Program & Abstracts
Dear Participants,

As the Program Chair, it is my pleasure to welcome you to attend the 41th International Narcotics Research Conference (INRC) in Malmö Sweden. This is an international meeting collecting scientists dealing with various aspects within a broad field of opioid research. Internationally well recognized scientists active as leaders at the absolute frontline of research directed to opioid-related drugs with impact on chronic pain and drug addiction will present hot topics and recent advances from their currently ongoing projects.

The meeting venue The Hilton Hotel in the center of Malmö offers first-class facilities for a conference like INRC. The conference starts with a reception in the Town House and ends with a banquet dinner at Kastellet close to The Oresund Bridge at the Swedish side of the bridge abutment.

Among the invited guest-speakers and plenary lecturers are a core of universally known top-scientists, all of them are opinion leaders in their respective fields. The conference opens by the Director of The Swedish Medical Product Agency Christina Åkerman.

INRC is an annual Head Meeting on opioid research and over the years it has represented the place there most break-throughs in the opioid field first have been communicated. I do hope that you will enjoy this meeting and also the city of Malmö, an exciting spot at the southern part of Sweden.

Fred Nyberg  
Professor and Conference Chair
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Fred Nyberg (Chair)

**INRC Executive Committee**
Lakshmi Devi, INRC president/Executive-Secretary, USA
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Ian Kitchen, UK
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Pao-Luh Tao, Taiwan
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Eija Kalso (Finland)
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Minoru Narita (Japan)
Ingrid Nylander (Sweden)
Toni Shippenberg (USA)
Lei Yu (USA)

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Torsten Gordh (Sweden)
Johan Franck (Sweden)
Mathias Hallberg Sweden)
Ingrid Nylander (Sweden)
Claes Post (Sweden)
Myron Zaluha (Sweden)
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<td>Symposium 8 Opioid addiction</td>
<td>Symposium 10 Opioids in addiction to alcohol and central stimulants</td>
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**14.00 Symposium 11** Endogenous opioids and addiction to gambling

**14.45 NIDA-symposium** Endogenous opioids and addiction to gambling

**16.15 Coffee Break**

**16.45 Business Meeting**

**19.30 Banquette dinner**
Hotel Accommodations

Hilton Hotel
Triangeln 2
SE-20010 Malmö, Sweden
Tel: +46-40-693-4700
Fax: +46-40-693-4711
General Information

The International Narcotics Research Conference 2010, Malmö, Sweden

Conference Venue:
Hilton Hotel - Malmö, Sweden

Badges
Every registered participant will receive a name badge that must be worn to gain access to the scientific sessions and meals/coffee breaks onsite.

Registration Desk
The personnel in the registration desk will assist in all conference needs. The registration desk will be open from Sunday, July 11 to Friday, July 16

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On Sunday, July 11, the registration will be located on the first floor. On Monday, July 12 through Friday, July 16, the registration will be located on the third floor.

Meals
Lunch are provided for all delegates and social registrants according to the following schedule:

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<td>Sunday July 11</td>
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<td>Thursday July 15</td>
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<td>Banquette dinner at Luftkastellet near the Öresunds bridge. Bus transport from Hilton at 19.00</td>
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Instructions for Presenters

Posters
To accommodate all posters on display for the duration of the meeting, poster boards are 250 cm by 100 cm but will be shared by four posters, two on each side.

Poster size: 70 cm high by 100 cm wide
Pushpins to mount the posters will be provided.
You will find your abstract number in this abstract book.

Oral Communications
The acceptable formats are Microsoft PowerPoint (Mac version 2008 and Windows version 2007), Apple Keynote ‘09, OpenOffice 3.2 and Adobe Acrobat.

All talks must be loaded on the conference computer prior to the Plenary och Founder’s lecture between 8:00-8:30.

Technical assistance and uploading of files can be done at the reception desk on the third floor.
INRC 2010 Program

Sunday July 11, 2010

15.00 - 19.00 Registration
19.00 - 21.00 Reception

Monday July 12, 2010

08.30 Welcome/announcement

Plenary Lecture (P1):
Professor Tomas Hökfelt (Stockholm, Sweden): Multiple messenger systems: Focus on neuropeptides and pain

Symposium #1: 09.30 - 10.30
Novel opioid peptide agonists and antagonists
Chairs: James Zadina & Aleksandra Misicka
Luca Gentilucci (Bologna, Italy): Interactions between atypical opioid agonists and MOR
Andrzej Lipkowski (Warsaw, Poland): Opioid-tachykinin chimeric peptides for chronic pain treatment
James Zadina (New Orleans, USA): Novel opioid peptide derived agonists

Coffee-break 10.30 - 11.00

Symposium #2: 11.00 - 12.30
Transcription and epigenetics of the opioid genes
Chairs: Georgy Bakalkin and Li-Na Wei
Jaques Drouin (Montreal, Canada): Genomic landscape regulating pituitary POMC expression
Li-Na Wei (Minneapolis, USA): Epigenetic control of the opioid receptor genes
Rui Yue and Gang Pei (Shanghai, China): Epigenetic regulation of gene transcription by β-arrestin
Hot topic #1 - Luda Diatchenko (Chapel Hill, USA): Expansion of the human µ opioid receptor gene architecture: Novel Functional variants

Discussion

Lunch 12.30 - 14.00

Symposium #3: 14.00 - 16.00
Ligand-directed Signaling and Functional Selectivity
Chairs: Lakshmi Devi & Brigitte Kieffer
Laura Bohn (Irvine, USA): Biased agonism at GPCRs with respect to βarrestin2 regulation of signaling
Stéphane Allouche (Caen, France): Extracellular signal-regulated protein kinase 1/2 (ERK1/2) activation after human delta-opioid (hDOP) receptor stimulation: Study of G protein receptor kinase 2 (GRK2) involvement in the neuroblastoma SK-N-BE cell line.
Amynah Pradhan (Montreal, Canada): Ligand-directed trafficking of the delta opioid receptor in vivo: two paths to analgesic tolerance
Graciela Pineyro (Montreal, Canada): Functional selectivity determines post-endocytic sorting and tolerance potential of delta opioid receptor (DOR) ligands
Hot topic #2 - Ivone Gomes (New York, USA): Novel peptide endocannabinoids reveal agonist directed signaling by cannabinoid receptors.

Discussion

Coffee-break 16.00 - 16.30

Poster session 1: 16.30 - 19.00
Tuesday July 13, 2010

08.30 - 09.30

Plenary Lecture (P2):
Professor Tony L Yaksh (San Diego, La Jolla, USA): Spinal opiates and analgesia - A real bench to bedside story

Symposium #4: 09.30 - 10.30
Opioids in neuropathic pain
Preclinical part
Chairs: Lei Yu & Hiroshi Ueda
Hiroshi Ueda (Nagasaki, Japan): Epigenetic control of opioid receptor gene expression in neuropathic pain model
Zsuzsanna Wiesenfield-Hallin (Stockholm, Sweden): Studies of anti-hyperalgesic effect of various opioids in a rat model of mononeuropathy
Masahiro Ohsawa (Tokyo, Japan): Changes of opioiergic systems in diabetic painful neuropathy

Coffee-break 10.30 - 11.00

Symposium #5: 11.00 - 12.30
Opioids in neuropathic pain
Clinical part
Chairs: Eija Kalso & Torsten Gordh
Torsten Gordh (Uppsala, Sweden): Opioid sensitivity in chronic pain patients correlates with the expression level of opioid peptide systems
Setsuro Ogawa (Tokyo, Japan): Use of opioid analgesics and its potential problem in patients with neuropathic pain
Inna Belfer (Bethesda, USA): Genetic basis for individual variations in pain perception and association studies of neuropathic pain
Oleg Kristhal (Kiev, Ukraine): “Big dynorphin” induces profound conductance changes in the plasma membrane: a putative mechanism of cell signaling
Eberhard Weihe (Marburg, Germany): Plasticity of opioid peptides in specific glutamatergic pathways in experimental pain

Lunch 12.30 - 14.00

Symposium #6: 14.00 - 16.00
Opioids and stem cell research - Recent progress in the stem cell biology and adult neurogenesis
Chairs: Minoru Narita and Kurt Hauser
Pamela Knapp (Richmond, USA): Stage specific effects of opiates and HIV on CNS progenitor function
Minoru Narita (Tokyo, Japan): Role of G-protein-coupled receptors in the differentiation from embryonic stem cells
Amelia Eisch (Dallas, USA): Postnatal hippocampal neurogenesis and opiates
Hot topic #3 - Kurt F. Hauser (Richmond, USA): CNS exposure to morphine and HIV-1 TAT disrupts oligodendrocyte structure and survival in inducible transgenic mice.
Hot topic #4 - Shilpa Buch (Omaha, USA): Morphine accelerates the neuropathogenesis of SIV infection in Rhesus Macaques.

Discussion

Coffee-break 16.00 - 16.30

Poster session 2: 16.30 - 19.00
Wednesday July 14, 2010

08.30 - 09.30

Founders Lecture:
Masamichi Satoh (Hiroshima, Japan): The fruits of opioid research in Japan -- A personal view --

Symposium #7: 09.30 - 10.30
Opioids, Incentive Drive and Habit
Chairs: Toni Shippenberg and Florence Noble
Nigel T Maidment (Los Angeles, USA): Licking, liking and longing: opioid modulation of goal-directed actions .
Agustin Zapata (Baltimore, USA): Kappa opioid receptors and cocaine seeking habits
Serge Ahmed (Bordeaux, France): Modeling heroin addiction in animals: effects of extended drug access on heroin consumption, motivation and preference.

Coffee-break 10.30 - 11.00

Symposium #8: 11.00 - 12.30
Opioid addiction – Clinical aspects
Chairs: Claes Post and Mats Berglund
Johan Kakko (Stockholm, Sweden): Methadone assisted treatment vs. a stepped strategy utilizing buprenorphine and methadone: results of the Swedish 3G study.
Mary Jeanne Kreek (New York, USA): Human molecular genetics and methylation of DNA related to heroin addiction.
Mahmoud S Ahmed (Galveston, USA): Role of human placenta in regulating fetal exposure to opiate medications.
Hot topic #6 - Amy Chang (San Francisco, USA): Mu opioid receptor trafficking and responsiveness to drugs of abuse.
Hot topic #7 - John Mendelson (San Fransisco, USA): The effects of 6B-naltrexol in opiate dependent subjects.

Discussion

Afternoon free
Thursday July 15, 2010

08.30 - 09.30

Plenary Lecture (P3):
Dan Larhammar (Uppsala, Sweden): Evolution of the opioid system

Symposium #9: 09.30 - 10.30
Opioids in alcohol addiction
Preclinical research
Chairs: Ingrid Nylander & Christine Gianoulakis:
Ingrid Nylander (Uppsala, Sweden): The impact of early environmental factors on ethanol-induced effects on opioid peptides in adult male rats
Christine Gianoulakis (Montreal, Canada): Effects of ethanol on opioid peptide release in CNS regions related to addiction
Andreas Zimmer (Bonn, Germany): Endogenous opioids and the commonalities of alcohol and nicotine

Coffee-break 10.30 - 11.00

Symposium #10: 11.00 - 12.30
Opioids in addiction to alcohol and central stimulants
Clinical research
Chairs: Mary Jeanne Kreek & Johan Franck
Karl Mann (Heidelberg, Germany): Searching for the naltrexone responder – the PREDICT Study
Anders Håkansson (Malmö, Sweden): Criminals with polydrug use – a comparison with heroin and amphetamine users
Johan Franck (Stockholm, Sweden): Naltrexone treatment for amphetamine dependence

Discussion

Lunch 12.30 - 14.00

Symposium #11: 14.00 - 14.45
Endogenous opioids and addiction to gambling
Chair: Fred Nyberg
Mark Potenza (New Haven, USA): The neurobiology and genetics of impulse control disorders: relationships to drug addictions.

