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan, [5]Faculty of Food Culture, Kurashiki Sakuyo University,, Kurashiki, Japan, [6]Laboratory of Pharmacology, Faculty of Pharmacy, Osaka Ohtani University, Osaka, Japan

Previously, we have demonstrated that the activation of the GPR40/free fatty acid receptor 1 (GPR40/FFAR1) signaling may play an important role in the regulation of the descending pain control system. Here, we examined the involvement of hypothalamic GPR40/FFAR1 signaling in the development of chronic pain. We used GPR40/FFAR1 knock out (GPR40KO) mice or wild type (WT) mice. A plantar incision was performed in mice. The complete Freund's adjuvant (CFA) was intraplantary injected in mice. Mechanical allodynia and thermal hyperalgesia were evaluated using von Frey filaments and plantar test, respectively. The repeated administration of GW1100, a GPR40/FFAR1 antagonist, CFA or incision-induced mechanical allodynia compared to vehicle treated mice. The repeated GW1100 treated mice significantly increased phosphorylated ERK in the spinal cord after low threshold touch stimulation. The level of the hypothalamic docosahexaenoic acid, a GPR40/FFAR1 agonist, significantly increased at 2 days after surgery compared to sham group. Furthermore, GPR40KO mice were exacerbated incision-induced mechanical allodynia, but not thermal hyperalgesia compared to WT mice. Our findings suggest that the dysfunction of this signaling pathway may be associated with the development of chronic pain.

M1.46

Modulation of morphine-induced antinociception in acute and chronic treatment by ketamine and its metabolites norketamine and 6-hydroxynorketamine

TO Lilius[1], EH Kangas[1], V Jokinen[1], H Viisanen-Kuopila[1], J Laitila[2], M Niemi[2,3], E Kalso[1,4], P Rauhala[1]

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

Ketamine attenuates morphine tolerance at subanaesthetic doses via the antagonism of N-methyl-D-aspartate receptors (NMDARs). Norketamine, the main metabolite of ketamine, is also an NMDAR antagonist with less potency. The suggested antidepressive

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effects of the NMDAR-inactive metabolite 6-hydroxynorketamine may result from other mechanisms. We compared the ability of ketamine, norketamine, and 6-hydroxynorketamine to augment morphine antinociception in acute and chronic models of morphine administration.

Male Sprague-Dawley rats were used. Subcutaneous ketamine, norketamine, and hydroxynorketamine were administered acutely alone, in combination with acute subcutaneous morphine, and after the development of morphine tolerance. Subcutaneous minipumps administering 9.6 mg morphine daily induced tolerance during 6 days. Tail-flick, hot plate, and paw pressure tests were used to assess nociception. Motor coordination was monitored with the rotarod test.

Norketamine (10 or 30 mg/kg) or hydroxynorketamine (30 mg/kg) did not enhance antinociception or cause rotarod impairment in acute treatment either alone or combined with morphine (2.5 mg/kg). In morphine-tolerant rats, 10 mg/kg ketamine abolished morphine tolerance for 120 minutes, whereas the effect of 30 mg/kg norketamine lasted for 150 minutes. In morphine-tolerant rats, acute ketamine caused marked rotarod impairment up to 15 min after administration, whereas norketamine had no acute rotarod effects. Acute hydroxynorketamine did not attenuate opioid tolerance.

Norketamine is interesting for further development as it did not produce rotarod disturbance at a dose that reversed morphine tolerance. This advantage may originate from its lower NMDAR antagonist potency. Hydroxynorketamine did not abolish morphine tolerance, which suggests that the NMDAR inhibition is critical for the drug effect.

M1.47

The Effect of OPRM1 A118G and FAAH P129T Polymorphisms on Analgesic Oxycodone and Metabolite Concentrations

Kristiina Cajanus[1], Mari A. Kaunisto[3], Mikko Neuvonen[2], Pertti J. Neuvonen[2], Mikko Niemi[2], Eija Kalso[1]

[1]Department of Anaesthesiology, Intensive Care and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [2]Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland

Oxycodone is increasingly used to alleviate postoperative pain. Pharmacokinetic as well as pharmacodynamic factors may play an important role in oxycodone analgesia. In this study, we investigated the effect of single nucleotide polymorphisms (SNP) in *OPRM1* and *FAAH* genes on the analgesic plasma concentrations of oxycodone and its metabolites.

1,000 women undergoing breast cancer surgery were recruited to the study. The patients had either breast conserving surgery or mastectomy with or without axillary clearance of lymph nodes. Postoperatively, the research nurse assessed the pain intensity of the patients every 5 min and administered i.v. oxycodone until the patient reported satisfactory analgesia (NRS ≤3). At this point, a blood sample was drawn for oxycodone and metabolite concentration measurements, and later, when the patient needed a new dose of oxycodone. One OPRM1 (rs1799971/A118G) and one FAAH SNP (rs324420/P129T) were genotyped from genomic DNA.

OPRM1 A118G polymorphism associated with noroxycodone plasma concentrations at the 1st state of satisfactory analgesia (P = 0.01) and when the patient needed a new dose (P = 0.006). The rare allele homozygotes had the highest noroxycodone concentrations. FAAH P129T associated with the oxymorphone concentration when the patient needed a new dose of oxycodone (P = 0.02). Neither of the studied SNPs showed statistically significant associations with oxycodone concentration at either of the time points.

Our results suggest a possible novel association between OPRM1 A118G genotype and noroxycodone analgesic plasma concentration.

M1.48

Lack of analgesic efficacy of opioids and gabapentin on acute experimental pain responses in methadone maintained patients

Bridin Murnion[1,4], Suzanne Nielsen[2,3], Gonzalo Rivas[2], Nicholas Lintzeris[2,4]

[1]Sydney Local Health District, Sydney, Australia, [2]South Eastern Sydney Local Health District, Sydney, Australia, [3]National Drug and Alcohol Centre, University of New South Wales, Sydney, Australia, [4]University of Sydney, Sydney, Australia

Aim: To identify the efficacy of different analagesic combinations on experimental pain tolerance in methadone maintained patients.

Methods: Study design was a randomised, controlled, double-blinded, double-dummy, within-subject crossover study examining cold-pressor tolerance (CPT) testing under 4 analgesic conditions of (a) usual methadone (UM) (b) usual methadone plus 30% extra methadone (UMM) (c) usual methadone plus oxycodone equivalent to 30% of usual methadone (UM0) or (d)usual methadone plus gabapentin 600mg (UMG). 8 participants stable on methadone maintenance treatment (MMT) were recruited. Cold pressor tolerance was assessed 2 hours after methadone dosing.
RESULTS: No difference in mean pain tolerance between the groups was identified. Mean pain tolerance was 25.9 (SD (14.7) sec for UM, 26.7 (SD 21.0) sec in UMM, 25.9 (12.0) sec in UM0 and 26.9 (SD 16.0) sec UMG. Interindividual variability in response to analgesic was observed. Individual CPT increased during the study.

Discussion: These data show similar CPT in methadone maintained patients compared to previous studies. Unlike chronic gabapentin treatment, acute gabapentin administration had no impact on CPT. In this small sample, overall additional methadone or oxycodone had no impact on CPT. However in some patients, prolonged CPT with increased opioid was identified.

Conclusion: These data suggest inter-individual variability in response to increasing opioid, and limited benefit of acute addition of gabapentin to manage acute pain in MMT patients. Further studies are required to identify variables that predict good or poor analgesic response.

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M1.49

Impact of efficacy and dose ratio on the antinociceptive effects of opioid/cannabinoid mixtures

D Maguire, C France

University of Texas Health Science Center as San Antonio, San Antonio, Texas, USA

Although opioids (e.g., morphine) are used to treat pain, they have unwanted effects limiting their clinical utility. Cannabinoid receptor agonists (e.g., THC) enhance the antinociceptive effects of opioids; by combining an opioid with a non-opioid, smaller doses of the opioid are required to produce the desired therapeutic effect while avoiding unwanted effects associated with large doses. It is unclear whether efficacy at the mu opioid or cannabinoid receptors impacts opioid/cannabinoid interactions in rats and which proportions of opioid to cannabinoid produce optimal therapeutic effects. This study examined antinociceptive effects of opioid/cannabinoid mixtures in rats using a warm water tail withdrawal procedure. Administered alone, the opioids morphine (1.78-17.8 mg/kg) and etorphine (0.0032-0.01 mg/kg) as well as the cannabinoids THC (3.2-32.0 mg/kg) and CP55940 (0.032-1.0 mg/kg) increased tail withdrawal latency from 50°C water. In one group (n=8), all ratios of morphine to THC (3:1, 1:1, and 1:3) were additive. The 3:1 and 1:1 ratios of etorphine to THC were additive and the 1:3 ratio was sub-additive. In another group (n=8), the 3:1 and 1:1 ratios of morphine to CP55940 were greater than additive (synergistic) and the 1:3 ratio was additive; all ratios of etorphine to CP55940 were additive. These results extend previous studies on opioid/cannabinoid mixtures and demonstrate that opioid/cannabinoid interactions depend, in part, on the drugs in the mixtures (e.g., whether they contain a high or low efficacy cannabinoid receptor agonist) as well as the ratio of opioid to cannabinoid. Supported by USPHS grant K05DA017918.

M1.50

Elevated agmatine in spinal cord following gene transfer of human arginine decarboxylase attenuates tolerance to opioid analgesia

C Fairbanks, C Churchill, C Peterson, S Erb, H Verma, K Kitto, J Waataja, R McIvor, L Vulchanova, G Wilcox

University of Minnesota, Minneapolis, USA

Background: Decarboxylated L-arginine (agmatine) prevents or reduces the development of tolerance to repeated opioid administration when given by systemic and central routes of administration. Since agmatine is an endogenous substance, overexpression of the synthetic enzyme for agmatine, arginine decarboxylase (ADC), should also affect induction of opioid analgesic tolerance. We report that spinal gene transfer of human arginine decarboxylase in mouse results in production of endogenous agmatine and reduction of morphine- and endormorphin-2-induced analgesic tolerance.

Methods: ICR-CD1 male mice were pre-treated with either AAV-ADC vector or saline eight-ten weeks before testing. The tail flick immersion test (52.5°C) was used. Morphine or saline was repeatedly delivered subcutaneously over 3 days; a single high intrathecal dose of endormophin-2 (10 nmol, i.t.) or saline was delivered in a separate experiment. Probe dose-response curves for each opioid were constructed in each of four pre-treatment groups (saline-saline, saline-opioid, AAV5-ADC-saline, AAV5-ADC-opioid). Additionally, the impact of immunoneutralization of endogenously produced agmatine was assessed by intrathecal pre-treatment with anti-agmatine IgG (compared with normal IgG) in the endo-2 tolerance experiments. Spinal cords were extracted, heat-stabilized and subjected to HPLC analysis for agmatine content.

Results: Spinal agmatine was elevated in subjects pre-treated intrathecally with AAV-hADC. Analgesic tolerance was induced in both morphine- and endo-2-treated control subjects but not in subjects pre-treated with AAV-ADC. Immunoneutralization of agmatine with anti-agmatine IgG restored analgesic tolerance to endo-2 in AAV-ADC-treated mice.

Conclusion: Expression of human arginine decarboxylase in the mouse sensory system prevented both morphine- and enodo-2-induced tolerance likely due to production of agmatine.

M1.51

No sexual dimorphism in gliosis-induced modulation of reward or hyperalgesia in opioid-dependent states

Anna Taylor[1], Sarah Pickens[2], Jeanette Chin[2], Edmund Ong[2], Sadaf Mehrabani[1], Catherine Cahill[2]

[1]UCLA, Los Angeles, CA, USA, [2]UCI, Irvine, CA, USA

Activated microglia contribute to pain hypersensitivity following peripheral nerve injury. While microglia are sufficient to induce chronic pain in male rodents, this is not true for females. Hence, microglial inhibitors did not prevent the development of pain behaviors in female mice, suggesting than an alternative mechanism was responsible for chronic pain. Our previous research identified that microglia are responsible for opioid-induced hyperalgesia and blunted reward associated with chronic opioid use. In the present study, we asked whether there is also sexual dimorphism of microglia involvement in opioid dependent states. C57Bl/6 male and female mice were treated with escalating doses of morphine twice daily for 4 days with and without concomitant treatment of the microglial inhibitor minocycline. Withdrawal thresholds to a painful mechanical stimulus were evaluated using Von Frey filaments to assess morphine-induced hyperalgesia. Reward was determined by conditioned place preference to cocaine. Female opioid-dependent mice developed opioid-induced mechanical hyperalgesia and showed blunted reward to cocaine, similar to male mice. Microglial inhibition reduced Iba-1 immunolabeling in the spinal cord and ventral tegemental area of female mice treated with chronic morphine. Unlike, chronic pain, minocycline blocked opioid-induced hyperalgesia in female mice. Similarly, microglial inhibition also recovered cocaine-induced reward. These results suggest that while microglia contribute to pain hypersensitivities produced by either chronic pain or chronic opioid treatment, the mechanisms of microglial-induced effects in opioid dependence are not equivalent to those engaged by chronic pain. Further research is needed to understand neuroinflammatory regulation of these states.

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M1.52
Landscape of alternative splicing of delta-opioid receptor in human
M Piltonen, AJ Chabot-Doré, M Parisien, L Diatchenko
The Alan Edwards Centre for Research on Pain, McGill University, Montreal, QC, Canada

Delta-opioid receptor (DOR), mu-opioid receptor (MOR) and kappa-opioid receptor comprise the opioid receptor family, to which nociceptin receptor can also be included. Each receptor is coded by a single gene, which undergo alternative splicing (AS) to different degrees. The structure of OPRD1 gene for DOR is the least diverse: only one alternative transcript is known in mouse, none in human. AS offers an intriguing explanation for diversity of receptor responses, because it can profoundly alter the receptor structure, ligand selectivity, trafficking or specificity for signaling pathways.

Pharmacological characteristics have suggested two distinct DOR-profile, but the identities of the receptors have remained ambiguous. Many options have been listed from post-translational modification to heteromerization and AS. Because of low number of reports describing AS of OPRD1, we performed an extensive characterization.
By combining 5'RACE PCR with deep sequencing, we identified multiple OPRD1 transcripts, which were confirmed by RT-PCR. These transcripts had either a cassette exon between exons 1 and 2 introducing a premature stop codon or adding 21 amino acids to the first intracellular loop, or a 5' extension of exon 2 without exon 1. Five alternative transcripts code for truncated 6-transmembrane spanning (6TM) receptors where the start codon is situated either at the beginning of exon 2 or just before it.

When transiently transfected into cultured cells, 6TM DOR is expressed and can be seen on the plasma membrane. We are testing the functional effects of 6TM DOR activation with cAMP and nitric oxide release assays and intracellular Ca++ recordings.

M1.53
Molecular dynamics of opioid receptor and other GPCR-mediated transmembrane water movements
H Ueda, M Harada
Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

We are now interested in in silico design of better compounds for GPCR and opioid receptors. For this purpose, we are using the strategy of molecular dynamics (MD) based on crystal structure or homology-modeled structure. Recently we have obtained successful results (Scientific Reports, 5:13343, 2015), in which we made a molecular model for lysophosphatidic acid 1 receptor (LPA1R), which plays crucial roles in the initiation (Nature Medicine, 10, 712-718, 2004) and maintenance of neuropathic pain. The model made by the combination of homology of S1P1R transmembrane regions and ab initio model of N-terminal and C-terminal using I-TASSER, was in principle consistent with the crystal structure (Chrencik *et al.*, Cell, 161, 1633-1643, 2015). The most important finding in our MD study demonstrated that activated LPA1R mediates transmembrane water movement, and this activity is abolished in Lys39 point-mutation in the N-terminal capping structure. In our presentation, we will also show the MD of mu-opioid receptor based on crystal structure, and we will also demonstrate the receptor-mediated transmembrane water movement and its pharmacological regulation. (supported by Platform for Drug Discovery, Informatics, and Structural Life Science funded by Japan Agency for Medical Research and Development)

M1.54
Molecular Dynamics Simulations of the μ-Opioid Receptor
K Sutcliffe, E Kelly, R Sessions
University of Bristol, Bristol, UK

The μ-opioid receptor has attracted much attention in recent years for the development of biased ligands. There is the opportunity to develop drugs which bias the receptor towards analgesic pathways, whilst avoiding adverse effects. Achieving this requires a greater understanding of the underlying molecular mechanisms. Using both the antagonist-bound (1) and the recently solved agonist-bound (2) crystal structures of the μ-opioid receptor, we have conducted molecular dynamics (MD) simulations, both on the unliganded structures and in the presence of their co-crystallised ligands, BU72 and β-FNA.

We find that accelerated MD (3) over 1 μs simulations samples a greater range of conformations than conventional MD, and the two unliganded structures converge to occupy the same conformational space. Measurement of the distance between R165 and T279 reveals the breaking and formation of a hydrogen bond between the intracellular ends of helices 3 and 6 even in the absence of a ligand; indicative of spontaneous activation and inactivation. A sodium ion moves from the extracellular space into the allosteric sodium binding site in simulations of both the unliganded structures. BU72 and β-FNA bind stably in the ligand binding pocket. Binding of either ligand causes the helix 3-6 hydrogen bond to break and remain broken for the entire simulation.

These MD simulations will form the basis to investigate binding poses and receptor conformational changes for biased ligands.

1. Manglik A *et al.* (2012). *Nature* 485: 321-326.
2. Huang W *et al.* (2015). *Nature* 524: 315-321.
3. Pierce LCT *et al.* (2012). *JCTC 8*: 2997-3002.

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M1.55

Receptor residence time determines bias towards internalisation of the μ opioid receptor

A J Gillis[1], A Yousuf[1], S Sianati[1], M Canals[2], M Kassiou[1], M J Christie[1]

[1]University of Sydney, Sydney, NSW, Australia, [2]Monash University, Melbourne, NSW, Australia

μ-Opioid receptor (MOPr) agonists produce analgesia via G-protein signalling. However, additional pathways such as ß-arrestin dependent signalling & regulation contribute to their clinical effects. Signalling bias between these pathways has been extensively demonstrated but the mechanisms remain unclear. Agonist residence at the receptor is required to maintain the ß-arrestin bound receptor state and thus residence time at the receptor may contribute to G-protein/ß-arrestin 2 bias. We used a series of oxymorphone analogues with extended aliphatic substitutions at the 6-position to systematically investigate the relationship of receptor residence time to G-protein/ß-arrestin 2 bias at the MOPr. Studies were conducted on AtT20 cells stably transfected with mouse MOPr. MOPr-mediated potassium channel (GIRK) activation and rate of channel deactivation were assessed using patch clamp electrophysiology. A resonance transfer assay was used to determine degree of ß-arrestin 2 recruitment to the MOPr and rate of ß-arrestin 2 dissociation. MOPr internalisation was quantified with immunocytochemistry. Direct agonist affinity was quantified by [3H] DAMGO binding displacement. Increasing substituent length in the analogue series had little effect on agonist binding affinity or potency in GIRK assays but systematically slowed both decay of MOPr-mediated channel activation and ß-arrestin 2 dissociation indicating increased agonist MOPr residence time. Extension of aliphatic chain led to unchanged efficacy for G-protein activation but increased efficacy for ß-arrestin 2 recruitment and MOPr internalisation. The increase in ß-arrestin 2 bias was highly correlated with the increase in receptor residence time. Receptor residence time is therefore a factor determining G-protein/ß-arrestin 2 bias at the MOPr.

M1.56

Role of Probe Dependence and Na+ Ions in the Allosteric Modulation of the Delta Opioid Receptor

EM Schramm, KE Livingston, JR Traynor

University of Michigan Department of Pharmacology, Ann Arbor, MI, USA

The recent discovery and characterization of positive allosteric modulators (PAMs) targeting the mu and delta opioid receptors (MOPr and DOPr, respectively) has revealed a novel pharmacological approach for the treatment of pain and mood disorders. Allosteric modulators bind to a site on the receptor that is spatially distinct from the orthosteric site for endogenous ligands where they may influence the binding affinity and signaling profile of orthosteric ligands. BMS-986122 is a PAM for MOPr that functions by counteracting the inhibitory effect of Na+ ions on receptor activation. BMS-986187 is a recently identified PAM for DOPr that is structurally dissimilar from BMS-986122. To examine if the mode of allosteric modulation at MOPr and DOPr by these diverse compounds is the same, we examined the probe dependence and role of Na+ ions in the allosteric modulation of DOPr by BMS-986187. Radioligand competition binding assays with membranes from CHO-DOPr cells were performed for a variety of orthosteric ligands in the presence or absence of 100 mM NaCl or BMS-986187. GTPγ35S binding in membranes was used to examine the influence of BMS-986187 on receptor activation. There was profound probe dependence to the action of BMS-986187, for example, the affinity of the peptidic DOPr agonist DPDPE shifted 59-fold-whereas the potency of the small molecule agonist BW373U86 was only marginally affected. This probe dependence was found to be due to an allosteric antagonism between Na+ ions and BMS-986187, confirming the same mode of action as allosteric modulation at MOPr. Supported by R01 DA033396 and 5T32GM008597-19.

M1.57

A fluorescence-based membrane potential assay for evaluation of multi-opioid receptor ligands

T Günther, P Dasgupta, S Schulz

University Hospital Jena, Jena, Germany

The value of HTS-compatible assays in microplate format for screening novel GPCR ligands is uncontroversial. However, many of these techniques cannot be directly used to study ligands of $G_{i/o}$-coupled receptors. For some assays, cells have to be disrupted or require artificial stimulation with forskolin to obtain the second messenger content (cAMP assays). Other methods require expression of chimeric G proteins to "switch" the $G_{i/o}$ to G_q to generate a read out (calcium mobilization assays). Although the physiological role of these processes remains questionable due to their artificial set up they are still the gold standards in the field. Here, we present a novel technique to assess a more naturalistic pathway of G protein signaling. We use G protein-coupled inwardly-rectifying potassium channels (GIRK) as a common effector system shared by all members of the opioid receptor family (MOPR, KOPR, DOPR) including the nociception receptor (NOPR). We suggest a simple protocol to obtain the complete profile of novel ligands with this *in vitro* technique to receive a better understanding of the complex actions of multi-opioid receptor ligands.

M1.58

Kappa Opioid High and Low Affinity Binding Sites for Full and Partial Agonists and Antagonists on the Human Kappa Opioid Receptor: Differences Among Partial Agonists and Antagonists

J Bidlack, L Dorval, B Knapp, L Silver

University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

The goal of this study was to determine if sodium and guanine nucleotides differentially altered the binding of antagonists and partial agonists to the kappa opioid receptor. High affinity binding to the κ opioid receptor on membranes from CHO cells stably expressing the human κ opioid receptor was measured by using 50 mM Tris-HCl, pH 7.4, plus 5 mM $MgCl_2$ and the κ-selective agonist [^{3}H]U69,593. Low affinity binding to the κ receptor was measured incubating membranes with Tris buffer, containing 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT and 50 μM GDP, and the opioid antagonist [^{3}H]naloxone. Full κ agonists had the greatest affinity for the receptor in the high affinity state. Partial agonists had a higher affinity for the receptor in the high affinity state, but the partial

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agonists retained some affinity for the receptor in the low affinity conformation. The κ-selective antagonists, nor-BNI and JDTic, had a higher affinity for the receptor in the low affinity state than in the high affinity state. Also, there was a difference between the two selective antagonists. Nor-BNI had a greater than a 300% increase in affinity for the low affinity conformation of the receptor, while JDTic had only a 75% increase in affinity for the low affinity binding site. These results suggest that sodium and guanine nucleotides regulate κ antagonist binding in addition to the binding of κ full and partial agonists. (Supported by the Office of Graduate Education & Postdoctoral Affairs and the Paul Stark Professorship.)

M1.59

Molecular interaction between μ-opioid receptor and sigma-1 receptor chaperone

Tomohisa Mori[1], Minoru Narita[1,2]

[1]Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan, [2]Life Science Tokyo Advanced Research Center (L-StaR), Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan

It has been reported that sigma-1 receptor chaperone (Sig-1R), which locates in the endoplasmic reticulum, plays a role in opioid-related behaviors, and its ligand can modulate the μ-opioid receptor (MOR) signal transduction. However, molecular interaction between MOR and Sig-1R has not been fully elucidated. In the present study, we demonstrated the critical function of Sig-1Rs under the stimulation of MORs. To investigate the interaction between Sig-1R and MOR, we used MOR stably overexpressed CHO cells. Apoptosis was promoted after long-term treatment of morphine in Sig-1R-knockdown CHO cells. We found that non-glycosylated MOR, but not glycosylated MOR, was co-immunoprecipitated with Sig-1R, whereas Sig-1Rs remained to be located in the endoplasmic reticulum even after stimulation of MOR, indicating that Sig-1R may not directly associated with MOR at the cellular surface. On the other hand, naloxone treatment in long-term morphine-treated CHO cells induced the translocation of Sig-1Rs from the endoplasmic reticulum to the surface of nucleus, accompanied by morphological changes, in the Sig-1R antagonist-reversible manner. These findings suggest that the Sig-1R may be a player to protect the cells against the stimulation of MORs. Thus, Sig-1R located in the endoplasmic reticulum plays a role in the cell-adaptive response under the stimulation of MORs.

M1.60

Effect of κ-Opioid Agonists On Angiogenesis And Cancer Metastasis

Yoshinori Kato[1], Yusuke Hamada[2], Kohei Yamamizu[3], Atsunobu Sagara[2], Miho Kawata[2], Naoko Kuzumaki[2], Michiko Narita[2], Minoru Narita[1,2]

[1]Life Science Tokyo Advanced Research Center, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan, [2]Department of Pharmacology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan, [3]Department of Cell Growth & Differentiation, Kyoto University, Kyoto, Japan

The utility of κ-opioid agonists gains increasing attention for their dual pharmacological activity to relieve cancer pain as well as to manage tumor growth. The inhibition of angiogenesis is the main anticancer mechanism for κ-opioid agonists. However, antiangiogenic therapy is considered to be a trigger of hypoxia, leading to a higher chance of metastasis. The objective of this study is to investigate the effect of a κ-opioid agonist, TRK820 (nalfurafine), on cancer growth and metastasis. We used a highly-invasive murine B16 melanoma and a highly-invasive 4T1/luc2 murine breast cancer cell line. To test the antiangiogenic activity of nalfurafine, a standard tube formation assay was performed using human umbilical vein endothelial cells (HUVECs). *In vitro* scratch assay was also conducted to investigate the inhibitory effect of nalfurafine on the migration of HUVECs. For *in vivo* study, B16 melanoma cells were inoculated subcutaneously into the back of C57BL/6J mice. Nalfurafine significantly inhibited the tube formation and migration of HUVECs, indicating that nalfurafine has an antiangiogenic potential. Nalfurafine also delayed the tumor growths of B16 melanoma as well as 4T1/luc2 xenografts. We are currently working on optical imaging of breast cancer metastasis in mice bearing orthotopic 4T1/luc2 xenograft to investigate whether nalfurafine affects breast cancer metastasis. In summary, peripheral κ-opioid agonists may be a potential anticancer agent by inhibiting tumor angiogenesis, and elucidating the effect of κ-opioid agonists on cancer metastasis is under way.

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TUESDAY 12 JULY POSTER SESSION B ABSTRACTS

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T1.01

Abuse Liability of Buprenorphine/Naloxone in Opioid Abusers Maintained on Hydromorphone: Does the Dose Matter?

S Comer[1,2], J Jones[1,2], J Manubay[1,2], S Mogali[2], V Metz[2], G Madera[2]

[1]Columbia University, New York, NY, USA, 2NYSPI, New York, NY, USA

Although buprenorphine (Bup) is used effectively for the treatment of Opioid Use Disorder, the medication itself can be abused. To address this concern, a formulation of Bup that contains naloxone (Nx) was developed. Nx antagonizes the euphoric effects produced by Bup, and it precipitates withdrawal in individuals physically dependent on short-acting opioids such as hydromorphone. However, it is not clear whether the absolute dose of Nx in the Bup/Nx formulation affects its ability to deter abuse of Bup. The purpose of this 6.5-week inpatient crossover study was to examine the interaction between Bup and Nx in participants (N=13 completers) maintained on oral hydromorphone (40 mg/day; 10 mg QID). The effects of intravenously (IV) administered Bup/Nx (1.51/0.44, 2.16/0.61, 6.15/1.71, 8.64/2.44mg) were examined. These doses were compared to IV placebo (2ml saline), heroin (25mg), Nx (0.3mg), and each active dose of Bup alone (1.51, 2.16, 6.15, and 8.64mg). Only heroin was self-administered significantly more than placebo. The two largest doses of Bup alone significantly increased positive subjective effects, which were attenuated by Nx. Bup alone failed to produce robust aversive subjective effects. All Bup/Nx combinations significantly increased ratings of "Bad" drug effect and opioid withdrawal symptoms. A dose-response relationship was observed for many negative subjective effects elicited by Nx. This study further demonstrates the ability of the Bup/Nx combination to deter IV use of this medication. It appears, however, that formulations with larger doses of Nx produce a greater degree of withdrawal, and therefore may be less abused.

T1.02

Opioid gene expression in non-classical reward structures depends on early life conditions and ethanol intake

Linnea Granholm[1], Aniruddah Todkar[2], Sofia Bergman[2], Kent Nilsson[3], Erika Comasco[2], Ingrid Nylander[1]

[1]Dept. Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [2]Dept. Neuroscience, Uppsala University, Uppsala, Sweden, 3Västerås Centre for Clinical Research, Uppsala University, Uppsala, Sweden

Brain development is associated with high sensitivity to environmental influences that can cause long-term changes in neuronal function, possible through altered gene expression. The endogenous opioid system continues to mature after birth and since it is highly involved in reward, an inadequate maturation of this system could lead to a subsequent susceptibility for alcohol use disorder. The aim was to investigate the possible interaction between early life conditions and adult voluntary intake of ethanol on opioid gene expression. Male Wistar rats were exposed to different early life conditions (conventional rearing, 15, or 360 minutes of daily maternal separation) and randomly assigned to voluntary drinking of ethanol or water in adulthood (1). Rats exposed to early life stress (360 minutes of maternal separation) had an increased expression of the opioid receptors (i.e. Oprm1, Oprd1 and Oprk1) in dorsal striatum. Ethanol drinking was associated with lower striatal expression of Oprd1 and Oprk1but only in rats exposed to early life stress. Furthermore, rats exposed to early life stress had high basal Pomc expression in the amygdala and responded with decreased expression after ethanol. Our results demonstrate how adverse events early in life can influence the central molecular response to ethanol intake. These findings could help to understand why early life stress increases the risk for addition, and furthermore, why some individuals respond better to opioid-based pharmacological treatment for alcoholism than others.

(1) Vrettou, M. *et al. Addict biol,* (2015) doi:10.1111/adb.12331. *Epub ahead of print*

T1.03

Identification of a major QTL influencing oxycodone behavioral sensitivity and dependence

L Goldberg[1], S Kirkpatrick[1], N Yazdani[1], K Luttik[1], M Mulligan[2], C Bryant[1]

[1]Boston University School of Medicine, Boston, MA, USA, [2]University of Tennessee Health Science Center, Memphis, TN, USA

Opioid addiction is heritable, yet its genetic basis remains poorly understood. Mice are valuable for identifying novel genes that contribute to variation in addiction-associated phenotypes including acute psychomotor stimulation and conditioned reward. The closely related C57BL/6J and C57BL/6NJ strains exhibit limited genetic diversity, yet show significant strain differences in several addiction-associated traits, including oxycodone-induced (OXY) locomotor activity and naloxone conditioned place aversion. Quantitative Trait Locus (QTL) mapping in these substrains drastically reduces the number of segregating genetic variants from millions to thousands, accelerating the identification of the causal genetic factors. We conducted QTL mapping for oxycodone conditioned place preference (CPP, N=212), and naloxone conditioned place aversion (CPA, N=209), along with saline-treated mice as controls (SAL, N=213). We utilized a 9 day (D) CPP/CPA protocol. Mice received drug (1.25 mg/kg OXY, 4 mg/kg NAL, or SAL, i.p., suspended in SAL) on D2 and D4, and SAL on D3 and D5. Mice were assessed for drug-free CPP/CPA (D8) and drug state-dependent CPP/CPA (D9). Mice were genotyped at 96 informative markers and QTL mapping was performed in R/qtl (scanone, 1000 permutations). We identified a major genome-wide significant QTL for D2 and D4 locomotor (Chr. 1 72.43 cM, LOD= 9.79) that co-mapped to the same region as a QTL for anxiety-like opioid withdrawal behavior in the elevated plus maze (chr. 1 77.33 cM; LOD=5.33). High priority candidate genes within this locus include Rgs7 and Akt. We are currently conducting striatal transcriptome analysis via RNA-seq to aid in candidate gene identification and neurobiological mechanisms.