Discussion

NIDA-symposium 14.45 - 16.15
Chair: Rao S. Rapaka (NIDA/NIH)
David Shurtleff (NIDA/NIH, USA): Basic Neuroscience Research at NIDA: An Update
Danielle Piomelli (UC Irvine, USA): Neural lipidomics and human diseases
Alexandros Makriyannis (Boston, USA): Focused Proteomics Approaches for investigations of the endocannabinoid system

Coffee-break 16.15 - 16.45

16.45 Business Meeting

19.30 Banquette dinner
Friday July 16, 2010

Symposium #12: 09.00 - 10.30
Opioids in neuroendocrinology – abuse of steroids
Chairs: Ruth Wood & Mathias Hallberg
Andrea Bedini (Bologna, Italy): Genomic and non-genomic effects of anabolic steroids on opioid receptor gene expression in neuronal cells
Ruth Wood (Los Angeles, USA): Anabolic-androgenic steroid dependence: overlap with opioid systems.
Discussion

Coffee-break 10.30 - 11.00

Symposium #13: 11.00 - 12.30
Opioid and cannabinoid interaction (joint session with the ICRS)
Chairs: Rafael Maldonado & Walter Fratta
Rafael Maldonado (Barcelona, Spain): Advances in the field of cannabinoid--opioid cross-talk.
Liana Fattore (Cagliari, Italy): Cannabis and reward – interaction with opioids
Charles P France (San Antonio, USA): Interactions between my agonists and delta9-tetrahydrocannabinol in Rhesus monkeys: Selective enhancement of antinociception.

End of conference

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(4,6), L. Stone (3,4,5) and G. Pineyro (1,2,3,5). Depts (1) Pharmacology and (2) Psychiatry, Univ.
de Montréal; Depts (3) Anesthesia, (4) Neurology and (5) Pharmacology; McGill Univ., Qc, Canada.

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Chairs: Toni Shippenberg and Florence Noble

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Program Abstracts

PLENARY LECTURE #1

MULTIPLE MESSENGER SYSTEMS: FOCUS ON NEUROPEPTIDES AND PAIN


Clearly the most important peptidergic system related to pain is the opioid peptides and their receptors, underscored by the still unsurpassed efficacy of morphine as pain killer. Also other peptides, such as substance P, somatostatin and calcitonin gene-related peptide (CGRP), may however play a role. It has been recognized that lesion to the peripheral branch of dorsal root ganglion (DRG) neurons, mimicking the cause of neuropathic pain, induces dramatic changes in regulation of a large number of molecules, including neuropeptides (vasoactive intestinal polypeptide, VIP, galanin and neuropeptide Y, NPY). Much efforts have gone into understanding the significance of these regulations, since this may lead to new ways to treat neuropathic pain. Thus it has been proposed that, for example, antagonists or agonists at both galanin and NPY receptors cause antinociception. Also molecules involved in signal transduction, e.g. PLC\(b\)3, are potential targets. Thus, an inhibitor of this enzyme causes a long lasting increase (>48 hours) in pain threshold in the spared nerve injury model. Still, opioid receptors remain in center of interest. One issue concerns the role of delta-opioid receptors (DORs) in view of their possible unique subcellular localization in large dense core vesicles, the storage organelles for neuropeptides, and their relation to mu-opioid receptors, an issue that will be further detailed in our presentation. Taken together, neuropeptides, the largest group of messenger molecules in the nervous system, and their receptors continue to be targets for research aiming at improving treatment of pain, in particular neuropathic pain.

SYMPOSIUM #1 - Novel opioid peptide agonists and antagonists

Chairs: James Zadina and Aleksandra Misicka

INTERACTIONS BETWEEN ATYPICAL OPIOID AGONISTS AND MOR


Endomorphins (EMs) are endogenous peptides with high selectivity for MOR; they induce strong antinociception by binding to both central and peripheral MOR but, unlike morphine, they are effective in reducing neuropathic pain and their analgesic effect seems to be dissociated by immunomodulatory, cardiovascular and respiratory effects. As native EMs show poor bioavailability and rapid degradation in vivo, we designed and assayed novel EM-1 (YPWF-NH\(_2\)) derivatives bearing chemical modification aimed to improve their application as analgesics. The ionic bond between a protonated amine and a conserved Asp in the third TMH of the opioid receptor is considered the driving force for ligand-receptor interaction of all opioid agonists, being the amine of Tyr the key pharmacophore for opioid peptides. The removal or derivatization of this pharmacophore usually transforms agonists into inactive compounds or antagonists, with only few compounds maintaining an agonist behaviour when deprived of such amino group. Recently, we discovered the novel EM-1 derivative c[YpwFG]; it displayed good affinity to MOR (K\(_i\) 34 nM), is an effective and potent analgesic for visceral pain when administered peripherally (i.p ED\(_{50}\) 1.25 mg/kg; s.c. ED\(_{50}\) 2.7 mg/kg), and retains central analgesic effects (tail-flick test) only at high doses (20 mg/kg). Interestingly, it triggers MOR internalization similarly to DAMGO but displayed an opposite effect on MOR transcription. This cyclopeptide is a structurally atypical opioid agonist, being deprived of the key pharmacophore, therefore we performed investigations by 2D-NMR, conformational analysis, and molecular docking to provide insights into its interaction with and activation of MOR. The resulting receptor-bound structure served as a general model to design new MOR-active compounds containing the sequence wF, to optimize ligand-receptor interactions. This search lead to c[YGwFG], which showed a 10-fold higher affinity for the MOR (Ki 3.6 nM) as well as good analgesic properties in vivo. Finally, we
verified the predictive power of the general model by designing a non-EM-like opioid compound, the cyclic tetrapeptide c[dl(1-NH\textsubscript{2})\textbeta-AwF]. In summary, these results suggest that alternative interactions might duly replace the electrostatic interaction of the protonated nitrogen with the Asp residue, which has not to be considered a conditio sine qua non for opioid receptor activation.

**OPIOID-TACHYKININ CHIMERIC PEPTIDES FOR CHRONIC PAIN TREATMENT**

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Our group proposed to develop new chimeric analgesics in which opioid pharmacophores are covalently hybridized with other types of pharmacophores that positively modulate effects of the opioid part. Synergistic enhancement of opioid analgesia and/or decrease of unwanted side-effects should result from such hybridization. It is generally accepted, that opioids and tachykinins are classified as functional antagonists. However, their spectrum of interactions is much more complicated. Series of new opioid agonist-tachykinin antagonists and opioid agonist-tachykinin agonist conjugates have been synthesized and tested. Hybridization of opioids with tachykinin receptor ligands resulted with new properties that are dependent on their agonist or antagonist nature. In general, hybridization of opioid agonists with tachykinin antagonists resulted in strong analgesia evidencing synergistic interaction between opioids and tachykinin elements. In contrary, the tachykinin agonists may partially reduce opioid analgesic potency of chimeric compounds, but its presence strongly reduces side effects of opioids. In conclusions, both types of hybridized opioid-tachykinin ligands are interesting but with different prospective clinical applications. Presented work has been partially supported by EC 6FP STREP grant “Normolife” (LSHC-CT-2006-037733).

**NOVEL OPIOID PEPTIDE DERIVED AGONISTS**

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A vast majority of natural agonists for G-protein coupled receptors (GPCR), including opioid receptors, are peptides. While their natural form has co-evolved for optimal activation of the receptor, their metabolic instability limits their usefulness as analgesic pharmaceuticals. Stable analogs that retain advantages of the natural compound may have improved pharmacologic properties. We have prepared a series of cyclized, D-amino acid containing analogs of the endogenous mu-selective opioid receptor agonists, the endomorphins, of sufficient stability to produce effective antinociception after peripheral administration to rodents. We also tested their effectiveness in producing antinociception relative to other effects, generally considered unwanted side-effects. Based on earlier studies showing separation of antinociceptive and rewarding properties of endomorphin-1 (EM1) in the conditioned place preference (CPP) test, the comparison of antinociceptive to rewarding (CPP) effects was a particular focus in these tests. The lead compound, a cyclized, D-Lysine (k) containing analog designated ck1 or CYT-1010, showed higher affinity for the cloned human mu opioid receptor than the parent compound while retaining selectivity for mu over delta and kappa receptors. It was highly resistant to in vitro enzymatic degradation in blood (4 days vs 6 min half-life compared to the parent compound) and produced potent antinociception by intracerebroventricular (i.c.v.), intrathecal (i.t.), and intravenous (i.v.) administration. Oral administration of a dose unexpectedly low for a peptide-based compound (0.3mg/kg) produced 80% maximum possible effect (MPE) in the tail flick assay in mice. In the spared nerve injury model of neuropathic pain, i.t. CYT-1010 was over 3 times more potent than morphine in alleviating tactile allodynia. Antinociceptive dose-response curves were generated for morphine and CYT-1010 after icv administration to minimize potential pharmacokinetic differences between the compounds. Doses producing antinociception matched for maximum possible effect (%MPE) and duration were then compared in the CPP test. In this test, which correlates with abuse potential, morphine produced significant CPP while CYT-1010 did not. Potential mechanisms of this apparent functional selectivity will be discussed. **Acknowledgements:** Supported by the VA, ONR, LA Board of Regents, and Cytogel Pharma LLC. Contents do not represent the views of the VA or the US Government.
GENOMIC LANDSCAPE REGULATING PITUITARY POMC EXPRESSION

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Pituitary expression of the POMC gene is largely driven by a proximal promoter that is conserved between species. This promoter is active in both corticotrope and melanotrope cells, with a preference for the later in transgenic assays. Using genome-wide ChIP-chip and ChIP-seq analyses of genomic organization, we identified a novel enhancer that preferentially directs expression to corticotropes during development. This enhancer is different from the neural enhancer and does not appear to confer hormonal regulation. Transcription factors discovered for their role in POMC expression also regulate differentiation of POMC lineages. Indeed, Tpit was shown to be a switch between POMC (corticotrope/melanotrope) and gonadotrope cell fates. However, current data do not account for cell type specificity of corticotropes compared to melanotropes. Genome-wide temporal- and lineage-restricted expression profiling led us to identify a transcription factor that is responsible for cell fate switching between these two cell types. Thus, corticotropes and melanotropes share a basic genetic program that is modulated by lineage-restricted regulatory factors acting through diverse regulatory domains of the POMC locus.

EPigenetic REGULATION OF Opioid RECEPTOR GenES – MOLECULAR MECHANISMS

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Three opioid receptors, MOR, DOR and KOR, are encoded by three distinct genes, which share a highly conserved genomic structure and promoter features but are differentially expressed. Transcriptional regulation of these genes engages both basal and regulated transcriptional machineries and employs activating and silencing mechanisms. In retinoic acid-induced neuronal differentiation model, these genes undergo drastically different chromatin remodelling processes, and display varied patterns of epigenetic marks. At the chromatin level, both MOR and KOR gene promoters undergo continuous and dynamic chromatin remodeling processes in differentiating cells. KOR gene exhibits a biphasic remodeling process, initiated with retinoic acid-induced chromatin condensation and nucleosome assembly, and followed by nerve growth factor-induced epigenetic alteration on KOR gene promoter. MOR gene is initially silenced in stem cells, maintained by DNA methylation and a highly condensed chromatin configuration. Its increased transcription in differentiated neurons involves RA-stimulated chromatin remodeling to open up its chromatin configuration. I will present our current understanding of how the three genes are differentially regulated by varied chromatin remodeling processes and epigenetic triggers. Acknowledgement: This work is supported by NIH grants DA11190, DA11806, DK54733, DK60521 and K02-DA13926 to LNW.