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T1.04

Regulation of RTP4, a Chaperone for MOPr-DOPr Heteromers

W Fujita[1], I Gomes[2], A Gupta[2], H Ueda[3], L Devi[2]

[1]Department of Frontier Life Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, USA, [3]Department of Pharmacology and Therapeutic Innovation, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Mu (MOPr) and delta (DOPr) opioid receptors associate to form heteromers; these heteromers exhibit unique intracellular signaling and are thought to be involved in important physiological functions including antinociception and anti-diarrhea. Little is known about the mechanisms regulating MOPr-DOPr heteromer levels. Previous studies revealed a chaperone molecule, receptor transport protein 4 (RTP4), protects MOPr-DOPr from proteasomal degradation and facilitates heteromer trafficking to the plasma membrane suggesting an important role for RTP4 in modulating MOPr-DOPr heteromer levels.

Here, we investigated the regulation of RTP4 expression by opioid receptors. Treatment of Neuro2a cells expressing MOPr-DOPr heteromers with deltorphin II (DOPr ligand), DAMGO (MOPr ligand), or Eluxadoline (MOPr-DOPr ligand) led to a significant increase in RTP4 mRNA levels. However, in cells expressing DOPr alone deltorphin II did not affect RTP4 levels suggesting a requirement for MOPr-DOPr in the deltorphin II-mediated regulation of RTP4 expression.

RTP4 represents the most abundant member of RTP family with differential distribution in the mouse brain. Investigation of *in vivo* regulation of RTP4 expression by chronic morphine administration revealed a significant increase in RTP4 expression in select brain regions. In these regions there was also a significant increase in the levels of MOPr-DOPr heteromers and of MOPr but no changes in the levels of DOPr, CB_1 cannabinoid or D_2 dopamine receptors. Together these results suggest that opioid ligands selectively regulate RTP4 expression and this leads to modulation of MOPr-DOPr heteromers levels *in vivo*.

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T1.05

Inhibition of the Sonic Hedgehog pathway prevents morphine tolerance.

Stephanie Puig, Howard B. Gutstein

University of Pittsburgh - UPMC, Pittsburgh, PA, USA

Morphine is the gold-standard treatment for chronic pain. However, with repeated dosing, the analgesic effect of opioids decreases. The mechanisms underlying opioid tolerance remain poorly understood. We recently made the groundbreaking discovery that Cyclopamine, an inhibitor of the Sonic Hedgehog (SHh) signaling pathway can block the development of morphine tolerance centrally and in the periphery. Because Cyclopamine is known to have off-target effects, this study aims at confirming the expression of SHh effectors in the pain processing structures and their involvement in the development of morphine tolerance.

Male Sprague-Dawley rats were injected intrathecally (i.t.) with morphine in combination with a specific inhibitor of the SHh signaling pathway components (Robotnikinin: SHh inhibitor, Vismodegib: Smoothened inhibitor, or GANT58: Gli inhibitor). The development of tolerance was assessed by measuring the thermal paw withdrawal latency for 5 consecutive days. Using immunohistofluorescence coupled to confocal microscopy imaging, we also defined the specific types of cells expressing the smoothened receptor in the dorsal root ganglia (DRG) and the substantia gelatinosa (SG) in basal conditions.

We found that inhibiting SHh, smoothened or Gli individually, completely eliminated morphine tolerance. We also discovered that smoothened is expressed in the pain-processing structures that are known to express the mu-opioid receptor.

We confirmed that the SHh signaling pathway is an important component in the development of morphine tolerance to analgesia. We hope that the advances generated by this study, could impact not only our understanding of the molecular and cellular mechanisms underlying opioid tolerance, but also the treatment of chronic pain.

T1.06

The accumbal dynorphin-kappa opioid system mediates pain-induced alterations in natural and opioid reward seeking

Nicolas Massaly[1], Adrianne Wilson-Poe[1], Ream Al-Hasani[1], Lucia Hipolito[3], Tamara Markovic[1], Dionnet Bhatti[1], Catherine Cahill[2], Michael R. Bruchas[1], Jose A. Moron[1]

[1]Dept of Anesthesiology - Washington University, St. Louis, MO, USA, [2]Dept of Anesthesiology - University of California, Irvine, CA, USA, [3]University of Valencia, Valencia, Spain

Persistent inflammatory pain induces changes in mood and the rewarding value of natural reinforcers and drugs. Although the circuitry and mechanisms underlying these adaptations are not fully understood, a likely factor is altered dopamine release in the nucleus accumbens. Dopamine release is tightly regulated by endogenous opioids and their receptors. Adaptations in the expression and function of these opioid receptors can alter dopaminergic transmission in the nucleus accumbens and could explain our previously observed rightshift in dose-response for heroin self-administration during inflammatory pain (Hipolito *et al.*, J. Neurosci, 2015). The kappa opioid system mediates dysphoria and this has been strongly linked to increasing the risk for drug abuse. The kappa opioid system negatively regulates dopamine release in the nucleus accumbens so we hypothesized that this system may play a key role in pain-induced alterations in intake and motivation for rewards. To assess this, we used a combination of electrophysiology, behavioral pharmacology, chemogenetics and optogenetics, to dissect the kappa opioid system involvement in pain-induced alterations in the rewarding properties of opioids and natural reinforcers. Our multi-modal approach revealed that inflammatory pain enhances the dynorphin-kappa opioid system in the nucleus accumbens, which then negatively impacts the motivation to self-

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administer sucrose in rats. Thus, targeting this dynorphin/KOR system might restore dopaminergic transmission and the rewarding value of reinforcers during pain. The goal of our ongoing work is to determine how the kappa opoid system alters the rewarding properties of opioids during pain and to characterize its involvement in the development of opioid addiction.

T1.07
Therapeutical Potential of Neuropeptide S in opioid addiction
P Ghazal
COMSATS, institute of information technology, Islamabad, Pakistan

Neuropeptide S has been the most recently deorphanized of all the neuropeptides. In 2004, Neuropeptide S (NPS) was identified to be the cognate ligand of the previously discovered orphan receptor GPCR 154, now termed as NPS receptor (NPSR). Since, then a wealth of data has elucidated the unique behavioral profile of this peptidergic system in modulation of numerous physiological function, including anxiety, reward and motivation. Neuropeptide S protective role has been documented in rodent models of opioid addiction. Due to rather small size and highly selective pharmacological profile, NPS system can serve as a potential therapeutical target for drug addiction. A number of peptide and non-peptide NPSR ligands have been synthesized with good selectivity and efficacy and have been tested in a range of psychiatric conditions including alcohol and cocaine dependence. The identification of a SNP in NPSR1 gene as a risk factor for many psychiatric conditions including, alcohol addiction, highlights the translational value of this peptide system. This talk will discuss the therapeutical potential of NPS in oppioid addiction in the light of data obtained up-to-date on this system.

T1.08
Development and Evaluation of a Specialist Multidisciplinary Opioid Reduction Clinic for Patients with Persistent Non-malignant Pain on High Dose Opioid Therapy
Fauzia Hasnie, Joanne Smart, Beth Guildford
INPUT Pain Management Centre, Guy's and St. Thomas' NHS Foundation Trust, London, UK

Introduction: With the escalation in prescription opioid use over the last 20 years, opioid-related morbidity and mortality (addiction; endocrine and immune dysfunction; and opioid-induced hyperalgesia) has increased (1). However, there is no evidence for the efficacy of high dose opioid therapy in management of persistent non-malignant pain (2). It is therefore necessary to change the pattern in which opioids are prescribed and improve patient outcomes. This clinical need has led to the development of a dedicated multidisciplinary outpatient service aimed at rationalising high dose opioid medication in chronic pain patients.

Methods: The 'Opioid Reduction Clinic' is led by a Pain Physician, Nurse and Psychologist. Patients are educated on opioid-related risk and non-opioid strategies for pain management. Clinical outcomes relating to function and quality of life; psychological co-morbidity and risk of aberrant drug-related behaviour were evaluated by validated questionnaires at assessment and at the end of a tailored weaning programme. Biochemical evidence of hypogonadism was assessed and correlated with clinical impact.

Results: Patients demonstrated 75% impairment in function and quality of life; with 60% suffering severe depression; and 62% being high risk for aberrant drug-related behaviour. In those patients who successfully tapered opioids, there was 50-60% improvement in outcomes globally. Biochemical evidence of hypogonadism did not correlate with clinical impact.

Discussion: There is a clinical need, that is currently unmet, to effectively manage chronic pain patients on high dose opioid therapy with greater support in a multidisciplinary setting.

(1) Okie S (2010). *N Engl J Med* **363:** 1981-1985.
(2) https://www.fpm.ac.uk>opioids-aware

T1.09
Oxycodone self-administration in male and female rats
Maria Mavrikaki[1], Marco Pravetoni[2], Sarah Page[1], David Potter[1], Elena Chartoff[1]
[1]Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, MA, USA, [2]Departments of Medicine and Pharmacology, University of Minnesota, Minneapolis, MN, USA

The prescription opioid oxycodone is one of the most widely prescribed painkillers in the US. However, its use is complicated by high abuse potential. Since sex differences have been described in most stages of drug addiction, the present study tests if there are sex differences in oxycodone intravenous self-administration. Male and female Sprague-Dawley rats were implanted with jugular vein catheters and trained to self-administer oxycodone (0.03mg/kg/infusion). Rate of acquisition and maintenance of self-administration behavior on fixed ratio 1 (FR1), FR2, and FR5 schedules of reinforcement were measured. In addition, sensitivity to the reinforcing effects of oxycodone (dose response), and motivation to work for oxycodone (progressive ratio) were measured. In a separate cohort of rats, distribution of oxycodone to plasma and brain were measured after intravenous delivery. On an FR1 schedule of reinforcement, male rats self-administered more oxycodone than females. On FR2 and FR5 schedules, no significant sex differences in drug intake were observed, although females had significantly more inactive lever presses than males. In the dose response experiment, females tended to self-administer more oxycodone across doses, but this effect was not significant. Similarly, there was a trend for females to work harder for oxycodone in the progressive ratio experiment. No significant sex differences were observed in plasma or brain oxycodone levels, suggesting that sex differences in oxycodone self-administration behavior are not due to pharmacokinetics. Taken together, our results suggest that there are sex differences in abuse liability of oxycodone, which has ramifications for the treatment of oxycodone dependence and abuse.

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T1.10

Oxytocin Efficacy in Treating Detoxified Heroin Dependent Individuals: A Randomised Double Blind Placebo Controlled Pilot Trial

Carol Weber[2], Christos Koumtsidis[3], Julia Boyle[2], Vicky Revell[2], Susanna Hourani[2], Ramin Nilforooshan[3], Alexis Bailey[1,3]

[1]St. George's University of London, London, UK, [2]University of Surrey, Surrey, UK, [3]Surrey and Borders Partnership NHS Trust, Surrey, UK

The biggest hurdle in treating recovering heroin addicts is the maintenance of a long term drug free or abstinent state, as around 70% of addicts relapse back to their drug-taking following 1 month detoxification. Preclinical evidence from our laboratory has revealed that administration of oxytocin analogue carbetocin is able to reverse the negative emotional aspects that opioid dependent individuals experience during abstinence from the drug and to prevent relapse to drug seeking (1).

We are currently conducting a pilot study which aims to assess the efficacy of intranasal oxytocin administration on heroin relapse prevention, on completing detoxification and on anxiety, depression and social anxiety levels in post-detoxified heroin dependent individuals undergoing an inpatient detoxification program at Windmill House, Surrey, UK. Detoxified heroin dependent individuals are given 6 insufflations (24 IU) intranasal oxytocin (Syntocinon) (n=15) or placebo (n=15) twice a day for 14 days. These subjects also undergo a 30 day post treatment follow-up. Relapse rates and retention in rehabilitation unit are monitored. Anxiety, depression, social anxiety and sleep disturbances, measured through validated standardised questionnaires and psychological tests are assessed at baseline and at specific time points throughout the study and at follow up. We anticipate the first results of the study in March 2017.

(1) Zanos P *et al.* (2014) *Neuropsychopharmacology* 39 (4) 855-865.
(2) Heinrichs M. *et al.* (2003) *Biological Psychiatry* 54 1389-1398.

T1.11

Oxycodone self-administration induces changes in expression of axon guidance molecules in the caudate putamen of adult male mice

V Yuferov[1], Y Zhang[1], Y Liang[2], C Zhao[3], M Randesi[1], MJ Kreek[1]

[1]The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY, USA, [2]Hospital Informatics, the Rockefeller University, New York,NY, USA, [3]Genomic resource Center, the Rockefeller University, New York, NY, USA

Axon guidance molecules such as semaphorins and ephrins and their receptors, plexins and neuropilins, modulate the formation of axon-target connections and synapse formation. The aim of this study is to investigate the expression of axon guidance genes in the dorsal striatum of mice following oxycodone self-administration using RNAseq technology. The caudate putamen was isolated from C57BL/6J mice after self-administration of oxycodone (0.25 mg/kg/infusion) 4h/day for 14 consecutive days or from saline controls. RNA-Seq libraries were prepared using Illumina's TruSeq® Stranded Total RNA LT kit. Illumina HiSeq 2500 was used to obtain 100 bp reads. DESeq2 was applied to estimate the expression fold change in the oxycodone vs saline groups. FDR p-values of less than 0.05-0.1 were used to select genes that have a significant expression change. RNA-seq data revealed upregulation of integrin alpha L and beta 2, and down-regulation semaphorin receptor plexin C1 (Plxc1). RT-PCR validated expression of two genes, Itgal and Plxdc1 in the CPu. Further examination of oxycodone-induced changes in expression of axon guidance genes may provide better understanding in mechanisms of oxycodone addiction.

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T1.12

Oxycodone-Induced Conditioned Place Preference is Reduced in a Humanized Fatty Acid Aromatic Hydrolase (FAAH) Knock-in Mouse Line.

Derek Simon[1], Francis Lee[2], Mary Jeanne Kreek[0]

[1]Rockefeller University, New York, NY, USA, [2]Weill Cornell Medical College of Cornell University, New York, NY, USA

There is evidence of bi-directional regulation of the endocannabinoid and opioid systems. However, the role of endocannabinoids in the addictive properties of opioids is unknown but a topic of critical importance. Fatty acid aromatic hydrolase (FAAH) is the primary enzyme that degrades the endogenous CB1 receptor agonist anandamide (AEA). The FAAH C385A (P129T) polymorphism is has been correlated to alcohol and drug use in humans. This mutation results in dysfunction of the FAAH protein enzymatic activity and subsequent sustained elevation of AEA levels throughout the brain. We examined oxycodone addictive-like behaviors in a knock-in mouse line expressing the mutant FAAH (homozygote: $FAAH^{A/A}$; heterozygote: $FAAH^{C/A}$). We observed a statistically significant reduction in conditioned place preference (CPP) for oxycodone in adult $FAAH^{A/A}$ and $FAAH^{C/A}$ compared to $FAAH^{C/C}$ mice. While $FAAH^{C/C}$ mice exhibited locomotor sensitization to oxycodone, this effect was blunted in the mutants. We identified an upregulation in the expression CB1 mRNA, but not MOPR mRNA, in the CPu of $FAAH^{A/A}$ and $FAAH^{C/A}$ mice. However, no differences in gene expression of CB1 or MOPR mRNA were detected in the NAc. Also, no differences in endocannabinoid content in the NAc were found. Interestingly, we observed no significant differences in CPP or locomotor sensitization between genotypes for adolescent animals. We are currently examining oxycodone-induced analgesia in adult vs adolescent mice. These results suggest adult but not adolescent carriers of the C385A allele may be resistant to opioid reward. Funding: Kopf Family Postdoctoral Fellowship (DPS), Dr Miriam and Sheldon G. Adelson Medical Research Foundation (MJK).

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T1.13

A Novel Heroin Conjugate Vaccine Induces High Affinity Antibodies to Heroin and Abrogates Nociceptive and Behavioral Effects of Heroin

GR Matyas[1], R Jalah[1,2], JFG Antoline[3,4], OB Torres[1,2], F Li[3,4], AE Jacobson[3,4], KC Rice[3,4], Z Beck[1,2], CR Alving[1,2]

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

Background: An effective heroin vaccine needs to induce high levels of antibodies that bind heroin and its metabolites with high affinity. Antibody-bound heroin cannot cross the blood-brain, thereby, blocking the effects of heroin. We developed a novel heroin conjugate vaccine that elicits significantly higher antibody affinities to heroin than previously reported haptens. This new vaccine protected rodents from intravenous heroin challenge.