EPigenetic REGULATION OF GENE TRANSCRIPTION BY β-ARRESTIN

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β-Arrestin plays a fundamental role in G-protein-coupled receptor (GPCR) desensitization and internalization in an agonist dependent manner. In addition, this multifunctional molecule can also mediate the cross-talk between GPCR and a variety of signaling pathways. Recently, β-arrestin is identified as an epigenetic regulator of gene transcription through interacting with multiple transcription factors. Under δ- or κ-opioid receptor stimulations, β-arrestin1 specifically accumulates at p27 and c-fos promoters through scaffolding CREB and histone acetyltransferase p300, enhancing local histone H4 acetylation level and gene transcription. A similar mechanism was also observed during T cell activation, where nuclear β-arrestin1 promotes Bcl-2 expression and significantly increases disease severity in a mouse model of multiple sclerosis. Moreover, β-arrestin1 sequesters the Polycomb recruiter protein YY1 to promote the activation of cdx4-hox pathway during zebrafish embryogenesis, which specifies the hematopoietic lineage commitment. Thus, emerging role of β-arrestin in epigenetic regulation represents an important mechanism for the interplay between
environment and genetics, and sheds light on the dynamic regulation of disease and development.

Hot topic #1

EXPANSION OF THE HUMAN MU-OPIOID RECEPTOR (MOR) GENE ARCHITECTURE: NOVEL FUNCTIONAL VARIANTS

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Opioids produce their therapeutic effects by binding to MORs, which are 7 transmembrane domain (7TM) GPCR, and inhibiting cellular activity. The analgesic efficacy of opioids is compromised by side-effects such as analgesic tolerance, dependence and opioid-induced hyperalgesia. In contrast to opioid analgesia these side effects are associated with cellular excitation. We recently discovered a new human alternatively spliced isoform of MOR (MOR1K) that is 6TM GPCR variant. To characterize the pattern of cellular transduction pathways activated by this human MOR1K isoform, we conducted a series of pharmacological and molecular experiments. Results show that, in contrast to stimulation of MOR1, stimulation of MOR1K with morphine leads to excitatory cellular effects. Furthermore, immunoprecipitation experiments reveal that unlike MOR1, which couples to the inhibitory Galpha(i/o) complex, MOR1K couples to the stimulatory Galpha(s) complex. Thus, the major MOR1 and the alternative MOR1K isoforms mediate opposite cellular effects in response to morphine, with MOR1K driving excitatory processes. Supported by NIDCR, NINDS and NCRR grants RO1-DE16558, U01-DE017018, NS41670, and PO1 NS045685.

SYMPOSIUM #3 - Ligand-directed signaling and functional selectivity

Chairs: Lakshmi Devi and Birgitte Kieffer

BIASED AGONISM AT GPCRS WITH RESPECT TO βARRESTIN2 REGULATION OF SIGNALING.

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G protein coupled receptors are subject to regulation by beta-arrestins and the type of regulation can be determined by the agonist-induced confirmation of the receptor which can also be dependent upon the environment in which the receptor is expressed. Studies in our laboratory have focused on how GPCR responsiveness to both drugs and neurotransmitters can be altered by βarrestin interactions in vivo. We have found that beta-arrestin2 plays a significant role in regulating morphine responses at the mu opioid receptor in such a manner that morphine-induced analgesia is enhanced and tolerance suppressed in beta-arrestin2 knockout mice suggesting a negative regulatory role of beta-arrestins in this system. If certain other opioid agonists are tested, the analgesia and opioid tolerance is comparable between genotypes. We propose that this may reflect an agonist bias, whereas the nature of the agonist dictates the contribution of beta-arrestin2 to the physiological response. We have seen similar examples of agonist bias in when we have looked at serotonin 2A receptor (5HT2AR) function in the beta-arrestin2 knockout mice. For example, we have found that serotonin induces 5HT2AR internalization and ERK activation that utilizes a beta-arrestin2 dependent mechanism while DOI, a hallucinogenic 5HT2AR agonist internalizes the receptor and activates ERK independent of beta-arrestin2. We have also found that a ligand bias exists between serotonin and its endogenous N-methylated tryptamine metabolites such that serotonin activates a beta-arrestin2/PI3-K/Src/AKT cascade in frontal cortex and in cortical cultures while N-methyltryptamines do not. The use of the beta-arrestin2 knockout mouse model has been useful for elucidating the contribution of beta-arrestin2 to agonist-directed signaling in vivo. Funded by DA14600; DA18860 and DA025158 to LMB and DA 219522 to KMR.
EXTRACELLULAR SIGNAL-REGULATED PROTEIN KINASES 1/2 (ERK1/2) ACTIVATION AFTER HUMAN DELTA-OPIOID (hDOP) RECEPTOR STIMULATION: STUDY OF G PROTEIN RECEPTOR KINASE 2 (GRK2) INVOLVEMENT IN THE NEUROBLASTOMA SK-N-BE CELL LINE.

L. Coulbault, V. Hanoux, Ph Jauzac †, S. Allouche Laboratoire de biologie cellulaire et moléculaire de la signalisation. UPRES-EA3919. Caen University Hospital, Normandy, France

We reported previously that hDOP receptor is differentially regulated after peptidic or alkaloid agonist treatment in SK-N-BE cells and that GRK2 can regulate hDOP receptor only after etorphine (alkaloid agonist) treatment. In the present study, we explored a putative role of GRK2 in hDOP receptor dependent ERK1/2 activation. SK-N-BE cells were transfected with the wild type GRK2 or its dominant negative mutant GRK2-K220R ; then ERK1/2 activation was determined after etorphine or DPDPE ([D-Pen(2,5)] enkephalin treatment using western-blot experiments. Results: GRK2 or GRK2-K220R overexpression does not affect ERK1/2 activation after etorphine or DPDPE treatment. Although preliminary, our results do not suggest a role for GRK2 in ERK1/2 activation after hDOP receptor stimulation in SK-N-BE cells. These results allow us to better understand mechanisms involved in DOP receptor dependent ERK activation.

LIGAND-DIRECTED TRAFFICKING OF THE DELTA OPIOID RECEPTOR IN VIVO: TWO PATHS TO ANALGESIC TOLERANCE

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Distinct agonists acting at the same G protein coupled receptor may engage different signaling responses. This concept, known as biased agonism, has important biological and therapeutic implications. Ligand-biased responses are well-described in cellular models; however, demonstrating the physiological relevance of biased agonism in vivo remains a major challenge. We used knock-in mice expressing fluorescent delta-opioid receptors to correlate ligand-biased receptor internalization in live neurons with receptor function in vivo. We examined agonists with similar binding and analgesic properties, but high-(SNC80) or low-(ARM390) internalization potencies. A single SNC80 -but not ARM390- administration triggered acute desensitization of the analgesic response. However, daily injections of both compounds over five days produced full analgesic tolerance. SNC80-tolerant animals showed receptor downregulation, and generalized tolerance to analgesic, locomotor and anxiolytic effects of the agonist. In contrast, ARM390-tolerant animals showed no change of receptor function, and tolerance developed for analgesia only. Hence, ligand-directed receptor trafficking of the delta-opioid receptor engages distinct adaptive responses, either at receptor or at systems level. This study reveals a novel aspect of biased agonism in vivo. Research supported by CNRS, INSERM, the Université de Strasbourg, the French ANR grant IMOP and the US NIH NIDA grant #DA05010. AP was supported by INSERM-FRSQ. CN was supported by the Fondation pour la Recherche Médicale (FRM).

FUNCTIONAL SELECTIVITY DETERMINES POST-ENDOCYTIC SORTING AND TOLERANCE POTENTIAL OF DELTA OPIOID RECEPTOR (DOR) LIGANDS.

N. Audet (1), E. Archer (1), O. Mnie-Filali (1), M. Amraei (1), M. Millecamps (3,6), A-J. Chabot (4,6), L. Stone (3,4,5) and G. Pineyro (1,2,3,5). Depts (1) Pharmacology and (2) Psychiatry, Univ. de Montréal; Depts (3) Anesthesia, (4) Neurology and (5) Pharmacology; McGill Univ., Qc, Canada.

Opiate tolerance has been related to desensitization. Within this context we hypothesized that functionally selective ligands stabilizing a DOR conformation which is preferentially sorted for recycling should allow to preserve receptor signalling and display longer lasting analgesic properties. This assumption was tested using DPDPE and SNC-80. Bioluminescence Resonance Energy Transfer (BRET) assays showed that sustained incubation with each agonist modified DORs Gbg interaction in a ligand-specific manner. barr recruitment was also distinct: while SNC-80 recruited barr to the vicinity of DORs and Gbg, DPDPE induced barr interaction only with DORs, resulting in a more stable DOR-barr complex for the former than the latter. In keeping with this observation, only DPDPE allowed recycling and full resensitization of the receptor signalling. Recycling differences for both ligands were also present in primary neuronal cultures. Finally, the use of an inflammatory pain model revealed development of acute analgesic tolerance for SNC-80 but not DPDPE.
Hot topic #2

NOVEL PEPTIDE ENDOCANNABINOIDS REVEAL AGONIST DIRECTED SIGNALING BY CANNABINOID RECEPTORS


We recently identified hemoglobin-derived peptides as endogenous CB1 cannabinoid receptor (CB1R) agonists. These peptides, termed longer hemopressins, activate a signal transduction pathway distinct from that of classical CB1R agonists. For example, lipidic endocannabinoids or classical CB1R agonists activate Gαi-mediated signaling whereas longer hemopressins activate additional Gαi independent signaling such as robust mobilization of intracellular Ca2+ levels (that is not seen with endogenous lipid agonists). To further explore this ligand directed signal specificity we used a combination of reverse phase protein array consisting of 60 different signaling molecules and graph-theory inspired network analysis. These analysis revealed that longer hemopressins activate a distinct signaling network characterized by stimulation of p70S6 kinase, tuberous sclerosis 2 (TSC2), GSK3 phosphorylation indicating that CB1R employs agonist directed signaling to expand its functional repertoire. Supported by NIH grants DA01952; DA08863 and GM071558 to LAD

PLENARY LECTURE #2

OPIATE REGULATION OF SMALL AFFERENT TERMINAL SENSITIVITY: A LINK IN THE CHAIN.

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The perception of pain with tissue injury depends on information received from encoding by spinal systems. Evidence from frog to human show that opiates alter this organized pain behavior by effects upon spinal sensory input. Diverse studies emphasize that spinal opiates act upon small, substance P(+) (sP) primary afferents, where a limited distribution of opioid receptors is identified. Using neurokinin 1 (NK1) receptor internalization as a marker of sP release, it is possible to assert a number of properties of the presynaptic opiate receptor regulation of this link. Several examples with numerous ramifications can be noted. 1) IT agonist/antagonist structure activity show that mu (DAMGO, morphine vs b-funaltrexamine ) and delta (DPDPE, SNC 80 vs naltrendole), but not kappa (U50488) receptors regulate this peptidergic terminal excitability at dose which correspond to the block of evoked pain behaviors. This inhibition is directly on the terminal as evidenced by the ability of spinal mu agonists to block intrathecal capsaicin evoked release. In contrast to the potent opiate effects, the release is poorly regulated by even 2 x MAC volatile anesthetics. 2) Synergy shown between different spinal agents, such as mu opiate and gabapentin is manifested on pain behavior and on afferent terminal release. 3) This terminal action of spinal opiates leading to a reduced release is subject to a well defined time and dose dependent development of tolerance and dependence, parallelling the behavioral profile. Thus chronic exposure to morphine yields no change in resting or evoked release after 6 days (e.g. tolerance with no hyperexcitability) but shows a prominent increase in release with naloxone (withdrawal). 4) Finally, release of sP is suppressed by doses of intrathecal agonists which correspondingly attenuate pain behavior. In contrast, block of sP release by systemic morphine occurs at doses greater than those required to block pain behavior, an insight into functional differences between systemic and spinal analgesic drug effects on downstream processing. This simple system thus permits one to define in vivo with some precision the properties of opiate receptor linked regulation of small afferent terminal excitability, an important element in the regulation of spinofugal outflow and to correlate this with behaviorally defined effects upon nociceptive processing. (NIH DA02110).
EPIDEMIOLOGIC CONTROL OF OPIOID RECEPTOR GENE EXPRESSION IN NEUROPATHIC PAIN MODEL

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Peripheral nerve injury causes a chronic neuropathic pain, which is often resistant to morphine. Previously we have demonstrated that peripheral, but not central, morphine analgesia is lost after sciatic nerve injury, accompanying the long-lasting down-regulation of mu opioid receptor (MOP) in the dorsal root ganglion (DRG). However, its underlying transcriptional mechanisms remain elusive. Considering that MOP gene contains the neuron-restrictive silencer element (NRSE), we assessed the role of neuron-restrictive silencer factor (NRSF) in the resistance to morphine in neuropathic pain model. Here we found that nerve injury up-regulates NRSF mRNA and protein expression in the DRG neuron. Moreover, chromatin immunoprecipitation analysis showed that nerve injury induces histone hypoacetylation at MOP-NRSE site with an increase in direct NRSF binding. Finally, NRSF-knockdown blocked nerve injury-induced MOP down-regulation and loss of peripheral morphine analgesia, suggesting the critical contribution of NRSF-mediated epigenetic mechanisms. In addition to MOP, we found that NRSF-knockdown blocks nerve injury-induced down-regulations of Na1.8 and two transient receptor potential channels (TRPM8 and TRPA1) as well as C-fiber hypoesthesia, but not A-fiber hypersensitization, thermal hyperalgesia and mechanical allodynia. Taken together, these data strongly suggest that NRSF-mediated epigenetic gene silencing is crucial for resistance to morphine and hypoesthesia in neuropathic pain.

STUDIES OF ANTI-HYPERALGESIC EFFECT OF VARIOUS OPIOIDS IN A RAT MODEL OF MONONEUROPATHY

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The effects of morphine, methadone and buprenorphine were compared in Sprague-Dawley rats with ischemic injury to the sciatic nerve, which causes sever hyperalgesia to mechanical stimuli for a number of weeks. The anti-hyperalgesic effect of systemically administered opioids was tested. Both morphine and methadone dose-dependently alleviated mechanical allodynia in the nerve injured rats. However, upon repeated administration the duration of methadone’s anti-hyperalgesic effect was longer than that of morphine. Twice-daily treatment with methadone reversed allodynia for 9 d, whereas the effect of morphine became reduced after 3 d, indication the development of tolerance. Incomplete cross-tolerance was observed between methadone and morphine. The anti-hyperalgesic effect of methadone was stronger in rats tolerant to morphine than the effect of morphine on methadone tolerant rats (1). The effect of the partial mu-opioid agonist buprenorphine was also examined and compared with morphine. Buprenorphine produced strong antinociception in normal rats. Buprenorphine also caused strong anti-hyperalgesia in sciatic nerve injured rats (2). Tolerance to the hyperalgesic effect of morphine developed more rapidly than for buprenorphine in nerve injured rats. In morphine tolerant animals buprenorphine completely restored its anti-hyperalgesic effect. In contrast, morphine had a weaker antihyperalgesic effect in rats repeatedly exposed to buprenorphine. The role of genetic factors in pain sensitivity, analgesia and anti-hyperalgesia have been recognised (3). The antinociceptive effect of morphine, methadone, buprenorphine and codeine was tested on two sub-strains of Sprague-Dawley rats. The antinociceptive effect of morphine and methadone was stronger in one of the sub-strains, whereas buprenorphine and codeine has a similar effect in both strains. Thus, sensitivity to various opioid analgesics may also differ between patient populations. Acknowledgement: The Swedish Research Council. References. 1. A Bulka, A Plesan X-J Xu, Z Wiesenfeld-Hallin, Reduced tolerance to the anit-hyperalgesic effect of methadone in comparison to morphine in a rat model of mononeuropathy Pain 95 (2002) 103-109 2. PF Kouya, J-X Hao, X-J Xu, Buprenorphine alleviates neuropathic pain-like behavior in rats ater spinal cord and peripheral nerve injury Europ J Pharmacol 450 (2002) 49-53 3. JS Mogil, M Devor,Introduction to pain genetics In JS Mogil (Ed.) The Genetics of Pain Seattle: IASP Press,(2004) pp. 1-17 4. A Bulka, PF Kouya, Y Böttiger, A-O Svensson, X-J Xu, Z. Wiesenfeld-Hallin, Comparison of the antinociceptive effects of morphine, methadone, buprnorphine and codeine in two sub-strains of Sprague-Dawley rats Europ J Pharmacol 492 (2004) 27-34.
Changes of Opioidergic Systems in Diabetic Painful Neuropathy


Neuropathy is one of the most common complications in diabetes mellitus and is frequently painful. Previous research has revealed several factors involved in diabetic neuropathy, whereas the agents that affect these factors failed to deliver convincing results in clinical trials. We have previously indicated that the antinociceptive effects of μ-opioid receptor agonists in diabetic mice were less than those seen in non-diabetic mice. On the other hand, δ-opioid receptor agonists produced a greater antinociceptive effect in diabetic mice than in non-diabetic mice. Therefore we suggest that the functions of opioid receptors are influenced by diabetes mellitus. We recently demonstrated that intrathecal (i.t.) treatment with the Substance P (SP) fragment SP1-7 attenuates thermal hyperalgesia in diabetic mice, which was suggested to be mediated through the naloxone- and (+)-pentazocine-sensitive σ1-receptor. In similarity to SP1-7, the tail-flick latency was prolonged by i.t. treatment with SP1-7-amide in both non-diabetic and diabetic mice. The increase in tail-flick latency induced by SP1-7-amide was more pronounced in diabetic mice than in non-diabetic mice. In both groups of mice, the prolongation of tail-flick latency was attenuated by naloxone and the σ1 receptor agonist (+)-pentazocine but not by selective μ-, δ- or κ-opioid receptor antagonists. These results agree with data previously obtained for SP1-7, suggesting that SP1-7, and its amidated analogue attenuate the thermal hyperalgesia in diabetic mice through a pathway in the spinal cord, involving a σ1 receptor system. We have previously synthesized peptide analogues that bind to the SP1-7 binding site with high affinity. One of them, the dipeptide Phe-Phe-NH2, has a five-fold higher affinity for the binding site of SP1-7 compared to the native heptapeptide. Therefore we examined the effect of Phe-Phe-NH2 on the thermal hyperalgesia in diabetic mice. I.t. treatment with Phe-Phe-NH2 produced prolongation of tail-flick latency in both diabetic and non-diabetic mice, which also seem to be mediated by inhibition of the σ1 receptor system. These results suggest that thermal hyperalgesia in diabetic mice may be mediated by a mechanism involving a σ1 receptor system. I.t. treatment with σ1 receptor antagonist BD-1047 increased the tail-flick latency in diabetic mice, which is reversed by the treatment with σ1 receptor agonist. On the other hand, the expression of σ1 receptors in the spinal cord was not changed in diabetic mice. It was concluded that thermal hyperalgesia in diabetic mice may be, at least in part, due to activation of the σ1 receptor system in the spinal cord of diabetes mellitus and that this could be attenuated by SP1-7 and related peptides.
peptide β-endorphin in 80 chronic low back pain patients with differential sensitivity to the opioid analgesic remifentanil and in 56 healthy controls. The results indicated a relation between pain protection and the minor allele of OPRM1, more opioid-related side effects and gender differences in patients with the minor allele of the ABCB1 gene and a correlation between increased opioid sensitivity and the major CACNA2D2 allele. Our results will be reviewed in the perspective of other relevant data from the field of opioid genetics.

USE OF OPIOID ANALGESICS AND ITS POTENTIAL PROBLEMS IN PATIENTS WITH NEUROPATHIC PAIN

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Use of opioids for the treatment of non-cancer pain including neuropathic pain has been increasing recently in Japan. IASP Guideline for Opioids Use on Non-cancer Chronic Pain says that opioids should be used: 1. only when patients’ pain can not be controlled with other analgesics, 2. under correct observance of contraindications, 3. under control by a fixed doctor, 4. under complete informed consent, 5. according to a contract, 6. basically through oral administration, 7. avoiding “on demand administration”, but by the clock, 8. under observation of patients’ behavior. Unfortunately it is a fact that this Guideline is not fully complied in the field of clinical practice, leading to appearance of undesirable complications. Only two opioids, morphine hydrochloride and codeine, had been permitted by Japanese government to use for non-cancer pain before 2009. This means that patients should had taken the opioids several times a day because of their short duration of action. In 2010, transdermal fentanyl patch have become available to manage severe non-cancer pain. In this case, we must have a lecture by “e-learning” which consists of several contents concerning how to use opioids for non-cancer pain and these are followed by tests. Doctors who wish to use fentanyl patch must pass the tests, after then they are authorized by a pharmaceutical company. This situation is insisted by Japanese government. Now, we are creating our own guideline for the use of chronic opioid therapy in chronic non-cancer pain. Several problems and issues about the use of opioids for non-cancer chronic pain, especially for neuropathic pain, will be presented in this symposium.

GENETIC BASIS FOR INDIVIDUAL VARIATIONS IN PAIN PERCEPTION AND ASSOCIATION STUDIES OF NEUROPATHIC PAIN

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Recent studies have established that both clinical and experimental pain perception are influenced by genetic factors. Since major injuries or disease exposures rarely occur together in sibling pairs, association studies in unrelated subjects are the standard method to identify genetic determinants of neuropathic pain. Several studies used a candidate gene approach to elucidate genetic contribution to neuropathic pain phenotypes; however, the data is limited and inconsistent. Possible reasons include: sample heterogeneity, underpowered study design, population admixture, poor phenotyping, genotyping errors, and statistical analytical mistakes. This presentation discusses current strategies to optimize population-based association studies of human neuropathic pain focusing on selection of genetic markers, genotyping data analysis and follow-up approaches. New powerful methods of molecular epidemiology and unbiased interrogation of the whole genome based on systematically grouped well-phenotyped patients possess enormous potential for progress in understanding neuropathic pain and may lead to personalized pain medicine.