Methods: The heroin hapten (6-AmHap) was synthesized and conjugated to tetanus toxoid (TT). The TT-6-AmHap vaccine, adjuvanted with liposomes containing monophosphoryl lipid A, was compared to a morphine-like hapten (TT-MorHap) vaccine in mouse and rat models for antibody titer and affinity to heroin, its metabolites and other opioids. Efficacy was assessed by subcutaneous and intravenous heroin challenge.

Results: The TT-6-AmHap vaccine significantly reduced heroin-induced antinociception and locomotion behavioral changes following subcutaneous and repeated intravenous heroin challenges in mice and rats. The vaccine elicited very high IgG levels of ~1.2 mg/mL. Competition ELISA demonstrated that 6-AmHap-induced antibodies had significantly higher affinities than TT-MorHap-induced antibodies to heroin and its metabolites, 6-acetylmorphine (6AM), morphine, morphine-3-β-glucuronide and morphine-6-β-glucuronide. Using equilibrium dialysis with UPLC-MS/MS quantification, the K_d values of the 6-AmHap-induced antibodies to 6AM and morphine were ≤0.5 nM and the % heroin bound was ≥90, while MorHap-induced antibodies had 10-fold higher K_d and had low heroin binding. In addition, 6-AmHap antibodies crossreacted with abused prescription opioids like hydrocodone, hydromorphone, oxymorphone, codeine and levorphanol.

Conclusions: TT-6-AmHap is an improved vaccine candidate that may be developed into a therapeutic for heroin and opioid abuse.

T1.14

Cocaine-induced regulations of the endocannabinoid system in reward-related brain regions

K Befort[1,2], D De Sa Nogueira[1,2], D Filliol[1,2], M Fonteneau[1,2], P Romieu[1,2], P Anglard[1,2], J Zwiller[1,2]

[1]Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Université de Strasbourg, Strasbourg, France, [2]CNRS UMR 7364 LNCA, Strasbourg, France

Drug addiction is a complex pathology inducing a long-term neuroplasticity. Although many individuals are exposed to drugs of abuse, only a subset experience the loss of control over drug use and compulsion for drug seeking and taking that defines the addicted state. Thus, understanding the neurochemical mechanisms underlying the reinforcing effects of drugs of abuse is critical for reducing the burden of drug addiction in society. Among the neurobiological mechanisms involved in addictive behaviors, epigenetic processes are emerging as crucial mediators of the long-term adaptations produced by drugs of abuse. The endogenous cannabinoid system is involved in the modulation of drug reward, in particular in cocaine addiction. Whether this occurs through epigenetic process is not well described.

Using a genome-wide methylation analysis in the prefrontal cortex of rats self-administering cocaine, we identified methylation regulation of a cannabinoid-related gene, cnrip. We therefore further analyzed transcriptional regulations of various components of the endocannabinoid system including enzymes for the endocannabinoids synthesis and degradation and two well-characterized receptors, cannabinoid receptors CB1 and CB2. We explored the effects of acute or chronic passive injections as well as a voluntary cocaine administration paradigm. Analysis of protein regulation and functional adaptations were also conducted. We focus our study on brain regions related to reward circuits, including striatum, prefrontal cortex, and hippocampus. Interestingly, cocaine produced significant changes in the level of endocannabinoid-related genes and proteins. Overall, this project will clarify the role of the endocannabinoid system in neuroadaptative processes involved in response to cocaine, leading to dependent state.

T1.15

Antagonism at the nociceptin/orphanin FQ opioid peptide receptor (NOP) decreases nicotine taking in a rat model of nicotine and alcohol co-administration

A Cippitelli[1], J Schoch[1], G Debevec[1], G Brunori[1], NT Zaveri[2], L Toll[1]

[1]Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA, [2]Astraea Therapeutics, Mountain View, CA, USA

Alcohol and nicotine are often co-abused. Although the N/OFQ-NOP receptor system is considered a potential target for development of drug abuse pharmacotherapies, especially for alcoholism, little is known about the role of this system in nicotine dependence. Furthermore, the effect of prior history of nicotine dependence on subsequent nicotine and alcohol taking is understudied. Using an operant co-administration paradigm, in which rats concurrently self-administer nicotine and alcohol, we found that nicotine dependent rats increased nicotine self-administration over time as compared to non-dependent animals, while patterns of alcohol lever pressing did not change between groups. Pretreatment with the potent NOP receptor agonist AT-202 (0.3, 1.0, 3.0 mg/kg, i.p.) increased nicotine lever pressing of both dependent and non-dependent groups, whereas the selective antagonist SB612111 (1, 5, 10 mg/kg, i.p.) elicited a clear reduction of nicotine responses, in both dependent and non-dependent rats. In parallel, AT-202 only produced minor changes on alcohol responses and SB612111 reduced alcohol taking at a dose (10 mg/kg) that also reduced locomotor behavior. Results indicate that a history of nicotine dependence affects subsequent nicotine- but not alcohol-maintained responding, and that NOP receptor antagonism, rather than agonism, blocks nicotine self-administration, which strongly suggests a critical role for the endogenous N/OFQ in the modulation of nicotine reinforcement processes.

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T1.16

Substance Abuse among Female Sex Workers in Osogbo, Nigeria

A Adelekan[1], P Omoregie[2], E Edoni[3]

[1]University of Ibadan, Ibadan, Oyo State, Nigeria, [2]Society for Family Health, Uyo, Akwa-Ibom State, Nigeria, [3]Niger-Delta University, Yenagoa, Bayelsa State, Nigeria

Female Sex Workers (FSWs) who inject drugs experience an elevated burden of HIV and other negative health outcomes compared to sex workers who do not inject drugs [1]. This study therefore designed to explore substance abuse among FSWs in brothels in Osogbo, Nigeria.

The study was a descriptive qualitative study that utilized in-depth interviews. All consenting 38 out of 46 identified FSWs who use drugs were selected using snowball sampling technique. Data was recorded with tape recorder. Interview transcripts were coded by question topics, and respondents' spontaneous and prompted comments were analyzed for content concerning subthemes and questions posed during the interviews. Data analysis was performed using thematic approach.

Age of respondents ranged from 18 to 45 with mean age of 24.4 ± 8.4 years. Some (33.1%) of the respondents were single and 8.2% had no formal education. All the respondents had ever abused codeine in cough syrups and tramadol while cocaine and codeine were most currently consumed drugs reported among respondents. Others are heroin, Captain black and Marijuana. Majority of the respondents reported sharing needles and injecting equipment to inject drugs and reasons adduced for substance use included to escape from intense burden of sex work, to become bold and confident to negotiate with clients, and to be strong in bed to attract more clients.

Substance abuse among respondents was high and they attributed this to the nature of their work. Integrating drug education into existing health programme for sex workers should be encouraged to ameliorate this problem.

T1.17

NOP Receptor Antagonism Decreases Alcohol Consumption: A Preclinical Evidence

G Brunori[1,2], G Scuppa[1], A Cippitelli[2], M Gorman[2], NT Zaveri[3], G Calò[4], L Toll[2], R Ciccocioppo[1]

[1]University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy, [2]Torrey Pines Institute for Molecular Studies, Department of Neuropharmacology, Port Saint Lucie, Florida, USA, [3]Astraea Therapeutics, LLC, Mountain View, California, USA, [4]University of Ferrara, Department of Medical Science, Section of Pharmacology and National Institute of Neuroscience, Ferrara, Italy

The Nociceptin/Orphanin FQ (N/OFQ) peptide and its cognate receptor (NOP) are widely distributed in brain areas involved in reward and motivation, supporting a role of the N/OFQ-NOP system in the regulation of addiction-related behavior. Activation of NOP receptors has been repeatedly demonstrated to blunt the reinforcing and motivating effects of various abused drugs, including alcohol. Interestingly, recent evidence shows that administration of NOP antagonists can also attenuate alcohol consumption. In the present study we used pharmacological and genetic manipulations of the NOP system to further explore its role in alcohol abuse. When exploring binge-like alcohol consumption in C57BL/6J mice using the "drinking in the dark" (DID) paradigm, we found that the potent agonist AT-202 (0, 0.3, 1.0, 3.0 mg/kg, i.p.) failed to reduce excessive alcohol consumption. Conversely, treatment with the selective antagonist SB-612111 (0, 3, 10, 30 mg/kg, i.p.) decreased binge-like alcohol drinking. SB-612111 also reduced alcohol preference over water in a two-bottle DID model, while leaving sucrose intake unaltered. NOP knockout [NOP(-/-)] rats were used to study the motivation for alcohol self-administration in the absence of N/OFQ function. NOP(-/-) rats showed reduced propensity to self-administer alcohol compared to NOP(+/+). This effect did not appear to be linked to disruption of reward mechanisms, as saccharin self-administration did not differ between genotypes. Pharmacological blockade of NOP receptors by SB-612111 (0, 3, 30 mg/kg, os) attenuated alcohol self-administration in NOP(+/+) but not NOP (-/-) rats. Altogether, these results support a role for NOP antagonism as a potential treatment for alcohol abuse.

T1.18

Characteristics of successful medication free prolong abstinence of subjects with opioid disorder

E Peles[1,2], E Levy-Gigi[3], S Schreiber[1,2], M Adelson[1]

[1]Dr Miriam & Sheldon G. Adelson Clinic for Drug Abuse, Treatment & Research, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel, [2]Sackler Faculty of Medicine Tel Aviv University, Tel Aviv, Israel, [3]University of Haifa, Haifa, Israel, Israel

Background: Opioid addiction is best treated by methadone maintenance treatment (MMT). Still, there are few (<10%) opioid addicts, a minority rarely studied, that succeed to live without maintenance medication.

Methods: We compared between 55 MMT patients for 10 or more years with negative urine tests for drug abuse (heroin, cannabis, amphetamines, benzodiazepine, cocaine) for 2 or more years, and 99 former opioid addicts who have been opioid medication-free for ≥10 years. Comparison included demographic, addiction history, lifetime psychiatric disorders, sleep indices, and cognitive state (including novel reinforcement learning task test measuring context and cue reversal learning).

Results: Groups were comparable in age and education, but the medication-free subjects were younger when having started opioids, with more severe addiction scores. MMT patients presented with a higher proportion of psychiatric comorbidity and chronic pain. Their scores of perceived sleep quality and cognitive state were poorer than the medication-free individuals. Both groups were equally able to acquire and reverse positive and negative outcomes in conditions of neutral context. However, MMT patients showed a selective deficit in reversing the outcomes of positive stimulus in drug-related context.

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Conclusions: The reason why the MMT patients failed to maintain prolonged abstinence could be attributed to their higher psychiatric comorbidity, chronic pain, poor sleep, or selective difficulty to learn negative outcomes when exposed to a drug, but not neutral, related environment. Each of the differences could be related to a genetic predisposition. The differences may serve as "biomarkers" to enable identify the minority who may succeed prolonged abstinence.

T1.19

Astrocytes-targeted TLR4 Knockdown by lentiviral vectors in the brainstem periaqueductal gray reduced chronic morphine physical withdrawal through blocking TNFα release.

Shue Liu, Qiaofeng Liu, Hyun Yi, Takafumi Iida, Gerald Zhuang, Roy C Levitt, David A Lubarsky, Shuanglin Hao
University of Miami, Miami, FL, USA

Physical dependence (withdrawal syndrome) is a major cause of compulsive drug-taking behavior and short term relapse. Preventing the transition from drug use to drug dependence has significant implications. Recent studies show that chronic morphine activates glial toll-like receptor 4 (TLR4) and induces the release of proinflammatory cytokines (e.g. tumor necrosis factor alpha (TNFα)). Functional studies implicate the periaqueductal gray (PAG) in the midbrain as an important locus that mediates many of the physical/behavioral manifestations of opioid withdrawal. In this study, we used Lentiviral vectors to astrocytes-selectively knockdown the expression of TLR4 in the PAG and tested the effect of the lentiviral vectors on morphine withdrawal (MW). MW induced overexpression of glial GFAP, TLR4, NF-kB, and TNFα in the PAG. TLR4 and TNFα were expressed in the glial cells. Microinjection of glial inhibitors (MIF and fluorocitrate) into the PAG inhibited the MW behavioral response. Microinjection of TLR4 selective agonist KDO2 into the PAG induced MW-like behavioral response. Microinjection of selective TLR4 inhibitor LPS-RS, reduced MW response and TNFα. Astrocytes-targeted TLR4 knockdown by lentiviral vectors expressing TLR4 shRNA into the PAG, suppressed MW response. The results suggest that astrocytes TLR4 activity and increased proinflammatory factors are involved in the MW response, and reduction of glial activity/proinflammatory molecules inhibits MW, providing a new approach to treating opioid dependence. Acknowledgments: Supported by NIH R03DA26734, R21DA25527, R01NS66792, and R01DA034749 to S.H.

T1.20

C7beta-Methyl analogues of the orvinols, demonstrating potential therapeutic application for relapse prevention in poly-habitual drug abuse

A Disney[1], M Ostovar[1], T Hillhouse[2], J Hallahan[2], JR Traynor[2], SM Husbands[1]
[1]University of Bath, Bath, Somerset, UK, [2]University of Michigan, Ann Arbor, Michigan, USA

A combination of buprenorphine and the MOR antagonist naltrexone has been demonstrated to be effective in reducing relapse to both opioid and cocaine use [ ]. A single ligand that could mimic this combination would be of significant interest as a drug abuse therapy.

We have evidence that an analogue of buprenorphine, BU127 bearing an aryl ring in place of the buprenorphine C20 t-butyl group, displays MOR and KOR antagonism while also exhibiting similar to buprenorphine affinity for NOP receptor in-vitro.

We have demonstrated moving the C-20 methyl group to the C-7(beta) position provides a series of compounds with a desirable poly-pharmacological profile.

Many of the ligands show higher efficacy and equivalent or better potency at NOP receptors when compared to buprenorphine and importantly possess the desired low MOR efficacy as well as KOR antagonist activity as measured by the [35S]GTP gamma S assay. Molecular modelling using the crystal structure of the human KOR explains the very low efficacy of this series of compounds at this receptor. In-vivo evaluation of lead compounds confirms the in-vitro profile, with MOR, KOR, DOR antagonism and NOP partial agonism. Further in-vivo evaluation including measures of relapse to drug taking will be presented. This work was supported by NIDA grant, DA07315.

[1] Gerra, G.; Fantoma, A.; Zaimovic, A. J. *Psychopharmacol.* 2006, 20, 806-814.

T1.21

Polypharmacology by heroin users: influence of other abused drugs on opioid depression of respiration

A Lyndon, S Audrey, M Hickman, G Henderson
University of Bristol, Bristol, UK

In fatal opioid overdose other drugs are often found along with opioids at post-mortem. Such drugs include alcohol, crack cocaine, benzodiazepines, and increasingly pregabalin/gabapentin [1].

Qualitative interviews were conducted at the Bristol Drugs Project to establish the motivations behind polydrug use among active heroin users. Interviews revealed that the majority of heroin users co-use heroin alongside crack cocaine in the same injection; termed a “snowball”. The interviews also shed some light on heroin users concerns about the use of pregabalin along with heroin. Participants stated that pregabalin turned individuals into zombies and the concoction often felt like being drunk and when used alongside heroin it was suggested that the combination could be deadly.

Respiration was measured in mice using whole body plethysmography [2]. Using tail vein injection we compared the effect of a combination of cocaine (5 mg/kg) and morphine (7.5 mg/kg) to morphine (7.5mg/kg) alone. The combination did not produce any additional respiratory depression than morphine alone. Pregabalin (40-400 mg/kg ip) depressed respiration. When given along with morphine (10 mg/kg ip) the drugs acted addictively to depress respiration. Pregabalin did not reverse morphine tolerance in morphine tolerant animals.

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While ethanol reverses morphine tolerance to respiratory depression [2], pregabalin acts addictively with morphine to increase the risk of fatal overdose, while cocaine has no effect on respiratory depression.

1. Hickman M. *et al.*, 2008 *Addiction* 103: 1060-1062
2. Hill R. *et al.*, 2015 *Neuropsychopharmacology* 41:762-773

T1.22

Investigating The Roles Of Kappa Opioid Receptors In Neurochemical Changes Caused By Stress And Drugs Of Abuse

Q Ma, S Wonnacott, S Bailey, C Bailey
University of Bath, Bath, UK

Stress is a well-known risk factor for the development of drug addiction and relapse back to drug-taking. There is growing evidence that kappa opioid receptors are involved in both stress responses and addiction-related behaviour (1).

We are using an immunohistochemical approach to investigate brain regions that are activated by stress and drugs of abuse, and to disclose the role of kappa opioid receptors in these responses. Expression of the immediate early gene c-Fos is a neuronal marker of recent neural activity. We are using c-Fos-GFP transgenic mice, where expression of green fluorescent protein (GFP) is driven by the activation of c-Fos (2). This provides an index of brain regions activated in these mice following an acutely stressful event (forced-swim test, or restraint). Following perfusion, expression of c-Fos and c-Fos-driven GFP, across multiple brain regions, is then assessed in brain sections using infra-red fluorescence immunohistochemistry (3). The involvement of kappa opioid receptors to these c-Fos expression changes is examined by administering kappa opioid receptor antagonists during stress induction.

The findings will provide insight into the effects of stress and drugs of abuse in different brain regions, and the role of kappa opioid receptors in mediating these effects.

1. Bruchas MR *et al.* (2010) Brain Res 1314:44-55.
2. Barth AL *et al.* (2004) J Neurosci 24:6466-75.
3. Eaton SL *et al.* (2016) J Anat 228:203-13.

T1.23

Investigating Brain Region-Specific Synaptic Plasticity Induced by Conditioned Place Preference to Morphine

JC Palandri[1], DJ Heal[2], S Wonnacott[1], CP Bailey[1]
[1]University of Bath, Bath, UK, [2]RenaSci, Nottingham, UK

In opiate addiction, one factor contributing to the high risk of relapse is drug-paired learning: Pavlovian conditioning where environmental cues are linked with the drug of abuse (1). This can be modelled using conditioned place preference (CPP). In this study we are using a combination of immunohistochemistry and western blotting to assess synaptic plasticity changes across multiple brain regions, including the prefrontal cortex and hippocampus, following morphine-induced CPP.

CPP was induced in c-Fos-GFP transgenic mice (2) (male, 7-8 weeks old) by morphine (10 mg/kg, i.p.). Preference was extinguished, then reinstated using a priming dose of morphine (5 mg/kg, i.p.). Significant preference was seen at both the expression and reinstatement phases. Two hours following reinstatement, brains were taken and processed for immunohistochemistry or Western blotting. Using infrared fluorescence (3), cFos expression in multiple brain regions is monitored, acting as an indicator of recent neuronal activity. Following subcellular fractionation to give postsynaptic densities, Western blotting is used to assess levels of total and phosphorylated synaptic AMPA receptors, taken as an index of synaptic plasticity (4).

These complimentary approaches will provide a view of neuronal activation and plasticity changes that is both localised and comprehensive, at different stages of the CPP paradigm.

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1. Feltenstein MW and See RE (2013) Cold Spring Harb Perspect Med 3:a011916
2. Barth AL *et al.* (2004) J Neurosci 24:6466-75.
3. Eaton SL *et al.* (2016) J Anat 228:203-13.
4. Billa SK *et al.* (2009) Eur J Neurosci 29:55-64.

T1.24

Acquisition and reinstatement of ethanol intra-VTA induced conditioned place preference in rats: role of mu-opioid receptors.

L Hipólito, L Marti-Prats, Y Campos-Jurado, MJ Cano-Cebrián, T Zornoza, A Polache, L Granero
Dpt. Pharmacy and Pharm. Tech. University of Valencia, Valencia, Spain

Mechanisms underlying the motivational properties of ethanol are still a matter of debate. Ethanol-context learned associations play an important role in those motivational properties being conditioned place preference (CPP) a valid model to evaluate them. A considerable amount of data has shown that motivational properties of ethanol are related to activation of mu-opioid receptors (MORs) in the mesocorticolimbic system (MCLS). However the exact mechanism by which ethanol activates these receptors is not fully understood. Our previous data reveal that salsolinol, an ethanol metabolite, activates and induces dopamine-dependent behaviours when administered in the MCLS. More interestingly, these effects are probably mediated by direct interaction of salsolinol with local MORs. Here, we investigate the acquisition and reinstatement of ethanol induced CPP in Wistar rats when administered directly into the posterior ventral tegmental area (pVTA) and the role of the MORs in the observed behaviour. The administration of

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ethanol (0, 35, 70, 150 and 300 nmol, n=6-8) into the pVTA induced a dose dependent place preference being 70 nmol the most active dose. Furthermore, after extinction of ethanol-CPP, an active dose of salsolinol (30 pmol, n=5) reinstated the preference. Finally, we investigated the specific role of MORs located in pVTA by administering two doses of β-funaltrexamine (2,5 nmol, n=5). Our results show, for the first time, that ethanol dose-dependently induces CPP when administered locally in the pVTA through an activation of the MORs. Our data also supports the critical role of salsolinol in the motivational properties of ethanol.

T1.25

Modulation of dopamine release mediated by mu-opioid receptor in rat striatum: a regional-dependent study

L Hipólito, Y Campos-Jurado, L Martí-Prats, A Bahilo, T Zornoza, MJ Cano-Cebrián

Dpt. Pharmacy and Pharm. Tech. University of Valencia, Valencia, Spain

The nigrostriatal dopamine (DA) system is implicated in the regulation of motivation, reward and motor activity. It is widely known that DA release in the dorsal striatum (DS) is mainly controlled by the firing rate of DA neurons in the substantia nigra. However, linfluences at terminal level, such as those involving the activation of mu opioid receptors (MORs), can play a key role in determining DA levels in striatum. In fact, several studies have demonstrated this modulation. Nonetheless, published data also suggest that the effect of opioid drugs on DA levels may completely differ depending on the DS subregion analyzed. In this study, microdialysis in freely moving rats was used to explore this regional dependence of the modulating effect of MORs. Here we evaluate the changes in DA levels induced by local administration of DAMGO (a selective MORs agonist) in three different subregions of the DS defined along its rostro-caudal axis. Our results indicate that the administration of 10μM DAMGO (20 minutes, 3.5 μL/min) into the rostral and caudal DS significantly reduced DA levels. Conversely, the same dose of DAMGO significantly increased DA levels in the medial DS. These data reveal a regional-dependent MORs modulation of DA release in the DS, similar to that previously described by our group in the ventral striatum along its medio-lateral axis. These findings may drive us to a better understanding of the nigrostriatal DA system, which will help to improve the present pharmacological strategies aimed at restoring DS functioning.

T1.26

Mouse model of OPRM1 A118G has effects on basal neuropeptide and neuropeptide receptor gene expression in addiction-related brain regions

Devon Collins, Matthew Randesi, Yong Zhang, Mary Jeanne Kreek

The Rockefeller University, New York, New York, USA

A common single nucleotide polymorphism (SNP) in the human mu-opioid receptor (MOR) gene (*OPRM1*), A118G, has been demonstrated to alter mu-opioid receptor function, thereby significantly altering stress responsivity in healthy humans. Mice bearing an equivalent SNP, A112G, in the mouse MOR gene were generated, characterized, and studied (Mague *et al.*, 2009). We have demonstrated genotype-dependent differences in response to endocrine challenge in healthy human 118G carriers (Ducat *et al.*, 2011) and in opioid self-administration in 112G mice (Zhang *et al.*, 2015). MOR activation leads to a series of downstream effects, including changes in gene expression. Thus, the A118G variant may effect changes in MOR-modulated gene expression. We have previously developed an array of stress-modulating and neuropeptide genes that are associated with mu-opioid dependence and MOR function in humans and rodents. Here, we measure the baseline mRNA expression of these genes in the caudoputamen, nucleus accumbens, and hypothalamus in minimally handled, drug-naïve, male and female mice homozygous for either the 112A or 112G allele. In the caudoputamen, *Oprk1, Oprm1*, and *Pdyn* mRNAs were significantly upregulated in 112G mice versus 112A mice. In the nucleus accumbens, *Oprl1* mRNA levels were upregulated in 112G mice versus 112A mice. In the hypothalamus, *Avp, Oxt, Gal*, and *Penk* were upregulated in 112G mice versus 112A mice. Thus, the A112G SNP may cause changes in basal gene expression that alter MOR-associated behavior, including stress responsivity and the specific disease of mu-opioid addiction.

T1.27

Prevalence and Correlates of Nonmedical Prescription Opioid Use Among a Cohort of Sex Workers in Vancouver, Canada

E Argento[1,2], J Chettiar[2], P Nguyen[1], J Montaner[1,2], K Shannon[1,2]

[1]BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada, [2]University of British Columbia, Vancouver, BC, Canada

Introduction: Nonmedical prescription opioid use (NPOU) is a major public health concern, causing extensive morbidity and mortality. Canada has the second highest consumption rate of POs globally and data indicate NPOU is growing among key populations and increasingly available in street-level drug markets. Despite evidence documenting the rise of NPOU, few studies have systematically examined NPOU among sex workers. This study prospectively investigated the prevalence and correlates of NPOU among women sex workers in Vancouver, Canada over three-years follow-up.

Methods: Data (2010-2013) were drawn from an open prospective cohort of sex workers, AESHA (An Evaluation of Sex Workers Health Access). Participants were recruited through outreach to outdoor and indoor venues. Bivariate and multivariable logistic regression using Generalized Estimating Equations (GEE) were used to examine social and structural correlates of NPOU.

Results: Of 692 sex workers at baseline, nearly one-fifth (n=130, 18.8%) reported NPOU (injection or non-injection) in the last six months. In multivariable GEE analyses, factors independently associated with recent NPOU were: exchanging sex while high (AOR 3.26, 95%CI 2.29-4.64), police harassment/arrest (AOR 1.83, 95%CI 1.43-2.35), a drug injecting intimate partner (AOR 1.66, 95%CI 1.11-2.49), and recent physical/sexual intimate partner violence (AOR 1.65, 95%CI 1.21-2.24).

Conclusion: Findings demonstrate that social and structural factors play a critical role in NPOU among sex workers. The high prevalence of NPOU in this study underscores the urgent need for structural interventions tailored to sex workers. Integrated violence and couples-based intervention efforts must be implemented to ameliorate this growing concern.

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T1.28

Revisiting the Primary Reinforcing Effects of the Delta-Opioid Receptor Agonist SNC80

Emily Jutkiewicz[1], Ashleigh Matthews[1], Kenner Rice[2]

[1]University of Michigan, Ann Arbor, MI, USA, [2]NIDA, NIH, Bethesda, MD, USA

Studies investigating the abuse-related effects of nonpeptidic delta-opioid receptor (DOR) agonists have revealed inconsistent results. While the DOR agonist SNC80 fails to maintain self-administration behavior in rhesus monkeys (1), SNC80 produces conditioned place preference (2) and robustly stimulates locomotor activity (3) in rodents. Therefore, the present study evaluated the primary reinforcing properties of SNC80 in male Sprague-Dawley. Naïve rats were implanted with indwelling intravenous catheters and, following recovery, were placed in operant chambers 5 d/week for 60 min sessions. Responding on the active nosepoke resulted in delivery of SNC80 (0-0.32 mg/kg/infusion) under a FR1 schedule of reinforcement and illumination of the house light followed by a 10 sec timeout. SNC80 maintained responding in a dose-dependent manner yielding an inverted U-shaped function, consistent with other drugs of abuse. At a dose of 0.32 mg/kg/infusion, SNC80 maintained responding in all rats, such that they earned between 30-40 infusions during 60 min sessions. Interestingly, a single convulsion occurred during the first or second infusion during the initial self-administration session only but did not deter responding. As work requirements increased, SNC80 failed to maintain responding and, in the absence of SNC80, responding extinguished. These data suggest that the DOR agonist SNC80 has primary reinforcing effects in rats but likely is a weak reinforcer.

(1) Negus SS *et al.* (1998). J Pharmacol Exp Ther 286:362-375.
(2) Longoni R *et al.* (1998). Behav Pharmacol 9:9-14
(3) Jutkiewicz EM *et al.* (2004). J Pharmacol Exp Ther 309:173-181.

T1.29

Under-recognised Physiological and Socio-Psychological Mental Ill-Health Complications of Opiate Cravings, Addiction and Dependence in Students of Tertiary Institutions

Adenike Ogunshe

University of Ibadan, Ibadan / Oyo, Nigeria

Substance use disorders like cravings and physiological dependence lead to various health complications; however, complications of substance abuse is grossly under-recognised in Nigerian tertiary institutions. Questionnaire administration, oral and telephone interviews and informal, youth-friendly group discussions were combined to investigate physiological and socio-psychological status of opiate-related substance abuse among students of tertiary institutions. Most commonly abused opiate-related substances include codeine, heroin, rohypnol, tramadol, skunk, morphine; while non-opiate-related abused substances were marijuana, nicotine, diazepam, methylphenidate, cocaine, toluene abuse or glue sniffing, hashish. Peer- and entertainment-influence, academic pressures, internet-influence, feelings of euphoria, change in youth priorities / life-style patterns, weakness in domestic-control, gross / total lack of appropriate students' welfare systems, especially off-campus system of students' accommodation and lack of restrictions in purchasing over-the-counter drugs were mostly responsible for substance abuse. Reasons for indulgence in substance abuse were basically for academic enhancement, repression of physical (financial) and emotional (domestic) pressures and sexual performances. Academic stress-induced substance cravings and peer influence were generally more severe than sexual-influence but peer- and sexual-influence were more severe for substance cravings than academic stress-induced substance cravings by academically poor students. Substance abuse had significant influence on sexual assaults, fraternity clashes, thefts, youth crimes, like cyber-crimes. Social status and institutional regulations influenced restricted doses of abused drugs, which influenced easier withdrawal from substances after graduation among some users. Lack of rehabilitation facilities in Nigeria accounts for high morbidities and mortalities among the highly dependents on abused substances, while faith-based therapy was considered immense factor in substance abuse treatments.

T1.30

Selective Small-molecule Nociceptin Opioid (NOP) Receptor Agonists Reduce Ethanol-induced Conditioned Place Preference in C57BL/6 Mice

Nurulain Zaveri[1], Michael Meyer[1], Velvet Journigan[1], Paul Marquez[2], Ahmed Hamid[2], Kabirullah Lutfy[2]

[1]Astraea Therapeutics, Mountain View, CA, USA, [2]Western University of Health Sciences, Pomona, CA, USA

Alcohol-related disorders are major public health issues with a very limited number of pharmacotherapies available for treatment. Literature evidence suggests that the NOP receptor may be a potential target to treat alcohol addiction. Indeed, nociceptin/orphanin FQ, the endogenous peptide agonist for NOP, as well as a synthetic small-molecule NOP agonist Ro 64-6198 reduce ethanol-induced mouse conditioned place preference (CPP), an animal model of reward. We examined the effect of a series of novel, selective NOP agonists on ethanol-induced CPP. Mice were tested for preconditioning preference on day 1 and the amount of time mice spent in each chamber was recorded. On days 2-4, mice were treated with vehicle or a NOP agonist (AT-312, AT-328, AT-202 and a known NOP agonist SCH221510), followed by saline/ethanol (2g/kg) or ethanol/saline, and conditioned to the CPP chambers for 15 min. Mice were then tested under a drug-free state for postconditioning place preference on day 5. On test day, mice were placed in the central chamber of the CPP apparatus and allowed to explore all chambers for 15 min. Our results revealed that AT-312 and AT-328, high affinity, selective NOP agonists, abolished ethanol CPP in WT mice. The effect of these drugs were mediated via the NOP receptor, as these compounds failed to alter the CPP response in mice lacking the NOP receptor. Together, these data suggest that the NOP agonists may be promising as medications for alcohol-related disorders. (Supported by HHSN275201500005C(NTZ) and TRDRP 24RT-0023(KL)

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T1.31

Small-molecule Agonists Selective for the Nociceptin Opioid (NOP) Receptor Attenuate Cocaine-induced Conditioned Place Preference in C57BL/6 mice

Nurulain Zaveri[1], Paul Marquez[2], Abdul Hamid[2], Michael Meyer[1], Velvet Journigan[1], Kabirullah Lutfy[2]

[1]Astraea Therapeutics, Mountain View, CA, USA, [2]Western University of Health Sciences, Pomona, CA, USA

Orphanin FQ/Nociceptin, the endogenous ligand for the NOP receptor is known to reduce rewarding effects of cocaine and other addictive drugs, suggesting that NOP agonists could be developed for cocaine abuse pharmacotherapy. A series of selective NOP receptor agonists developed in our laboratory were tested for effects on cocaine-induced conditioned place preference (CPP) using wild-type and NOP-/- mice. Mice (both genotypes) were tested for baseline place preference on day 1. On days 2-4, mice were treated with vehicle or a NOP agonist (AT-202, AT-312 or AT-328), followed by cocaine (15mg/kg) and confined to one conditioning chamber for 30 min. In the afternoon of each conditioning day, mice were injected with the alternate treatment, and confined to the opposite chamber. On day 5, mice were tested for postconditioning preference in a drug-free state, placed in the central chamber of the CPP apparatus, and allowed to freely explore the chambers for 15min. Cocaine induced a robust CPP response in vehicle-treated WT and NOP -/- mice. AT-312 and AT-328 abolished the CPP response in wild-type mice, although AT-202 failed to alter cocaine-induced CPP. The compounds did not alter cocaine-induced CPP in mice lacking NOP receptor, confirming their action via the NOP receptor. Although it not clear why AT-202 failed to alter cocaine CPP, our *in vitro* data indicates that AT-312 and AT-328 are more selective for NOP compared to AT-202. Taken together, the present data suggests that the NOP receptor agonists may be promising medications for cocaine addiction. (Support: NIH R01DA027811(NTZ); CA-TRDRP 24RT-0023(KL)

T1.32

Distinct motivational states underly morphine self-administration in the presence and absence of nerve injury

A.G. Hohmann, T. Gutierrez, J.D. Crystal

Indiana University, Bloomington, IN, USA

We used an intravenous drug self-administration paradigm to evaluate the impact of a spared nerve injury (SNI) on morphine self-administration and mechanical paw withdrawal thresholds in rats. We hypothesized that different motivational states underlie morphine self-administration in the presence or absence of neuropathic pain. We asked, therefore, whether lever pressing behavior in naïve, neuropathic and sham-operated groups differed during extinction (i.e. when morphine was withheld). Self-administration of morphine, but not vehicle, attenuated nerve injury-induced allodynia. By contrast, mechanical withdrawal thresholds in sham and naive groups were unaltered by morphine self-administration. We used a dose of morphine that produced similar levels of active lever responding in neuropathic, sham and naive groups. Responding on the active (morphine-paired) lever was higher than responding on the inactive lever in all groups. However, the percentage of active lever responding for morphine was higher in neuropathic compared to either sham-operated or pooled control (naive and sham) conditions. In an extinction test, neuropathic groups showed perseveration in responding on the previously active lever compared to sham-operated or naïve groups with similar histories of morphine self-administration. Responding on the inactive lever did not differ between groups. Neuropathic animals also elicited more infusions of vehicle when morphine was withheld. Our results suggest that drug self-administration paradigms may be useful for evaluating both analgesic efficacy and motivational properties associated with opioid reinforcers in animal models of neuropathic pain.