BIG DYNORPHIN INDUCES PROFOUND CONDUCTANCE CHANGES IN THE PLASMA MEMBRANE: A PUTATIVE MECHANISM OF CELL SIGNALING


Little is known about interaction of basic “cell penetrating” peptides including big dynorphin (BD) with the plasma membrane. We have found that BD at 1 micromolar concentration induces transient increases in the plasma membrane conductance, from 10 pS to 300 pS with duration from 2 to 10 ms. Effects are evident in neurons and non-neuronal HEK cells, and are not blocked by naloxone suggesting their non-opioid nature. Induction of conductance changes occurs only at negative membrane voltages and is inhibited by increased external Ca++. Preliminary estimate of the pore diameter indicates that it may be roughly 10 times larger than that of the voltage-gated Na+ channel
pore. Therefore, the emerging pores are not probably ion selective. Both depolarization and Ca\textsuperscript{2+} entry via such pores may induce signaling in target cells. Indeed, BD triggers strong Ca\textsuperscript{2+} signals in both excitable insulinoma MIN6 and non-excitable HEK cells. We propose that direct interaction with the plasma membrane may represent a hitherto unknown mechanism by which BD can signal information between cell

**PLASTICITY OF OPIOID PEPTIDES IN SPECIFIC GLUTAMATERIC PATHWAYS IN EXPERIMENTAL PAIN**


Three vesicular transport proteins for glutamate (VGLUT1, VGLUT2, VGLUT3) have been recently identified. Previous work of our group and others has demonstrated pathway-specific expression patterns of VGLUTs which define specific subsets of glutamatergic neurons with unique intrinsic activities, trafficking patterns or regulatory properties of vesicular glutamate transport. Here we investigated the expression and regulation of proenkephalin and prodynorphin derived opioid peptides and other neuropeptides (substance P, PACAP, CGRP) in relation to nociceptive neurons with VGLUT-isoform specific codes along the neuraxis of pain under conditions of experimental inflammatory (collagen-induced arthritis) and neuropathic pain (Bennett model) in rodents. In order to elucidate the functional role of VGLUTs in pain perception, we applied gene silencing by means of isoform-specific RNA interference (RNAi). Isoform-specific co-expression of VGLUTs with opioid and neuropeptides were analyzed at the mRNA and protein level using dual in situ hybridization and confocal immunofluorescence microscopy. For specific suppression of VGLUT2 different siRNAs were designed and tested for their efficacy in transiently VGLUT2-transfected PC12 cells and rat primary dorsal root ganglion (DRG) cultures using real time RT-PCR. VGLUT2 was the most abundantly expressed isoform in spinal neurons. The majority of proenkephalin or prodynorphin expressing interneurons expressed VGLUT2. Proenkephalin and prodynorphin were coexpressed in allVGLUT2 coded projection neurons. Under chronic pain conditions, opiod peptides and other neuropeptides were specifically induced in VGLUT2-positive dorsal horn neurons and synapses. Application of VGLUT2 siRNA in vitro yielded a reduction of VGLUT2 immunopositive cells by more than 80%. Intrathecal injection of VGLUT2 siRNA in rats subjected to unilateral sciatic ligation resulted in a significant reduction in allodynic behaviour as compared to control siRNAs. Conclusion: These results indicate VGLUT-isoform-specific codes of opioid and other neuropeptides in nociceptive neurons and prove that the generated VGLUT2 siRNAs are well suited as pharmacological tools to reduce neuropathic pain. We suggest that endogenous opioids control the excitability of and glutamate release from spinal glutamatergic neurons selectively coding for VGLUT2 in balance with the actions of the neuropeptides PACAP and substance P. Supported by BMBF grant 01GG9819/0

**SYMPOSIUM #6 - Opioids and stem cell research – Recent progress in the stem cell biology and adult neurogenesis**

*Chairs: Minoru Narita and Kurt Hauser*

**STAGE SPECIFIC EFFECTS OF OPIATES AND HIV ON CNS PROGENITOR FUNCTION**

Y. K. Hahn (1), C. M. Bulle (1), K. F. Hauser (2) and P. E. Knapp (1,2). (1) Depts. Anatomy and Neurobiology, (2) Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, 23298, USA.

Injection drug abuse and HIV infection are interlinked epidemics, and there is abundant evidence that exposure to opiates can accelerate both the onset of AIDS and development of HIV-related neuropathology in the CNS. HIV infection is almost exclusively propagated in immune cells. Thus, CNS pathology is largely secondary to inflammatory processes driven by glial cells, or to toxic effects of released viral proteins. The potential for synergy between HIV proteins and opiates in the CNS is extensive since opioid receptors are widely expressed by neural and glial precursors, their mature derivatives, and also by microglia. Although CNS progenitors of neurons and glia are not thought of as classical HIV targets, our studies show that critical progenitor behaviors are adversely affected by exposure to HIV proteins and/or morphine, and that co-exposure is synergistic for certain outcomes. In vitro, the HIV-1 Tat protein, but not morphine, stimulates progenitor secretion of chemokines
RANTES, MIP-1a and MIP-1b. Tat-stimulated progenitors enhance microglial chemotaxis through interactions with CCR5 that are sensitive to both antibody-mediated and pharmacological blockade. HIV-1 Tat and morphine separately reduce progenitor motility, but the effects are not synergistic. There is, however, a robust interactive effect of Tat and morphine on proliferation and lineage progression among progenitors expressing Sox2 and Olig2. For the endpoints thus far examined, we have not observed sensitivity of progenitors to another neurotoxic HIV-1 protein, gp120. Preliminary in vivo studies, using inducible Tat-expressing transgenic mice, have confirmed certain of these findings. For example, Tat expression can reduce overall proliferation (Ki67+) in the striatum, largely through effects on the Olig2+ population. Overall, our findings show that many aspects of CNS progenitor function may be vulnerable to the individual or combined effects of Tat and morphine. Depending upon timing, such exposure may alter neuron and glial populations, affecting gliosis, inflammation and CNS repair. The findings may have special relevance to pediatric HIV patients, who have more rapid development and higher incidence of neurologic complications, and whose progenitor pools are highly dynamic and plastic. Support: NIH DA24461

ROLE OF G-PROTEIN-COUPLED RECEPTORS IN THE DIFFERENTIATION FROM EMBRYONIC STEM CELLS


Embryonic stem cells (ES cells) will be valuable resources for clinical therapies because of their unlimited self-renewal ability and potential to generate any differentiated cell type. On the other hand, monoamines, such as serotonin or dopamine, and neuropeptides appear in the embryo before cell differentiation, and may have any other functions than neurotransmission during embryogenesis such as differentiation and growth. G protein-coupled receptors (GPCRs) play key role in many complex biological processes, including development. However, the role of GPCRs in ES cell pluripotency and differentiation has received little attention. Here we focus on the effect of monoamines and neuropeptides bound to GPCRs on mouse ES cell differentiation including neural or glial differentiation from neural stem cells, and pluripotency. We profiled the expression of hundreds of GPCRs in undifferentiated ES cells and differentiated neural stem cells. We also demonstrated that the expression of brain-enriched microRNAs can be regulated by several monoamines and neuropeptides, and those microRNAs directly affect ES cell differentiation. This phenomenon could be, at least in part, regulated by epigenetic mechanisms such as DNA methylation and histone modification. These results suggest that G protein signaling pathway plays a crucial role in ES cell differentiation and pluripotency.

POSTNATAL HIPPOCAMPAL NEUROGENESIS AND OPIATES

A. J. Eisch, A. A. Arguello, P. Rivera, I. M. Bowen, S. E. Bulin, M. A. Johnson. Dept Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA.

Drugs of abuse, like morphine and heroin, produce robust changes in the birth and survival of neurons in the postnatal hippocampal subgranular zone (SGZ). This is intriguing since the hippocampus is central to many aspects of the addictive process, including drug-context associations and relapse to drug taking. Also, the functional integration of postnatally-generated neurons into hippocampal circuitry and the role of new neurons in memory formation raise the possibility that decreased adult SGZ neurogenesis may alter hippocampal function in such a way as to maintain addictive behavior or contribute to relapse. We will review our work on the impact of opiates on the different stages of postnatal neurogenesis, focusing on recent data exploring whether cells “born” during opiate exposure have altered dendritic processes and on animal models of morphine self-administration. Understanding the relationship between opiates and SGZ neurogenesis opens the possibility of understanding brain functions subserved by neurogenesis, such as memory, and also of harnessing neural stem cells for repair of the diseased and injured brain. Supported by the National Institute on Drug Abuse.
CNS EXPOSURE TO MORPHINE AND HIV-1 TAT DISRUPTS OLIGODENDROCYTE STRUCTURE AND SURVIVAL IN INDUCIBLE TRANSGENIC MICE


Although opiate abuse appears to exacerbate neuroAIDS, the cell targets and modes of action are unclear. We examined the effects of HIV Tat and morphine on the morphology and survival of oligodendrocytes (OL) using GFAP-driven, doxycycline-inducible Tat transgenic mice. Tat by itself induced caspase-3 activation in OLs, which was enhanced by morphine co-exposure. Co-exposure to Tat and morphine increased TUNEL+ OLs. Tat and morphine exposure caused a significant increase in Golgi-impregnated OLs with truncated, aberrant, and fragmented processes. Electron microscopy revealed increased OLs with vesiculated mitochondria and abnormal intracytoplasmic membranes with Tat induction; morphine co-exposure worsened Tat-induced myelin defects, associated axonopathies, and caused marked degeneration in some OLs, which displayed abnormally dense, marginalized nuclear heterochromatin or pyknotic nuclei. Thus, OLs appear to be targets of HIV Tat and Tat-induced OL degeneration is exacerbated by morphine. Support: NIH DA19398 & DA24461.

MORPHINE ACCELERATES THE NEUROPATHOGENESIS OF SIV INFECTION IN RHEUS MACAQUES

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Morphine modulates the immune system by enhancing expression of the TH1 cytokine IL-4 and suppressing the TH2 cytokine IFNγ. It also upregulates expression of CCR5, a major co receptor used by HIV for cell entry. However, it is unclear how these effects of morphine alter the outcome of infection. In this study we have used the SIV macaque model of HIV pathogenesis to investigate the effects of morphine on the immune responses, neuropathogenesis and disease progression in infected animals. Sixteen SIV infected rhesus macaques were divided into 2 groups; 4 were virus alone controls (V) and 12 others were administered morphine continually (M+V). Morphine group exhibited significantly higher mortality rates, succumbing to infection within 5 weeks post-infection. These rapid progressors exhibited high incidence of CNS complications. Viral loads in the plasma of most M+V animals were at least 10^5 copies/mL or higher. CSF viral load in the morphine group was at least a log higher than the V group. Immunohistochemical studies showed more SIV gp120 antigen in the basal ganglia of the morphine versus the V group. The SIV gp120-positive antigen also corresponded to regions staining positive for CD68 indicating that the majority of infected cells are macrophages or activated microglia. Ex vivo studies on CD14+ monocytes further corroborated the in vivo findings. Taken together these findings suggest that morphine plays a direct role of in the comorbidity of SIV encephalitis. Acknowledgement: This work was supported by grants from the National Institutes of Health (SB).

INRC 2010 FOUNDERS LECTURE

THE FRUITS OF OPIOID RESEARCH IN JAPAN -- A PERSONAL VIEW --

M. Satoh, Lab. of Pharmacol., Fac. of Pharm., Yasuda Women’s Univ., Hiroshima, Japan.

Dr. Hiroshi Takagi (1924 - ; professor emeritus of Kyoto University) was a pioneer and leader of opioid research in Japan and was my teacher in pharmacology. He joined the INRC at very early stage and was nominated as a member of the executive committee in 1972, so I regard him as a founder of the INRC. This presentation will include some products of opioid research originated from the laboratories of Prof. Takagi (and the successor) in Kyoto University and other groups in Japan: Early history revealing mechanisms of analgesic action of morphine; an enhancement of...
the bulbospinal descending inhibitory system and an involvement of the descending noradrenergic pathway, identification of kyotorphin (Tyr-Arg, an analgesic dipeptide) from the bovine brain, cloning of cDNAs for rat KOR and MOR, modulation of psychological dependence on morphine under inflammatory or neuropathic noceptive (painful) state (Dr. T. Suzuki and colleagues), discovery and development of a novel anti-itch (pruritus) drug, nalfurafine hydrochloride which is a selective KOR agonist (Dr. H. Nagase and colleagues).