T1.33

Mechanistic Insights into Opioid-induced Breast Cancer Cell Migration

S Tripolt, DA Fux

Institute of Pharmacology and Toxicology, Division Clinical Pharmacology, University of Veterinary Medicine Vienna, Vienna, Austria

Recent studies revealed that opioid treatment may be associated with tumour metastasis. The underlying signalling mechanism, however, is largely unknown. Epithelial-mesenchymal transition (EMT) is a central process by which malignant cells acquire increased migratory capability. We thus started to analyse whether opioids may drive tumour cell migration and thus metastasis by inducing EMT. EMT is initiated by internalization and down-regulation of E-cadherin, an cellular adhesion protein. To investigate the effect of opioids on E-cadherin, human breast cancer cells were incubated with [D-Ala^2, D-Leu^5]Enkephalin (DADLE) and examined for E-Cadherin expression by FACS and qRT-PCR analysis. The experimental approaches revealed that DADLE exposure is accompanied by both a loss of surface E-Cadherin protein and a significant reduction of E-cadherin mRNA. To further obtain insights into the cellular signalling mechanism we tested DADLE-exposed tumour cells for activation of Signal Transducers and Activators of Transcription STAT3, which is known to suppress the expression of E-Cadherin. Western blot analysis showed that incubation of breast cancer cells with DADLE led to prominent Tyrosine 705 STAT3 phosphorylation. Moreover, we observed that DADLE incubation was accompanied by an increase of N-cadherin mRNA and of transcription factors of the Snail family, which are further key factors of cell migration. As inhibition of STAT3 prevents DADLE-promoted cell migration, our data provides first evidence that opioids may promote cancer cell migration by STAT3 regulation. Our findings thus help to better understand opioid-triggered tumour metastasis and govern the ground for future therapeutic strategies to prevent tumour metastasis during pain management by opioids.

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T1.34

HIF-1alpha Regulation By Delta-Opioids In Human Breast Cancer Cells

A Schoos, F Holstein, DA Fux

University of Veterinary Medicine, Vienna, Austria

Studies revealed that morphine promotes breast cancer growth via stimulating tumour angiogenesis. However the underlying signalling mechanism is largely unknown. Stimulation of delta-opioid receptors (DOR) induces HIF-1alpha activation and VEGF expression in neurons. As VEGF is a key player in tumour angiogenesis, we were interested whether the opioid effect may also be seen for breast cancer cells.

Estrogen-receptor positive T47D and MCF-7 breast cancer cells were stimulated with 1µM [D-Ala^2, D-Leu^5]-enkephalin (DADLE) and analysed for HIF-1alpha activation by immunoblotting. In both cell lines, HIF-1alpha was activated in a time-dependent and naloxone (10 µM)-sensitive manner. In parallel, DADLE treatment enhanced VEGF-A expression as determined by qRT-PCR. Moreover, expression of MMP-9, a target gene of VEGF signalling, was also enhanced. Cell incubation with DADLE together with Echinomycin (10 nM) prevented VEGF-A and MMP-9 expression, indicating that opioid-mediated gene expression depends on HIF-1alpha. To obtain more insights into the mechanism of HIF-1alpha regulation, cells were examined for AKT and ERK1/2 activation by immunoblotting. An increase of phospho-AKT and phospho-ERK1/2 was detected in DADLE exposed T47D and MCF-7 cells. As Wortmannin (2,5µM), LY294002 (10µM), the AKT 1/2 inhibitor (100µM) and PD98,059 (10µM) impaired HIF-1alpha activation, AKT and ERK1/2 activity is suggested to participate in DADLE-induced HIF-1alpha regulation.

Our results show that stimulation of DORs in estrogen-receptor positive cells induces VEGF-A and MMP-9 expression by AKT and ERK1/2-dependent activation of HIF-1alpha. As VEGF-A and MMP-9 are key players in new vessel formation, our findings give first mechanistic insights into opioid-driven tumour angiogenesis.

T1.35

Polymorphism of the mu-opioid receptor gene OPRM1 as a predictive marker for survival of breast cancer patients

Leena Karhinen[1], Sofia Khan[1], Rainer Fagerholm[1], Tiina Tasmuth[2], Mari Kaunisto[3], Carl Blomqvist[2], Heli Nevanlinna[1,2], Eija Kalso[1,2]

[1]University of Helsinki, Helsinki, Finland, [2]Helsinki University Central Hospital, Helsinki, Finland, [3]Institute for Molecular Medicine Finland, Helsinki, Finland

Opioids are routinely given to breast cancer patients during surgery and for the management of severe cancer-related pain. However, pre-clinical studies propose that opioids may promote tumour growth and metastasis (1, 2).

Most clinically used opioids target the mu-opioid receptor encoded by the OPRM1 gene. Polymorphism of the mu-opioid receptor, in particular the single-nucleotide replacement A118G (SNP rs1799971) has been studied extensively. It results in a missense mutation and altered glycosylation of the mu-opioid receptor (3) and it has been shown to modify the clinical outcome of morphine treatment (4,5,6). Furthermore, the A118G variant has been proposed to be associated with the prognosis of breast cancer (7, 8) and of esophageal squamous cell carcinoma (9).

In this study we investigated the association of the A118G variant with the 5- or 10-year survival of breast cancer patients. We combined genotyping data from two different cohorts of Finnish women with breast cancer, all patients of the Helsinki University Central Hospital: one with 805 patients and the other with 1000 patients, ages 18 - 75 years. Our first results from the first cohort suggest that there is a significant correlation between the A118G variant and 10-year breast cancer specific survival. Further studies will shed light on whether the correlation can be confirmed in the second cohort and whether the A118G polymorphism may be used as a predictive marker for breast cancer survival.

T1.36

A novel regulatory role of RGS4 in δ-opioid receptor mediated neuronal outgrowth and differentiation

Z Georgoussi[1], P Pallaki[1], I Serafimidis[2], EM Georganta[1], MP Papakonstantinou[1], S Koutloglou[1], A Symeonof[1], M Gaitanou[3]

[1]Laboratory of Cellular Signaling & Molecular Pharmacology, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Ag. Paraskevi, Athens, Greece, [2]Laboratory of Developmental Biology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, [3]Laboratory of Cellular & Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece

RGS4 has been found to be a universal negative regulator of μ, δ and κ-opioid receptor signaling that confers selectivity for Gi/o protein coupling to these receptors by interacting directly with them (Georgoussi *et al.*, 2006, 2012; Leontiadis *et al.* 2009; Papakonstantinou *et al.*, 2015). It was also shown that δ-opioid receptor (δ-OR) forms a multicomponent signaling complex, consisting of Gi/Go proteins and the Signal Transducer and Activator of Transcription STAT5B, that leads to neurite outgrowth and neuronal differentiation via a phosphoryalted-STAT5B-Gαi/o pathway (Georganta *et al.*, 2010, 2013). Knowing that RGS4 is a multitask protein we wondered whether RGS4 could be implicated in neuronal differentiation and neurite outgrowth through a similar signaling pathway. Our data demonstrate that expression of RGS4 in HEK293 cells resulted in inhibition of δ-OR mediated-STAT5B phosphorylation and transcriptional activation. Measurements of [D-Ser2, Leu5, Thr6]-enkephalin (DSLET) mediated neurite outgrowth and differentiation was blocked upon overexpression of RGS4 in neuroblastoma cells. Additionally, DSLET treatment of primary neuronal cortical cultures from RGS4$^{-/-}$ mice, expressing a nonfunctional RGS4, exhibited significant alterations in neuronal sprouting as compared with the wild type RGS4$^{+/+}$. Collectively, our results demonstrate that RGS4 plays a significant regulatory role in neuronal effects via a non-canonical function implicating STAT5B activation by opioids. *Supported by the Excellence II-3722, "NO-ALGOS" to Z.G. ZG participates in the EU-COST Action CM1207 (GLISTEN).*

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T1.37

Opioid Precursor Protein Isoform is Targeted to the Cell Nuclei in the Human Brain

G Bakalkin[1], O Kononenko[1], I Bazov[1], H Watanabe[1], O Dyachok[2], K Alkass[3], H Druid[3], M Andersson[1], J Mulder[4], A Fex Svenningsen[5], G Rajkowska[6], T Yakovleva[1]

[1]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [2]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden, [3]Department of Forensic Medicine, Karolinska Institute, Stockholm, Sweden, [4]Department of Neuroscience, Science for Life Laboratory, Karolinska Institute, Stockholm, Sweden, [5]Institute of Molecular Medicine-Neurobiology Research, University of Southern Denmark, Odense, Denmark, [6]Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, USA

Neuropeptide precursors are traditionally viewed as proteins giving rise to small neuropeptide molecules. Prodynorphin (PDYN) is the precursor protein to dynorphins, endogenous ligands for the κ-opioid receptor. We here describe two novel splicing variants of human *PDYN* mRNA. Expression of one of them was confined to the striatum where its levels constituted up to 30% of total *PDYN* mRNA. This transcript may be translated into ΔSP-PDYN protein lacking 13 N-terminal amino acids, a fragment of the signal peptide. ΔSP-PDYN was not processed to mature dynorphins and surprisingly, was targeted to the cell nuclei in a model cellular system. This may be driven by bipartite nuclear localization signal (NLS) that is cryptic in the full-length PDYN molecule and becomes functional when signal peptide is truncated. Nuclear PDYN isoform was identified by western blot and radioimmunoassay in neuronal nuclei isolated from human striatum using fluorescence-activated nuclei sorting, and by immunofluorescence staining and confocal microscopy in the human caudate nucleus. These results along with the presence of putative NLS in other neuropeptide precursors raise questions of the nuclear localization is a general feature of neuropeptide precursor proteins, and their putative nuclear function.

T1.38

Synaptic mechanisms of OPRM1 A118G (MOR N40D) gene variants in human neurons

A Halikere, Z Pang

Child Health Institute of New Jersey, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, USA

Mu opioid receptor (MOR) signaling modulates synaptic transmission and thus plays a pivotal role in regulating reward behaviors relevant to addiction. The single nucleotide polymorphism (SNP) rs1799971 (OPRM1 A118G) has been linked to drug and alcohol use disorders. The A118G produces a non-synonymous amino acid substitution in the human MOR, replacing an asparagine with an aspartate at position 40 (MOR N40D). To investigate the molecular and cellular mechanisms of this SNP, we derived human neurons, using the induced neuronal (iN) cell technology, from induced pluripotent stem (iPS) cell lines generated from multiple human subjects carrying either homozygous N40 or D40 alleles. Our compelling preliminary data reveal that D40 MOR mediates stronger suppression of synaptic releases compared to N40 MOR in inhibitory human neurons. Interestingly, pre-exposure of these human neurons to DAMGO for 24 hours diminishes their sensitivity to DAMGO, possibly owing to the desensitization or internalization of membrane MORs following prolonged activation of the receptors. Moreover, N40 MOR carrying human neurons regained partial sensitivity to DAMGO during a 7-day pre-exposure paradigm, whereas D40 failed to re-sensitize. We thus hypothesize that human N40 and D40 MORs have differential membrane recycling dynamics. We are currently testing this hypothesis. Our data will provide important insights on the mechanisms by which MOR gene variants may alter synaptic transmission in a human neuronal context and will shed light on how MOR gene variants may impact drug abuse behavior in humans.

T1.39

Striatal orphan GPR88 inhibits delta opioid signaling through physical interaction

LP Pellissier[1], J Gandía[1], MA Ayoub[1], BL Kieffer[2], J Le Merrer[1], JAJ Becker[1]

[1]Physiologie de la Reproduction et des Comportements - INRA UMR0085 - CNRS UMR7247- Université Rablais - INSERM, Nouzilly, France, [2]Douglas Hospital Research Center - Faculty of Medicine, McGill University, Montreal, Canada

The orphan G protein coupled receptor (GPCR) GPR88 is the most highly expressed GPCR in the striatum. GPR88 is involved in a large repertoire of behavioral responses that engage motor activity, spatial learning and emotional processing (1). Gpr88 knockout mice show increased delta opioid receptor (DOR) activity in the striatum and chronic blockade of DOR using naltrindole partially improved their motor coordination and normalized their spatial navigation and anxiety.

Here, we analyzed the functional consequences of co-expressing GPR88 with DOR on various signaling pathways in heterologous cells. BRET between GPR88 and DOR display specific and saturated signals in contrast to mu opioid receptor (MOR) or other striatal GPCRs. Co-expression of GPR88 decreases G-protein dependent and independent signaling of DOR but not MOR. Our data provide new insights in orphan GPR88 function as an inhibitor of DOR signaling.

(1) Meirsman et al, Biological Psychiatry, in press

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T1.40

Engineered relocation of delta opioid receptors to the cell surface increases agonist efficacy

DJ Shiwarski[1], A Tipton[2], BF Schmidt[1], MD Giraldo[3], MS Gold[3], AA Pradhan[2], MA Puthenveedu[1]

[1]Carnegie Mellon University, Pittsburgh, USA, [2]University of Illinois at Chicago, Chicago, USA, [3]University of Pittsburgh, Pittsburgh, USA

The delta opioid receptor (DOR) is a promising alternate target for pain management, especially for modalities of pain not treated by current opioid analgesics, and for neuropsychiatric disorders. While DOR agonists can signal efficiently under experimental conditions, they show poor antinociceptive responses *in vivo*. This low efficacy has remained a major limitation in targeting DOR for antinociception. Here we show that this is largely due to limited surface expression of DOR in sensory neurons. Using direct visualization of DOR trafficking, we define a PTEN- and lipid-regulated checkpoint that retains DOR in the neuronal Golgi and limits DOR surface delivery. Manipulation of phosphoinositide conversion releases DOR from this checkpoint, stimulates DOR delivery to the neuronal surface *in vitro* and *in vivo*, and allows effective DOR-mediated antinociception *in vivo*. Our results resolve a key constraint in targeting DOR for pain management, and provide a proof of principle for a strategy to overcome this constraint.