SYMPOSIUM #7 - Opioids incentive drive and habit

Chairs: Toni Shippenberg and Florence Noble

LICKING, LIKING AND LONGING: OPIOID MODULATION OF GOAL-DIRECTED ACTIONS

K. M. Wassum (1), I. Cely (1), S. B. Ostlund (1), B. W. Balleine (2), N. T. Maidment (1). (1) Dept. of Psychiatry and Biobehavioral Sciences, UCLA, USA, (2) Brain and Mind Research Institute, University of Sydney, Australia.

Endogenous opioids may mediate some of the affective qualities of natural and drug rewards. However, evidence also points to their involvement in motivational processes. This distinction may be important considering that a failure to update the incentive value of, or “desire” for, a substance in the face of diminishing affective experience or overt negative consequences is a frequently cited feature of the addicted state. Using a sucrose-rewarded seeking-delivery instrumental chain paradigm that incorporated a licking frequency measure of reward palatability, we recently reported dual, dissociable roles for endogenous opioids in mediating palatability and the assignment of incentive value used to direct instrumental actions. Administration of naloxone into the basolateral amygdala (BLA) during re-exposure to the reward in an elevated hunger state blocked the increase in incentive value-driven reward-seeking actions observed in vehicle-treated animals when subsequently tested, off-drug, under non-rewarded conditions. Interestingly, intra-BLA naloxone was without effect on the palatability-enhancing impact of elevated hunger state. That is, naloxone selectively blocked translation of this increased ‘liking’ into increased incentive value. Follow-up experiments using antagonists/inverse agonists selective for mu, delta or kappa receptors identified the mu receptor as the mediator of this BLA opioid-dependent component of incentive learning. Furthermore, we observed that a downward shift in value attribution induced by re-exposure to the sucrose outcome in a relatively sated state was not affected by intra-BLA CTOP administration, indicating that opioid processes in the BLA mediate encoding of positive but not negative shifts in incentive value. The BLA-opioid-dependent nature of positive incentive learning is not restricted to value shifts induced by changes in hunger state. Repeated pairing of systemic cocaine administration with re-exposure to a previously trained sucrose outcome also enhanced apparent sucrose incentive value, and this effect of cocaine was similarly blocked by injection of CTOP into the BLA during the pairing. A dual role of endogenous opioids in both the affective experience associated with drug taking and the incentive learning process involved in reward seeking may underlie the intensely addictive property of opiate drugs as well as other substances, such as cocaine, that may induce release of endogenous opioid peptides. Funded by NIDA DA09359 and DA05010.

KAPPA OPIOID RECEPTORS AND COCAINE SEEKING HABITS

A. Zapata, Baltimore, USA

MODELING HEROIN ADDICTION IN ANIMALS: EFFECTS OF EXTENDED DRUG ACCESS ON HEROIN CONSUMPTION, MOTIVATION AND PREFERENCE

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Among the different behavioral criteria used to discriminate substance dependence (or drug addiction) from other non-disordered forms of drug use, drug intake escalation presents a number of unique features that makes it particularly suitable for modeling in non-human animals. This criterion has stood the passage of time despite major revisions of diagnostic systems, it is common to all known drugs of abuse and it can be readily and unambiguously operationalized in laboratory animals. I will present evidence showing that escalation to heavy consumption of heroin can be rapidly induced
in the majority of individual animals (i.e., rats) by increased drug availability. Such an escalation of
drug use is probably paralleled by an authentic escalation to drug addiction, as it is associated with
the co-occurrence of other addiction-like changes (i.e., increased motivation and demand for heroin
use; increased difficulty to abstain from heroin use; decreased sensitivity to negative consequences;
decreased sensitivity to natural reward; increased preference for heroin). In addition, during
escalation of heroin intake, most individual animals become increasingly responsive to drug- and
stress-primed reinstatement of drug seeking after extinction. Finally, following increased heroin use,
most individuals present selective cognitive dysfunctions (e.g., deficits in executive functions) that
may contribute to the establishment and/or persistence of addiction. Thus, the study of individuals
with escalating patterns of heroin use should provide a unique and valid approach to experimentally
investigate the behavioral and neurobiological mechanisms that underlie the progression to heroin
addiction. This work was supported by grants from the French Research Council (CNRS), the
Université Victor-Segalen Bordeaux 2, the Conseil Régional d’Aquitaine, the National Research
Agency (ANR), the Fondation pour la Recherche Médicale (FRM) and the Mission Interministérielle
de Lutte contre la Drogue et la Toxicomanie (MILDT).

SYMPOSIUM #8 - Opioid addiction – Clinical aspects
Chairs: Claes Post and Mats Berglund

METHADONE ASSISTED TREATMENT VS. A STEPPED STRATEGY UTILIZING
BUPRENORPHINE AND METHADONE: RESULTS OF THE SWEDISH 3G STUDY
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Methadone maintenance treatment (MMT) is effective, and is generally being considered as the “gold
standard” when treating heroin addicts. There are, however, concerns about safety issues, as well as
questions about side-effects (such as sedation, weight gain, sexual dysfunctions etc). Buprenorphine
maintenance (BMT) is also effective, and buprenorphine has potentially an advantageous safety
profile. It may, however, have a potentially lower potency / efficacy. There are few adequate head-
to-head comparisons, and no evaluation of a rational, flexible - adaptive approach. Research
questions addressed in the 3G-study were: Can a stepped strategy be developed that capitalizes on
the advantageous safety of BMT? That adaptively uses the two medications available? That overall
does as well as “gold standard” MMT? Can predictors for who needs what be found? The study was
a two center RCT, performed in Stockholm and Uppsala, Sweden. 96 heroin dependent subjects
were included, almost exclusively i.v. drug users. First month was a double blind setting, single blind
thereafter. The patients were either randomised to enhanced MMT (eMMT), or stepped treatment
(STEP). Everybody received flexible dosing based on structured clinical assessment, and behavioral
treatment: Group CBT+ individual counselling. A non-confrontative approach was used, including
Contingency management. The patients were monitored through urine toxicology twice weekly
for feedback only. There was a remarkably good overall outcome, both when looking at 6-month
retention, the proportion of drug clean urines during final month, as well as reduction of problem
severity. No predictors for who needs what were found. Virtually identical outcomes in both arms
indicate that the stepped strategy should be used for safety reasons. Enhanced, non-confrontative
and flexible approaches can markedly improve treatment outcomes.

HUMAN MOLECULAR GENETICS AND METHYLATION OF DNA RELATED TO
HEROIN ADDICTION
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Advances in studies of human molecular genetics of specific addictive diseases, and specifically
heroin addiction, have been numerous since we began our research in 1995. There are now
some epigenetic studies, that is methylation of DNA, of human genes related to heroin and other
addictions. Our laboratory over the last three years has conducted several types of studies: 1) further
mechanistic studies of functional polymorphisms of the mu opioid receptor and dynorphin genes,
2) further studies of specific genes identified to be involved in a process related to opiate addiction,
3) hypothesis-driven arrays of genes which may be involved in addictive diseases and affective
disorders, and 4) arrays with a small number of variants for genome-wide association studies. In our
hypothesis-driven studies, we have been able to conduct studies of very well characterized long-term
heroin addicts meeting criteria for methadone maintenance treatment. We have used a hypothesis-driven array to identify genes with variants associated with heroin addiction. In Caucasian subjects, we have identified SNPs from mu, delta, and kappa opioid receptors, as well as galanin, casein kinase 1 epsilon, and serotonin receptor 3B genes. In African American subjects, variants of different genes were identified with nominal significance for association. In genome-wide association studies using a 10K array, we identified several novel genes with a modestly high P value for association with heroin addiction. Variants of other genes hypothesized to be related to opiate addiction included the metabotropic glutamatergic receptor 6 and 8, and also the mu opioid receptor genes. We have been studying the extent of DNA methylation of the promoter region of the mu opioid receptor and found that the extent at specific CpG sites is significantly increased in Caucasian and Hispanic subjects with long-term heroin addiction. When studies were conducted in Caucasians, African Americans, and Hispanics, the patterns of increased extent of methylation were different across the three groups. The majority of subjects studied were in methadone maintenance treatment; it is not known whether these significant epigenetic differences between former heroin addicts and control subjects are due to some pre-drug exposure causes, to long-term addiction to heroin, or to methadone maintenance treatment. Funding for this work was provided by P60 DA05130 (MJK), R01 MH79880 (MJK), and R01 MH076537 (HC; MJK subcontract PI).

ROLE OF HUMAN PLACENTA IN REGULATING FETAL EXPOSURE TO OPIATE MEDICATIONS

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Introduction: The use of methadone (M) or buprenorphine (BUP) for maintenance of the pregnant opiate addict improves maternal and neonatal outcomes. However, a controversy exists on whether the dose of either opiate and its concentration in maternal circulation correlates with the incidence or intensity of neonatal abstinence syndrome (NAS). Our hypothesis, since 1999, is that NAS should correlate with the concentration of an opiate in the fetal circulation which is subject to its transfer across the placenta. During gestation, placental functions include supply of nutrients, exchange of gasses, elimination of waste products and synthesis of hormones. Human placenta also acts as a functional barrier that regulates fetal exposure to toxins and xenobiotics including medications. Therefore, the extent of M and BUP transfer to the fetal circulation is affected by the activity of placental metabolizing enzymes and efflux transporters that are also subject to single nucleotide polymorphisms in the fetal genome. Results: Both M and BUP cross human placenta. The extent for BUP transfer is < M because of its high retention by the tissue. M and BUP are bio-transformed to EDDP and norBUP, respectively and predominantly by cytochrome P 450 19 (CYP 19). However, the rate and extent of M and BUP biotransformation increases with gestation and formation of their metabolites varies widely between placentas. The efflux transporter P-glycoprotein (P-gp) is localized on the apical membranes of the syncytiotrophoblast and is responsible for the extrusion of M but not BUP. P-gp expression is down regulated with gestation but does not correlate with its activity (high expression does not equate with high (Vmax). Single nucleotide polymorphism (SNP) in the fetal MDR1 gene affects P-gp activity. A decrease in P-gp expression is associated with SNPs C3435T and G2677T/A while C1236T and C3245T homozygous variants are associated with increased activity. Conclusions: Human placenta regulates the extent of fetal exposure to M and BUP used in maintenance of the pregnant opiate addict. The activity of placental enzyme CYP 19 and the efflux transporter P-gp contribute significantly to the concentration of M in the fetal circulation. Therefore, the incidence and intensity of NAS should correlate with the concentration of M and BUP in the fetal, not maternal, circulation. Currently, the above factors are being investigated in a population of pregnant women under treatment with methadone and BUP. Supported by a grant from NIDA (DA-13431) to MSA.
Hot topic #5

ALTERED EXPRESSION OF OPIOID GENES DURING HEROIN WITHDRAWAL AND ASSOCIATED STRESS-INDUCED RELAPSE VULNERABILITY IN RATS

Y. Zhou (1), F. Leri (2), E. Cummins (2), A. Ho (1), M.J. Kreek (1). (1) Rockefeller Univ, NY, USA; (2) Univ of Guelph, GP, Canada.

The hypothesis that heroin addiction is characterized in part by a relative deficiency in the endogenous beta-endorphin system is supported by findings that chronic opiate exposure in rodents down-regulates pro-opiomelanocortin (POMC) gene expression in the hypothalamus (Hyp). We report here that there was decreased POMC mRNA in the Hyp of rats immediately after chronic (10-day) intermittent escalating-dose experimenter-administered heroin, during early (12-hour) spontaneous withdrawal, and after 9-day withdrawal from 7-day intravenous heroin self-administration (SA). Increased orexin mRNA was found in lateral Hyp of both groups of heroin withdrawn rats, implicating this peptide in response to withdrawal stress. We further examined whether individual vulnerability to relapse in stress-induced reinstatement of drug-seeking behavior after comparable heroin consumption was related to altered expression of preprodynorphin (ppDyn) gene. We divided heroin SA rats into high and low responders (HR and LR) to foot-shock stress-induced reinstatement during withdrawal. LR had higher ppDyn mRNA in nucleus accumbens core and shell than LR rats. Our results suggest that withdrawal from chronic heroin is associated with decreased Hyp POMC mRNA, and that increased accumbal ppDyn mRNA is involved in modulating vulnerability to stress-induced heroin relapse.

Support: NIH NIDA DA-P60-05130 (MJK); CIHR NET (FL).

Hot topic #6

MU OPIOID RECEPTOR TRAFFICKING AND RESPONSIVENESS TO DRUGS OF ABUSE

A. Chang, A. Madhavan and J.L. Whistler. Ernest Gallo Clinic & Research Center, Department of Neurology, University of California, San Francisco, Emeryville, CA USA.

The utility of morphine for the treatment of chronic pain is hindered by the development of analgesic tolerance and physical dependence. Morphine is unusual in its failure to promote desensitization and endocytosis of the mu opioid receptor (MOR). Recently we generated a knock-in mouse that expresses a mutant form of the MOR, RMOR that undergoes morphine induced desensitization, endocytosis and recycling. Mice expressing this mutant receptor develop reduced tolerance and dependence to morphine. More recently, we have examined whether altering trafficking of the MOR in the RMOR mice affects reward and addictive like behaviors, as well as synaptic plasticity in brain regions important for the reinforcing properties of morphine. We will report some of these findings here. Work supported by NIDA grant DA019958 and funds provided by the State of California for medical research through UCSF.

Hot topic #7

THE EFFECTS OF 6B-NALTREXOL IN OPIATE DEPENDENT SUBJECTS

J. Mendelson (1), K. Flower (1), M. Jang (1), C. Harris (1), W. Sadee (2), W. Snape (1), and G. Galloway (1). (1) CPMCRI, (2) Ohio State University, USA

Complications of opioid use include abuse and constipation. 6-beta-naltrexol (6BN), a neutral opioid antagonist, may decrease abuse liability and reverse opioid-induced constipation. In this proof-of-concept, placebo-controlled, blinded study, 3F and 1M opioid-dependent subjects (on 19-70 mg/day methadone) received ascending doses of 0.05, 0.15, 0.50 and 1.0 mg 6BN. Measures included vital signs, Visual Analog Scales (VAS), the Subjective and Objective Opioid Withdrawal Scales (SOWS and OOWS), oral-cecal transit times, and laxation. PK profiles were obtained with the 1.0 mg dose. 3 subjects (all F) received the maximal 1.0 mg of 6BN. The one M reached stopping criteria at 0.5 mg. No subject would have been advanced beyond 1 mg due to abdominal distress. Although 6BN produced abdominal discomfort, no significant changes in VAS measures of “Opioid Withdrawal” or “Sickness”, total SOWS, HR, or BP occurred. Doses greater than 0.5 mg produced
laxation within 20 minutes in 3/4 subjects. PK was consistent with prior data. 6BN did not produce substantial opioid withdrawal but did produce laxation. These features suggest that 6BN may be useful in opioid combination formulations. Supported by AIKO Biotechnology.

PLENARY LECTURE #3

EVOLUTION OF THE OPIOID SYSTEM

D. Larhammar, S. Dreborg, and G. Sundström, Dept. of Neuroscience, Uppsala University, Uppsala, Sweden

The many prominent roles of the opioid system in reward, pain, feeding etc mean that it is of crucial interest to find out how it has evolved. By combining sequence comparisons with information on the chromosomal location of the genes, we have found that the opioid peptide and receptor genes duplicated already before the origin of the jawed vertebrates more than 450 million years ago. During that period, the whole genome of the vertebrate ancestor doubled twice. Thereby, a single ancestral receptor gene gave rise to the four opioid receptors (delta, kappa, mu and orphanin receptor). The ancestral peptide gene generated two copies in these events and the fourth member arose by a local gene duplication. Interestingly, the ancestral peptide and receptor genes were located on the same chromosome. Additional gene duplications have taken place in bony fishes: some species have duplicated three of the peptide genes and two of the receptor genes, leading to a considerably more complex system than in mammals. In conclusion, these analyses show that an advanced vertebrate opioid system was already established in the first jawed vertebrates and that orphanin/nociceptin and its receptor belong to the opioid gene families on equal terms with the other members.

SYMPOSIUM #9 - Opioids in alcohol addiction, preclinical research

The many prominent roles of the opioid system in reward, pain, feeding etc mean that it is of crucial interest to find out how it has evolved. By combining sequence comparisons with information on the chromosomal location of the genes, we have found that the opioid peptide and receptor genes duplicated already before the origin of the jawed vertebrates more than 450 million years ago. During that period, the whole genome of the vertebrate ancestor doubled twice. Thereby, a single ancestral receptor gene gave rise to the four opioid receptors (delta, kappa, mu and orphanin receptor). The ancestral peptide gene generated two copies in these events and the fourth member arose by a local gene duplication. Interestingly, the ancestral peptide and receptor genes were located on the same chromosome. Additional gene duplications have taken place in bony fishes: some species have duplicated three of the peptide genes and two of the receptor genes, leading to a considerably more complex system than in mammals. In conclusion, these analyses show that an advanced vertebrate opioid system was already established in the first jawed vertebrates and that orphanin/nociceptin and its receptor belong to the opioid gene families on equal terms with the other members.

THE IMPACT OF EARLY ENVIRONMENTAL FACTORS ON ETHANOL-INDUCED EFFECTS ON OPIOID PEPTIDES IN RATS

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Numerous findings support a link between ethanol, endogenous opioids and early environmental factors. This line of research is continued in studies of the early environmental impact on ethanol-induced effects on opioids, and on the response to naltrexone treatment. The early environment has profound consequences for behaviour later in life. Exposure to adverse experiences early in life is related to increased propensity for drug addiction and, vice versa, early experiences can be protective and contribute to resilience for later abuse and addiction. However, compared to the increasing evidence for genetic influence on the neurobiological effects of ethanol and on pharmacotherapy of alcohol addiction, less is known about the environmental impact. The consequences of exposure to early environmental protective and risk factors are currently studied using the rodent model maternal separation (MS). Prolonged daily MS simulates early adversity and rats exposed to this risk environment develop a phenotype distinct from rats subjected to a more naturalistic rearing with short daily MS. Exposure to prolonged MS results in a sex-dependent outcome with males being more affected than females. Male rats displayed basal low levels of enkephalins, altered risk-taking behaviour, high ethanol consumption and preference for high ethanol concentrations. Furthermore, rats exposed to prolonged and short MS, respectively, respond differently to long-term voluntary ethanol consumption. Rats reared in the risk environment have enhanced opioid activation after ethanol drinking, whereas opioid peptides in rats subjected to short MS are essentially unaffected. Endogenous opioids have been linked to ethanol-induced reward and these results suggest altered reward mechanisms depending on early environmental experiences. In addition, the early environment had consequences for the effect of naltrexone. Rats exposed to prolonged MS responded to naltrexone treatment with reduced ethanol consumption while the rats that experienced short daily MS did not respond to naltrexone. These results provide evidence for involvement of endogenous opioids in the neurobiological basis for early environmental influence on adult behaviour and neurobiology. Further, these studies highlight the importance of not only considering genetics but also early environmental factors, both in the aetiology of alcohol addiction.
EFFECTS OF ETHANOL ON OPIOID PEPTIDE RELEASE IN CNS REGIONS RELATED TO ADDICTION.

C. Gianoulakis. Department of Psychiatry McGill University and Douglas Mental Health University Institute, Montreal, Quebec, Canada

Ventral tegmental area (VTA), nucleus accumbens (NAc) and central amygdala (CeA) are brain regions associated with the processes of reward and reinforcement of drugs of abuse, including alcohol. Studies with experimental animals and human alcoholics demonstrated that blocking the activity of the endogenous opioid system attenuated alcohol consumption, suggesting its implication in the processes of alcohol reinforcement. If we accept that endogenous opioids mediate, at least in part, some of the reinforcing effects of ethanol, then acute ethanol exposure should alter the activity of distinct components of the endogenous opioid system in brain regions associated with the processes of reward and reinforcement. Thus, our recent studies aimed in demonstrating evidence of dynamic interactions between ethanol and endogenous opioid peptides in brain regions associated with the processes of reward and reinforcement. In brief, using the in vivo microdialysis technique, the effect of acute exposure of ethanol naïve rats to various concentrations of ethanol on the release of endorphins, enkephalins and dynorphins at the level of VTA, NAc and CeA was investigated. Results demonstrated that ethanol increased the release of endorphin, enkephalin and dynorphin peptides in an ethanol dose, time and brain region dependent manner. These findings appear to support the hypothesis that endorphins and enkephalins, through their interactions with m and d opioid receptors at the level of VTA and NAc, may mediate some of the reinforcing effects of low and moderate doses of ethanol, while dynorphins, through their interactions with k opioid receptors, may mediate some of the aversive effects of high doses of ethanol. Endogenous opioids may mediate ethanol reinforcement through their interactions with the mesolimbic dopaminergic system. In CeA an early long lasting increase of beta-endorphin release supports the previously reported attenuation of alcohol consumption by local microinjections of opioid antagonists, as well as a role of the CeA beta-endorphin in alcohol consumption, though the exact mechanism(s) is not clear. Furthermore, acute ethanol increased the release of corticotrophin releasing hormone (CRH), while CRH receptor antagonists attenuated the ethanol-induced increase of beta-endorphin release. Thus, local activation of CRH receptors may partially mediate the enhanced release of beta-endorphin in response to ethanol at the level of CeA. Supported by grants from the Natural Science and Engineering Research Council of Canada.