T1.41

The β-galactosidase complementation assay – a tool to examine the regulation of the μ-opioid receptor

E Miess, S Schulz

Jena University Hospital, Friedrich Schiller University Jena, Institut of Pharmacology and Toxicology, Jena, Germany

Opioid drugs are the most potent analgesics, which are used in the clinic; however, by activating the μ-opioid receptor (MOR) they also produce several adverse side effects. There is substantial evidence suggesting that G protein-coupled receptor kinases (GRKs) and β-arrestins play key roles in regulating MOR signaling and responsiveness. We have previously shown that agonist-induced phosphorylation of MOR occurs at a conserved 10-residue sequence, ^{370}TREHPSTANT^{379}, in the carboxyl-terminal cytoplasmic tail. Morphine induces a selective phosphorylation of serine^{375} (S375) that is predominantly catalyzed by GRK5. By contrast, high-efficacy opioids not only induce phosphorylation of S375 but also drive higher-order phosphorylation on the flanking residues threonine^{370} (T370), threonine^{376} (T376), and threonine^{379} (T379) in a hierarchical phosphorylation cascade that specifically requires GRK2/3 isoforms. To investigate this mechanism further, we have adapted a β-galactosidase complementation assay for β-arrestin1/2 and the GRK2/3 isoforms. Using this assay, we were able to show that activation of MOR by high-efficacy agonists such as DAMGO results in recruitment of β-arrestin1/2 and also in GRK2/3, whereas activation by low-efficacy agonists such as morphine results only in detectable recruitment of β-arrestin2 but not β-arrestin1 and furthermore reduced the recruitment for GRK2/3. The morphine-induced β-arrestin recruitment was strongly enhanced by overexpression of GRK2 or GRK3. Conversely, siRNA knock down of GRK2 or GRK3 strongly inhibits DAMGO-induced β-arrestin recruitment. Mutation of S375 to alanine strongly inhibited β-arrestin1/2 and GRK2/3 recruitment. However, mutation of all 11 carboxyl-terminal serine and threonine residues of MOR was required to completely abolish interaction with β-arrestin and GRK.

T1.42

MOR-HA knock-in mouse: a new tool to study μ-opioid receptor regulation and expression

A Kliewer, S Schulz

Pharmacology and Toxicology, Jena, Germany

Opioid drugs exert nearly all of their clinically relevant actions through stimulation of MORs (μ-opioid receptors). The molecular biology of endogenous opioid peptides and their cognate receptors has been studied extensively *in vitro*. For MOR, signaling efficiency is tightly regulated and ultimately limited by the coordinated phosphorylation of intracellular serine and threonine residues. Morphine induces a selective phosphorylation of serine 375 that is predominantly catalyzed by G protein– coupled receptor kinase 5. As a consequence, the selective morphine-induced S375 phosphorylation does not lead to a robust beta-arrestin mobilization and receptor internalization. By contrast, high-efficacy opioid agonists such as fentanyl or etonitazene not only induce phosphorylation of S375 but also drive higher order phosphorylation on the flanking residues threonine 370, threonine 376, and threonine 379 in a hierarchical phosphorylation cascade that specifically requires GRK2 and GRK3 isoforms. As a consequence, multisite phosphorylation induced by potent agonist promotes both beta-arrestin mobilization and a robust receptor internalization. However, little is known about agonist-selective phosphorylation patterns *in vivo* after acute and chronic drug administration. To learn more about MOR regulation *in vivo* we have generated a new μ-opioid receptor knock in mouse with an N-terminal HA-tag. Using these mice, we were able to study *in vivo* phosphorylation of an endogenous G protein-coupled receptor using both mass spectrometry and phosphosite-specific antibodies. We were also able to address the question which of the many putative MOR splice variants detected on the mRNA level are indeed expressed as functional receptors in mouse brain.

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T1.43

Mechanisms involved in PKC mediated regulation of μ opioid receptor desensitization.

Arsalan Yousuf, MacDonald Christie

University of Sydney, Sydney, NSW, Australia

Utility of opioid analgesics is hampered by tolerance and dependence. Agonist induced desensitization of μ-opioid receptor (MOPr) is widely considered to be an initial step in the development of tolerance. Different opioids acting on MOPr can activate different regulating pathways leading to desensitization but exact mechanisms are still uncertain. In the current study we examined three different mutations of mouse MOPr C-terminal tail i.e. ^{355}TSST^{357}/A, ^{375}STANT^{379}/A, TSST+STANT/A transfected and expressed stably in AtT20 cells. The effects of MOPr activation and desensitization were examined using activation of GIRK channels with whole cell and perforated patch clamp electrophysiology at 37°C. Acute MOPr desensitization produced by 5 minutes exposure to met-enkephalin (10μM) did not differ from wild type for all three mutants in perforated patch mode but was abolished in STANT/A and TSST+STANT/A in whole cell mode. Using the selective PKC blocker, Calphostin-C (30nM) in perforated patch clamp mode, desensitization by met-enkephalin was reduced in STANT/A but completely blocked in TSST+STANT/A. By contrast, desensitization was maintained in wild type MOPr and all mutants upon 5 minutes exposure to morphine (10μM) in both perforated and whole cell patch mode. The PKC inhibitor. Calphostin-C reduced desensitization by morphine in perforated patch mode by ~50% in wild type and TSST/A but it was maintained in STANT/A and TSST+STANT/A mutants. Taken together these results indicate that phosphorylation in the region including ^{375}STANT^{379} of MOPr is crucial for switching the mechanism of desensitization from a GRK/β-arrestin to PKC mediated process.

T1.44

Agonist-induced NOP receptor phosphorylation revealed by phosphosite-specific antibodies

Anika Mann[1], Stefan Schulz[1], Nurulain Zaveri[2], Lawrence Toll[3]

[1]Institute for Pharmacology and Toxicology, Jena, Germany, [2]Astraea Therapeutics, California, USA, [3]Torrey Pines Institute for Molecular Studies, Florida, USA

The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is the fourth most recently discovered and least characterized member of the opioid receptor family (MOR, KOR and DOR) and modulates several physiological processes by its endogenous ligand nociceptin. The NOP receptor is a potential target for the development of ligands with therapeutic use in several pathophysiological states such as chronic and neuropathic pain. Consequently, there is increasing interest in understanding the molecular regulation of NOP receptor. Recently, we generated two phosphosite-specific antibodies directed against carboxyl-terminal residues serine^{351} (S351) and threonine^{362}/serine^{363} (T362/S363), which enabled us to selectively detect either the S351- or the T362/S363-phosphorylated forms of the receptor. Our results show that nociceptin, MCOPPB, SCH221510 and Ro64-6198 induce a stably phosphorylation at S351 and T362/S363 followed by a profound receptor internalization. The nociceptin-induced S351 and T362/S363 phosphorylation can be blocked by selective antagonists (J113397 or SB612,111). NNC63-0532, buprenorphine and norbuprenorphine failed to induce a phosphorylation at these sites. In the presence of nociceptin, S351 phosphorylation occurred at a faster rate than phosphorylation at T362/S363. Activation of PKC by PMA facilitated receptor phosphorylation only at S351, indicating that S351 can also undergo heterologous phosphorylation. Using NOP-GFP *knock in* mice, we detected NOP receptors in brain, spinal cord and dorsal root ganglia (DRG). We were also able to demonstrate a dose-dependent NOP receptor phosphorylation at T362/S363 in mouse brain *in vivo*. Together, these data provide new insights into the molecular regulation of the NOP receptor *in vitro* and *in vivo*.

T1.45

Mu opioid receptor-mediated disinhibition of neurones in the ventral tegmental area through β-arrestin2 and c-Src

Fiona Bull[1], Claire Sneddon[1], Daniel Baptista-Hon[1], Wendy Walwyn[2], Tim Hales[1]

[1]University of Dundee, Dundee, Scotland, UK, [2]University of California, Los Angeles, Los Angeles, CA, USA

The tyrosine kinase, c-Src, has a role in mu receptor (MOR)-mediated inhibition of voltage-activated Ca2+ channels in sensory neurons. The scaffolding protein β-arrestin2 (β-arr2) is implicated c-Src recruitment to MORs in dorsal root ganglion neurones. Mice that lack β-arr2 exhibit increased sensitivity to the reinforcing effects of morphine; however, it is not known whether β-arr2 and/or c-Src participate in the actions of opioids in neurons within the reward pathway. It is also unclear whether the actions of morphine are exclusively mediated through MORs or involve delta opioid receptors (DORs). We examined the involvement of MORs, DORs, β-arr2 and c-Src in morphine-evoked inhibition of GABAergic inhibitory postsynaptic currents (IPSCs) recorded from neurons in ventral tegmental area (VTA) containing brain slices. Morphine caused a concentration-dependent inhibition of spontaneous IPSC frequency. Most of the inhibition by morphine was mediated through MORs, with only a negligible effect remaining in MOR-/- neurons. However, a small reduction in the inhibition by morphine in DOR-/- VTA compared to WT neurons revealed a role for DORs. The inhibition of IPSCs by DAMGO was unaffected by the absence of DORs, while the inhibition by DPDPE was abolished. Inhibition of IPSCs by either morphine or DAMGO was reduced in β-arr2-/- VTA neurons. The application of the c-Src inhibitor PP2 to wild type VTA neurons also reduced inhibition by morphine, while the inactive analogue PP3 and the MEK inhibitor SL327 had no effect. These data suggest that MOR-mediated inhibition of IPSC frequency involves a β-arr2 / c-Src mediated mechanism.

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T1.46

Differential Receptor-mediated β-arrestin Recruitment by Opioid Peptides

Ivone Gomes[1], Salvador Sierra[1], Lindsey Lueptow[1], Wakako Fujita[2], Lakshmi Devi[1]

[1]Icahn School of Medicine at Mount Sinai, New York, USA, [2]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Opioid peptides are generated by the posttranslational processing of the three precursors, proenkephalin, proopiomelanocortin, or prodynorphin. These peptides are thought to exert their biological effects through activation of the three opioid receptors (δ, μ, or κ) and consequently Gαi-mediated signaling cascades. Recently studies showed that opioid receptors also activate G protein-independent signaling cascades. While many studies have reported that the G protein-independent signaling is mediated through β-arrestin recruitment, few studies have directly examined the ability of endogenous opioid peptides to recruit β-arrestin. Therefore we compared β-arrestin recruitment by the major endogenous opioid peptides to δ, μ, or κ opioid receptors using a functional complementation assay (with β-galactosidase activity as a readout). We find that enkephalins recruit β-arrestin to δ with higher potency and efficacy compared to μ or κ receptors. Unexpectedly, endorphins, putative μ receptor agonists, recruit β-arrestin to δ with higher potency and efficacy compared to μ or κ receptors. Most interestingly, dynorphins, putative κ receptor agonists, recruit β-arrestin to μ or δ receptors with greater potency and efficacy compared to κ receptors; at κ receptors dynorphin A is ~2-fold more efficacious than dynorphin B. Together, our results indicate that opioid peptides differentially recruit β-arrestin at the three opioid receptors with overlapping specificity; this could modulate spatio-temporal dynamics of receptor signaling in regions where the receptors are co-localized and/or the peptides are co-released.

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T1.47

Opioid Receptor Trafficking is Regulated by Postendocytic Processing of Opioid Peptides

Achla Gupta, Ivone Gomes, Salvador Sierra, Lakshmi Devi

Icahn School of Medicine at Mount Sinai, New York, USA

It is generally accepted that opioid receptors, upon activation, are endocytosed along with the agonist, and the latter if a peptide is processed in an endocytic compartment allowing for receptor recycling and resensitization. While much is known about the fate of the endocytosed receptors, little is known about the endocytosed peptides and the peptidases involved in their endocytic processing. We showed previously that endothelin converting enzyme 2 (ECE2) selectively hydrolyses neuropeptides including opioid peptides *in vitro*. We also showed that ECE2 inhibition decreases δ and μ receptor recycling and signaling without significantly affecting receptor internalization only for those agonists that are substrates of ECE2. In a recent set of studies we examined the modulation of κ receptor activity by ECE2. Using CHO cells expressing Flag-tagged κ receptors and HA-tagged ECE2, we examined the effect of ECE2 inhibition on receptor recycling and signaling by dynorphin peptides. We find that ECE2 inhibition impairs recycling in response to Dyn B (ECE2 substrate) and not Dyn A or Leu-Enk (non-substrates). In F11 cells expressing endogenous receptors, inhibition of native ECE2 impairs recycling by Dyn B but not U69,593 (non-substrate). Finally, we find that the effect of ECE2 inhibition on recycling impacts signaling since inhibition of ECE2 impairs receptor resensitization. Taken together these data reveal a novel role of ECE2 in modulating the κ opioid system by postendocytic processing of dynorphin peptides.

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T1.48

Blocking MOPr desensitization: studies towards small-molecule inhibitors of GRK2 and GRK3

M Ostovar[1], SM Husbands[1], CP Bailey[1], E Kelly[2], G Henderson[2], M Thomas[1]

[1]University of Bath, Bath, North East Somerset, UK, [2]University of Bristol, Bristol, Avon, UK

There is ongoing debate about the role of G protein–coupled receptor kinases (GRKs) in agonist-induced desensitization of the μ-opioid receptor (MOPr) in brain neurons. We have studied a novel membrane-permeable, small-molecule inhibitor of GRK2 and GRK3, **Compound 101** to elucidate the role of GRK2/3 in acute agonist-induced MOPr desensitization (1).

Improved synthesis of **Cmpd101** was developed in 9 steps in good overall yield. Pharmacological studies with **Cmpd 101** support the view that GRK2 and GRK3 do play a role in MOPr desensitization in LC neurons. What was surprising was that **Cmpd 101** only partially reversed the loss of MOPr function that underlies desensitization. The inability of this compound to fully reverse the loss of MOPr function could indicate either that there are two mechanisms of MOPr desensitization, one involving GRK-mediated phosphorylation (inhibited by **Cmpd 101**) and one that does not involve GRK2 and GRK3 or that, in intact neurons, for reasons that are unclear, **Cmpd 101** does not completely inhibit GRK2 and GRK3.

Encouraging biological results, prompted us to synthesise analogues of **Cmpd 101** using Wittig and Click chemistry. This will allow us to better define the pharmacophore responsible for affinity and selectivity within this series. For further support, molecular modelling studies were carried out by docking **Cmpd 101** and the analogues into a crystal structure of GRK2. These new compounds are currently under evaluation and results will be presented at the meeting.

(1) Lowe JD *et al.* (2015). Mol Pharm. **88:** 347-356.

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T1.49

Spinophilin is regulated by δ-opioid receptor activation to modulate receptor signaling

S Koutloglou, Z Georgoussi

Laboratory of Cellular Signaling and Molecular Pharmacology, Institute of Biosciences and Applications, National Center for Scientific Research Demokritos, 15310, Agia Paraskevi, Athens, Greece

Spinophilin is a neuronal multifunctional scaffold protein known to interact with the μ- and δ-opioid receptors to modulate their signalling (Fourla *et al.*, 2012). Observations have also shown that spinophilin can form a multi-protein complex consisting of RGS4, Gα and Gβγ subunits of G proteins within the C-terminal tail of μ-and δ-opioid receptors. Spinophilin can be phosphorylated in serine and/or tyrosine residues, modifications that alter its ability to interact with RGS proteins and reduce agonist-driven GPCR endocytosis and trafficking. Knowing that spinophilin is a multimeric protein, involved in cytoskeletal rearrangements, we wondered whether spinophilin can be phosphorylated by selective opioid agonists to modulate δ-opioid receptor signalling, and thus disrupt interactions with RGS4 and/or other signalling intermediates. Functional studies indicated that DSLET-activation of δ-opioid receptor in HEK293 cells, leads to tyrosine-phosphorylation of spinophilin, an effect which is abolished in the presence of c-Src inhibitors, suggesting that is mediated via c-Src kinase, which associates with spinophilin. Site-directed mutagenesis of specific tyrosine residues of spinophilin defined the responsible sites for this phosphorylation. Moreover, flow cytometry in HEK293 cells, indicated that spinophilin expression alters the levels of internalized δ-opioid receptors. Collectively, our results suggest that opioid agonists regulate spinophilin's function, implicated in δ-opioid receptor signalling.

T1.50

Internalization efficacy is not consistently predictive of tolerance potential of delta opioid receptor agonists.

Hanieh Bagheri Tudashki[1], Joseph Haddad[2], Graciela Pineyro[1,2], Réjean Couture[2]

[1]CHU St-Justine, Montréal, Québec, Canada, [2]Université de Montréal, Montréal, Québec, Canada

The magnitude and speed of analgesic tolerance of DOPr agonists has been associated to their internalization profiles. In this study we investigated whether this association could be extended to other agonists, while additionally controlling for factors as receptor reserve. Analgesic dose response curves were obtained in a rat model of diabetic polyneuropathy measuring anti-allodynic actions of two efficiently internalizing agonists, deltorphin II (DELT; peptidic) and SNC-80 (non-peptidic) and two with poor sequestration, SB 235863 (non-peptidic) and TIPP (peptidic). All drugs (injected i.t.) produced maximal analgesia but differed in potency. The response to TIPP was the most potent, SB 235863 was markedly right-shifted while curves for SNC-80 and DELT were superimposed located between those of the other two ligands. Rats were then treated with daily (6 days) injections of each drug (ED80). Homologous tolerance was evaluated by after each injection and heterologous tolerance was assessed by comparing the analgesic effect of DELT (ED50) observed one day before and one day after treatments were over. Nonpeptidic agonists induced heterologous tolerance while peptidic ones did not. Furthermore, DELT produced no homologous tolerance and TIPP maintained half of its analgesic effect throughout treatment while SNC-80 (t1/2 ~ 1 day) and SB235863 (t1/2 ~ 3 days) lost all analgesic action with different course. Thus, tolerance patterns were not associated with analgesic efficiency or internalization profiles. On the other hand, recycling was necessary to maintain reduced tolerance profiles observed for DELT and TIPP.

T1.51

Heat Shock Protein 90 Regulates Mu Opioid Receptor Signaling In Vitro and in Different Regions of Mouse Brain

W Lei[1], N Mullen[2], S McCarthy[2], C Brann[2], P Richard[2], J Cormier[2], K Lindros[2], K Edwards[2], E Bilsky[2], JM Streicher[1]

[1]University of Arizona, Tucson, AZ, USA, [2]University of New England, Biddeford, ME, USA

Heat shock protein-90 (Hsp90) is a ubiquitous regulator for many signaling pathways and proteins. Only 2 previous studies suggested that Hsp90 might be involved in the regulation of opioid receptor signaling, which is one of the primary targets for drug discovery for chronic pain. We studied Hsp90 in vitro by treating MOR expressing CHO, HEK, U2OS, and SH-SY5Y cells with an Hsp90 specific inhibitor (17-AAG) for 24 hrs followed by the MOR agonist DAMGO. We found that MOR, βarrestin2, and Akt expression was reduced in some cell types, while Hsp70 was increased in all cell types. The activation of ERK MAPK was also altered by Hsp90 inhibition in a cell context dependent manner - whether increasing DAMGO stimulated activation, increasing the baseline, or blunting activation. We then studied Hsp90 regulation in vivo by injecting CD-1 mice intracerebroventricularly (icv) with 17-AAG for 24 hrs, followed by icv injection of vehicle or DAMGO. We found an increased ERK signaling baseline and abolished DAMGO induction in the periaqueductal grey and brain stem, along with increased Hsp70 expression. Furthermore, 17-AAG treatment caused a modest 15.6% decrease in the acute anti-nociceptive tail-flick response to DAMGO, and increased urine and feces output after naloxone precipitated withdrawal in models of acute and chronic morphine dependence. These findings demonstrate that Hsp90 has a strong regulatory role in MOR signaling both in vitro and in vivo, and suggest that Hsp90 activators or other modulators could be potentially used as a co-therapy for improving the therapeutic index of opioid drugs.

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T1.52

A spectrum of bias analysis applied to a kappa opioid receptor agonist.

Edward L Stahl[1], Frederick J Ehlert[2], Laura M Bohn[1]

[1]The Scripps Research Institute, Jupiter, Florida, USA, [2]University of California-Irvine, Irvine, California, USA

The kappa opioid receptor (KOR) has provided a useful target for the development and analysis of functionally selective ligands. Specifically the kappa ligand, 6' guanidinonaltrindole (6'-GNTI), has been identified as a modestly but convincingly biased ligand when its agonist activity was compared across different effector systems. These response systems include the stimulation of heterotrimeric G protein and the agonist-mediated recruitment of β arrestin2 to the KOR. We have published a competitive model that was well suited to analyze the bias of 6'-GNTI.

In the studies presented here, we apply several different methods of quantitating bias that range from the standard activity method previously described to a novel Schild-type model. (It should be noted that this new method is named for the Schild plot; the Schild method is a common form of linearization used to analyze the effects of competitive ligands.) We utilize both Monte Carlo simulations and experimental evidence from the KOR and 6' GNTI to demonstrate the application and utility of each of these methods. These studies provide an example of the range of analysis possibilities available to researchers interested in comparing agonist activity across systems. Moreover, we demonstrate that the relative cost of the more rigorous "Schild-type" model is insignificant compared to the less rigorous methods considering that both methods can be employed with an equal number of data points. We conclude that each method is useful depending on the demands of the analysis. This work is supported by a grant from the National Institute on Drug Abuse (R01DA031927 LMB).

T1.53

Role of PKC in tolerance to opioid depression of respiration

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

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

PKC inhibitors have been reported to reverse tolerance to morphine antinociception [1]. The present investigation examined the effect of PKC inhibition on tolerance to morphine depression of respiration.

Respiration was measured by whole body plethysmography in male mice (CD-1) breathing 95% air/5% CO2 [2]. To measure changes in respiration, data for each mouse were normalised to pre-drug baseline and area under the curve calculated for a 30 min post-drug period.

Acute administration of morphine (10 mg/kg ip) rapidly depressed respiration. The PKC inhibitor calphostin C (15 µg/kg), and tamoxifen (0.6 mg/kg) which also inhibits PKC and vehicles did not decrease mouse respiration. Prolonged morphine, methadone or oxycodone was administered by osmotic mini-pumps to deliver 45, 7.5 and 120 mg/kg/day sc respectively for 6dys.

In all three drug treated groups, challenge with morphine (10 mg/kg) on day 6 caused significantly less depression of respiration than in mice implanted with saline delivering pumps. Pre-treatment with either calphostin C or tamoxifen for 30 min caused a significant enhancement of morphine-induced respiratory depression in morphine- and oxycodone-treated, but not methadone- or saline-treated mice. The degree of respiratory depression seen was greater in morphine pre-treated than oxycodone pre-treated mice.

These data show that inhibition of PKC can reverse tolerance to respiratory depression induced by morphine and oxycodone but not methadone. This supports our previous work demonstrating ethanol reversal of tolerance induced by morphine but not methadone.

1. Smith *et al.*, Br J Pharmacol, 128(1): 220-6.
2. Hill, R., *et al.*, Neuropsychopharmacology, 41(3): 762-73.

T1.54

Ethanol Reversal of Morphine Tolerance

R Hill1, E Kelly[1], W Dewey[2], G Henderson[1]

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

Acute low dose ethanol has been shown to reverse tolerance to morphine respiratory depression and antinociception [1, 2] The present investigation examined the potential role of the ethanol metabolite, acetaldehyde in tolerance reversal and the effect of chronic ethanol on morphine tolerance.

Respiration was measured in freely moving male mice (CD-1) using plethysmography [1]. Tolerance was induced by osmotic mini-pump infusion of 45 mg/kg/day morphine for 6 days. Prolonged treatment with ethanol for 14 days was achieved by inclusion of ethanol in the liquid diet.

After 6 days in morphine pump implanted mice the respiratory depression observed in response to acute morphine challenge (10 mg/kg ip) was attenuated compared to that in control, saline pump implanted animals i.e. tolerance had developed. An acute ethanol injection (0.3 g/kg ip) along with the morphine challenge significantly enhanced the respiratory depression i.e. ethanol reversed morphine tolerance. Pre-treatment with D-penicillamine (50 mg/kg ip), an acetaldehyde chelator, for 30min significantly reduced the ability of acute ethanol to reverse morphine tolerance.

Mice that had received prolonged ethanol treatment showed significantly greater respiratory depression during the morphine treatment (days 8 -14 of ethanol treatment). On challenge with acute morphine on day 14 ethanol treated animals again showed enhanced respiratory depression compared to controls.

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These results suggest an important role for acetaldehyde in acute ethanol reversal of morphine tolerance. Additionally, prolonged ethanol treatment prevents the induction of morphine tolerance

1. Hill *et al.*, Neuropsychopharmacology, 41(3): 762-73
2. Hull *et al.*, J Pharmacol Exp Ther. 345(3): 512-9.

T1.55

PLGA-Curcumin Attenuates Opioid Tolerance, Dependence, and Opioid-induced Hyperalgesia by Inhibiting CaMKIIα

Z. J. Wang, X Hu, M Szymusiak, Y Liu
University of Illinois, Chicago, IL, USA

PLGA-Curcumin Attenuates Opioid Tolerance, Dependence, and Opioid-induced Hyperalgesia by Inhibiting CaMKIIα

Xiaoyu Hu, Magdalena Szymusiak, Ying Liu, and Zaijie Jim Wang, University of Illinois, Chicago, IL, USA

Chronic use of opioid analgesics has been hindered by the development of opioid addiction, tolerance, and hyperalgesia. We have reported that curcumin, a natural flavonoid from the rhizome of Curcuma longa, attenuated opioid tolerance, although the underlying mechanism remains unclear. In this study, we tested the hypothesis that curcumin may inhibit Ca2+/calmodulin-dependent protein kinase II a (CaMKIIa). We used formulation technology to produce PLGA-curcumin nanoparticles to overcome poor solubility and bioavailability problems. PLGA-curcumin reduced the dose requirement by 10-30 folds. Curcumin or PLGA-curcumin did not produce antinociception by itself or affect morphine (1–10 mg/kg) antinociception. However, pretreatment with PLGA-curcumin (p.o.) prevented morphine tolerance and dependence in a dose-dependent manner, with ED50 values of 3.9 and 3.2 mg/kg, respectively. PLGA-curcumin also attenuated established opioid tolerance (ED50: 12.6 mg/kg), dependence (ED50: 3.1 mg/kg), mechanical allodynia (ED50: 11.2 mg/kg), thermal hyperalgesia (ED50: 9.9 mg/kg). Moreover, we found that the behavioral effects of curcumin correlated with its inhibition of CaMKIIa. These results suggest that curcumin may attenuate opioid tolerance, dependence, and hyperalgesia by suppressing CaMKIIa activity.

T1.56

Inhibition of the mammalian target if rapamycin complex 1 (mTORC1) attenuates morphine tolerance in naïve and neuropathic mice.

Amal Alsubayiel, JoãoPaulo Ferreira Bertacchi, Jeremy Signoret-Genest, Ilona Obara
Durham University, School of Medicine, Pharmacy and Health, Stockton on Tees, UK

Recently, it has become clear that opioid effectiveness is modulated by the mammalian target of rapamycin complex 1 (mTORC1), a kinase which controls protein synthesis. However, direct mTORC1 inhibitors are only used in limited clinical indications due to adverse effects. This study explored for the first time the effect of the widely used anti-diabetic drug metformin that inhibits mTORC1 through activation of the adenosine monophosphate-activated protein kinase (AMPK) on the development of morphine tolerance in naïve and neuropathic mice. Specifically, administration of morphine to naïve (20 mg/kg, i.p.) and neuropathic (40 mg/kg, i.p., spared nerve injury model - SNI) adult male C57BL/6J mice (n=6) twice daily resulted in tolerance to its analgesic effect after 6 days. When metformin (200 mg/kg, i.p.) was administered 24 hours before each morning morphine injection tolerance did not develop as measured by the tail-flick test in naïve and tail-flick, von Frey and acetone tests in SNI mice. Also, a single metformin dose injected on day 9 in morphine tolerant naïve or SNI mice fully restored the analgesic effect of morphine. Our parallel studies using the direct mTORC1 inhibitor CCI-779 (25 mg/kg, i.p.) showed that these effects were attributed to mTORC1 inhibition. This mechanism was confirmed by Western blotting showing inhibition of mTORC1 activity in the dorsal spinal cord after metformin and CCI-779 treatment in both naïve and SNI mice. Together, our results support the idea that targeting mTORC1 may offer a novel and clinically promising strategy for enhancing morphine analgesic efficacy, especially in neuropathic pain.

T1.57

Does tolerance to the analgesic effect of opioid drugs develop and if so, is it of clinical relevance?

E Luff
University of Bristol, Bristol, UK

For patients experiencing moderate to severe pain, either acutely or chronically, an opioid drug is one of the only options available to the treating clinician. They are generally highly effective, relatively safe and can be administered via a number of routes, depending on the individual case. However, evidence proposing the development of tolerance to the analgesic effect of these potentially compromises their use. Clinicians fear addiction, patients want to have effective analgesia when they really need it and many people may be left with inadequate pain relief, due to misconceptions and a lack of understanding. The evidence around tolerance is varied and human studies are sparse. A detailed review of current literature is required to ascertain the extent to which tolerance develops in the clinical environment and if it does, to determine whether it is of significant concern when using an opioid. The development of tolerance does seem to occur, but only in certain subsets of patients, with the majority not experiencing such a phenomenon. More clinical research is required in certain areas, to provide further evidence and solid conclusions. Education and understanding, both of patients and medical staff, will lead to improvements in pain control, compliance and patient satisfaction.

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T1.58

Induction of brain CYP2D-mediated activation of codeine to morphine increases the rate of codeine analgesic tolerance

DM McMillan[1,2], RF Tyndale[1,2]

[1]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health (CAMH), Toronto, Canada, [2]Department of Pharmacology & Toxicology, University of Toronto, Toronto, Canada, [3]Department of Psychiatry, University of Toronto, Toronto, Canada

Repeated opioid administration produces analgesic tolerance, a phenomenon mediated by action at the mu-opioid receptor. CYP2D enzyme in the brain activates codeine to morphine, a step required for codeine analgesia. Higher brain, but not liver, CYP2D is found in smokers and nicotine induces rat brain, but not liver, CYP2D. Nicotine induction of rat brain CYP2D increases central codeine metabolic activation and analgesia in vivo. We hypothesized that codeine tolerance would be related to brain morphine formation; inducing brain CYP2D would increase the activation of codeine to morphine and therefore accelerate tolerance. Rats (n=12/group) were pretreated with nicotine (brain CYP2D inducer; 1 mg/kg s.c.) or vehicle (saline; 1 ml/kg s.c.). Codeine (40 mg/kg p.o.) was administered daily to measure tolerance; peal antinociception was assessed daily at 30 min using the tail-flick reflex assay. Control pretreatment and treatment groups were included. After the first codeine injection, nicotine (vs. saline) pretreatment increased analgesia (1.32-fold change $AUC_{0\text{-}60min}$; $p<0.05$). Across the subsequent three days of codeine, animals pretreated with nicotine displayed a greater rate of loss in peak analgesia (slope = -11.42 percent per day) compared to vehicle pretreatment (= -4.20; $p<0.006$), suggesting a more rapid rate of onset of tolerance. From day four to seven, both nicotine and vehicle pretreated animals experienced a similar loss in analgesia ($p>0.1$). Variation in brain CYP2D activity (e.g. smoking) may contribute towards altered opioid efficacy, thereby influencing rates of developing opioid tolerance and potentially compensatory increases in dose.

T1.59

Ethanol Reversal of Oxycodone Tolerances

J Jacob[1], R Hill[2], K Lippold[1], K Sakakibara[1], G Henderson[1], H Akbarali[2], W Dewey[1]

[1]Virginia Commonwealth University, Richmond, VA, USA, [2]University of Bristol, Bristol, BS8 1TH, UK

We have previously shown that ethanol reverses tolerance to the antinociceptive and respiratory depressant effects of morphine. In this study, we examined in mice (Swiss Webster) if tolerance to oxycodone can also be reversed by ethanol. Tolerance developed to oxycodone (64 mg/kg po twice daily for 4 days) in both the tail flick (n=20) and locomotor activity tests (n=10). Tolerance to increased locomotor activity was fully reversed by 2g/kg ethanol po, but only partially to the antinociceptive effect. However when morphine (10 mg/kg sc) was administered as a challenging analgesic, 2 g/kg ethanol completely reversed this cross-tolerance. In mice (CD1) implanted with an osmotic minipump to deliver oxycodone (120 mg/kg/day sc) for 6 days cross tolerance to the respiratory depressant action of morphine developed and was partially reversed by acute injection of ethanol (0.3 g/kg ip). At the cellular level, we examined ethanol's effects on morphine and oxycodone-induced inhibition of neuronal excitability in dorsal root ganglia (DRG) neurons. Acute morphine [3 μM] or oxycodone [3 μM] (n=10) applied directly to the bath shifted the threshold to more positive potentials for the initiation of the action potentials, indicative of a decrease in excitability. 48 hour exposure [3 μM] or overnight exposure [10 μM] to morphine or oxycodone resulted in the development of tolerance. 20 minute pretreatment with 10 mM ethanol restored the hypoexcitability effects of morphine in these cells. These data suggest that the reversal effects of ethanol on morphine tolerance extend to that of oxycodone tolerance.

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