OPIOID PEPTIDES AND THE COMMONALITIES OF ALCOHOL AND NICOTINE


“Smokers drink and drinkers smoke” is not only a popular expression, but also an unfortunate well-documented fact. It has been estimated that more than 80% of alcoholics are also nicotine dependent and that, vice versa, the rate of alcoholism is substantially increased by a factor of 4 to 10 in the nicotine-dependent population. However, the cause for this very high degree of comorbidity is still largely unknown. At the molecular and cellular level, both drugs have very different mechanisms of action. Nicotine specifically activates ligand-gated ion channels in the brain, which are normally gated by acetylcholine, while alcohol interacts with various neurotransmitter receptors. Despite this diversity, both drugs seem to engage the endogenous opioid system as a modulator of some of its pharmacological effect. An acute exposure to nicotine or alcohol leads to a release of opioids peptides in specific brain regions, thus resulting in an activation of their corresponding receptors. If the brain is exposed repeatedly or chronically to these drugs, adaptive changes in the level and expression of opioid peptides and receptors occur. These adaptive changes are thought contribute to the homeostatic or allostatic adaptations of the brain, which have been associated with drug dependence. We have used mouse models with genetic deletions of the endogenous opioid peptide genes to further clarify the involvement of opioid peptides in specific aspects of alcohol and nicotine addiction. These animal experiments were complemented by human genetic studies. This work was supported by a grant from the German Ministry of Education and Research.
SYMPOSIUM #10 - Opioids in addiction to alcohol and central stimulants, clinical research

Chairs: Mary Jeanne Kreek and Johan Franck

SEARCHING FOR THE NALTREXONE RESPONDER – THE PREDICT STUDY

K. Mann, Central Institute of Mental Health Mannheim, University of Heidelberg, Germany

Naltrexone treatment of alcoholism is successful but effect sizes are in the low to moderate range. The heterogeneity of patients entering treatment trials is a potential explanation. Attempts have been made to subdivide patient groups for specific treatment approaches but psychopathological characteristics as basis for treatment matching have failed. The OPMR1 polymorphism is a first example where patients with a specific variant of the mu-opioid receptor gene do better with naltrexone. In the PREDICT study (Mann et al., 2009) 426 alcohol-dependent patients were randomized to either placebo, naltrexone or acamprosate. We used biological as well as psychopathological characteristics of patients in order to test their response to naltrexone (or acamprosate). Neuroimaging showed an increase in brain activity in the ventral striatum which was correlated to time to relapse. We also found support for our hypothesis concerning the rewarding characteristics of alcohol in a certain subgroup of patients: f-MRI BOLD response in the ventral striatum predicted a positive naltrexone response. In conclusion the endorphine system can be a target when it comes to a personalized approach in the treatment of alcoholism. Funded by BMBF (01EB0410). Medication provided by Bristol Myers Squibb and Merck/Lipha. K. Mann, F. Kiefer, M. Smolka, H. Gann, S. Wellék, and A. Heinz. Searching for responders to acamprosate and naltrexone in alcoholism treatment: rationale and design of the PREDICT study. Alcohol Clin Exp Res. 33 (4):674-683, 2009.

CRIMINALS WITH POLYDRUG USE – A COMPARISON WITH HEROIN AND AMPHETAMINE USERS

A. Hakansson (1), F. Schlyter (2), M. Berglund (1). (1) Clin. alc. research, Lund university, Malmö, Sweden (2) Swedish Prison and Probation Service, Norrköping, Sweden

This study in criminal justice clients assessed with the Addiction Severity Index compares primary polydrug users (n=1,183) to heroin (n=391) and amphetamine users (n=1,396), and compares polydrug users with (n=408) and without (n=775) opioid use. Compared to heroin users, polydrug users reported less heroin, methadone and injections, equally high frequency of other opioids, and higher frequency of most other substances, psychiatric medication, overdose, depression, cognitive symptoms, violent behaviour and parental substance use. Compared to amphetamine users, polydrug users had more psychiatric symptoms, higher frequency of most substances, but less injecting. In logistic regression, polydrug use with opioids (versus polydrug use without opioids), was associated with younger age, less alcohol, more tranquillisers and injections, overdoses and somatic medication. Polydrug use displays a more severe picture, and polydrug use with opioids appears more problematic and more closely connected to tranquilizers than to alcohol. The study was funded by the Swedish Prison and Probation Service and Malmö university hospital.

NALTREXONE TREATMENT FOR AMPHETAMINE DEPENDENCE

J. Franck, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Amphetamine abuse and dependence constitutes a significant and growing health problem. Despite the widespread abuse of this class of psychostimulant drugs there is no approved medication for the treatment of amphetamine dependence. Animal studies have shown that opioid receptor antagonists may reduce certain behavioural effects of amphetamine. We have recently shown that the opioid antagonist, naltrexone reduces drug seeking behaviour in experimental models of amphetamine dependence and modulates sensitized locomotor behaviour. In human laboratory models, naltrexone blunts the subjective effects of amphetamine in both healthy subjects and amphetamine dependent patients. The efficacy of naltrexone to increase weeks of abstinence in amphetamine-dependent patients was tested in a placebo controlled trial. Eighty individuals meeting DSM IV criteria for amphetamine dependence were randomized to either naltrexone or placebo treatment. All patients visited the clinic weekly to receive medication (50 mg naltrexone or identical placebo) and relapse prevention therapy. Urine samples were analysed twice weekly for illicit drug use. The primary outcome measure was abstinence from amphetamine use, as measured by the total number of negative amphetamine urine samples during 12 weeks of treatment. Overall, 55 patients (68.7%) completed
study. The intention-to-treat analysis showed that the naltrexone group had a significantly higher number of amphetamine negative urine samples, compared to the placebo treated group (p<0.05). There was a reduction in craving levels and self reported weekly consumption of amphetamine, over 12 weeks of treatment, in the naltrexone group compared to placebo. Naltrexone had minimal side effects and was well tolerated. The results suggest that naltrexone is efficacious in reducing relapse to amphetamine use in amphetamine dependent individuals.

SYMPOSIUM #11 - Endogenous opioids and addiction to gambling
Chair: Fred Nyberg

THE NEUROBIOLOGY AND GENETICS OF IMPULSE CONTROL DISORDERS: RELATIONSHIPS TO DRUG ADDICTIONS
M. N. Potenza, Yale School of Medicine, USA
The extent to which impulse control disorders like pathological gambling might best be conceptualized as non-substance or “behavioral” addictions has been debated. The categorization of impulse control disorders as addictions has multiple implications with respect to prevention and treatment of these disorders. This presentation will focus on epidemiological, phenomenological, genetic, behavioral and neuropsychopharmacological data into the relationships between an impulse control disorder (specifically pathological gambling) and other psychiatric disorders (particularly substance use disorders). The existing data suggest strong links between pathological gambling and substance use disorders. The implications of these findings with respect to prevention and treatment of impulse control disorders (e.g., with respect to the use of opioid antagonists) will be discussed.

NIDA-SYMPOSIUM
Chair: Rao S. Rapaka

BASIC NEUROSCIENCE RESEARCH AT NIDA: AN UPDATE
D. Shurtleff, NIDA/NIH, USA

NEURAL LIPIDOMICS AND HUMAN DISEASES
D. Piomelli, UC Irvine, USA

FOCUSED PROTEOMICS APPROACHES FOR INVESTIGATIONS OF THE ENDOCANNABINOID SYSTEM
A. Makriyannis, Northeastern University, Boston

SYMPOSIUM #12 - Opioids in neuroendocrinology – abuse of steroids
Chairs: Ruth Wood and Mathias Hallberg

GENOMIC AND NON-GENOMIC EFFECTS OF ANABOLIC STEROIDS ON OPIOID RECEPTOR GENE EXPRESSION IN NEURONAL CELLS
A. Bedini, G. Guarino, M. Baiula, S. Spampinato. Dept. Pharmacology, University of Bologna, Italy
Nandrolone and other anabolic androgenic steroids (AAS) can alter the expression and function of neurotransmitter systems. These effects can help to explain the behavioral changes, drug dependence and neurodegeneration observed in steroid abusers. Nandrolone (10^{-8} M–10^{-3} M) causes a time- and concentration-dependent downregulation of mu opioid receptor (MOR) transcripts in SH-SY5Y neuroblastoma cells. This effect is prevented by the androgen receptor (AR) antagonist hydroxyflutamide. Receptor binding assays confirmed a decrease in MOR of approximately 40% in nandrolone treated cells. Treatment with actinomycin D (10^{-5} M), a transcription inhibitor, revealed
that nandrolone mainly regulates MOR mRNA stability. In cells transfected with a human MOR luciferase promoter/reporter construct, nandrolone does not alter the rate of gene transcription. Conversely, it reduces delta opioid receptor (DOR) mRNA and the number of DOR binding sites in the neuronal hybrid cells NG 108-15 partly decreasing the rate of transcription of DOR mRNA: an effect insensitive to hydroxyflutamide. These results suggest that AAS may downregulate MOR and DOR expression through different transcriptional and post-transcriptional actions.

ANABOLIC-ANDROGENIC STEROID DEPENDENCE: OVERLAP WITH OPIOID SYSTEMS.

R. I. Wood. Dept of Cell & Neurobiology, Keck School of Medicine of USC, Los Angeles, CA, USA.

Anabolic–androgenic steroids (AAS) are drugs of abuse. They are taken in large quantities by athletes and others to increase performance, with negative health consequences. As a result, in 1991 testosterone and related AAS were declared controlled substances in the United States. However, the relative abuse and dependence liability of AAS have not been fully characterized. In humans, it is difficult to separate the direct psychoactive effects of AAS from reinforcement due to their systemic anabolic effects. However, using conditioned place preference and self-administration, studies in animals have demonstrated that AAS are reinforcing in a context where athletic performance is irrelevant. Furthermore, AAS share neurotransmitter systems in common with other drugs of abuse. In particular, recent evidence links AAS with opioids. In humans, AAS abuse is associated with prescription opioid use. In animals, AAS overdose produces symptoms resembling opioid overdose (hypothermia, bradypnea), which can be blocked by the opioid antagonist naloxone. Naltrexone also prevents AAS self-administration. In terms of brain sites of action, AAS activate Fos expression in the mesolimbic dopamine system, and modify the activity of the endogenous opioid system. One potential mechanism for the reinforcing effects of AAS involves the dynorphin-kappa opioid receptor (KOR) system in the nucleus accumbens (Acb). Activation of KOR in Acb is aversive, and previous studies have shown that AAS reduce KOR in Acb. Phosphorylation of cyclic AMP response binding element protein (pCREB) stimulates Acb production of dynorphin, the endogenous KOR ligand. We hypothesize that AAS may alleviate dysphoria through inhibition of pCREB, thereby reducing KOR activity in Acb. Together, these observations help elucidate AAS brain mechanisms of action, and the potential for AAS dependence. Supported by NIH (RO1-DA12843).

SYMPOSIUM #13 - Opioid and cannabinoid interaction (joint session with the ICRS)

Chairs: Rafael Maldonado and Walter Fratta

ADVANCES IN THE FIELD OF CANNABINOID--OPIOID CROSS-TALK

R. Maldonado, Barcelona, Spain

CANNABIS AND REWARD – INTERACTION WITH OPIOIDS

L. Fattore (1,2), W. Fratta (1,2,3). (1) CNR Neuroscience Institute - Cagliari, (2) Centre of Excellence “Neurobiology of Dependence”, (3) Dept Neuroscience, Univ Cagliari, Cittadella Universitaria of Monserrato, Italy

Opioids and cannabinoids strictly interact in modulating drug reward, craving and relapse. In our earlier works, we showed that acute cannabinoid primings reinstate responding for heroin in rats following 3-week extinction, an effect attenuated by administration of the cannabinoid CB1 receptor antagonist rimonabant (RIMO). In this follow-up study we verified the effect of the opioid antagonist naloxone (NX), alone or in combination with RIMO, on the reinstatement of heroin-seeking behaviour triggered by cannabinoid primings (reinstatement study). Results showed that cannabinoid-induced reinstatement of heroin-seeking is significantly attenuated by RIMO (3 mg/kg) and NX (1 mg/kg) and fully blocked by co-administration of sub-threshold doses of the two. Moreover, since craving for heroin was shown to increase over time, we also assessed the possibility that incubation of heroin-seeking may alter the hedonic value of cannabinoids, and hence facilitate the self-administration (SA) of the cannabinoid agonist WIN55,212-2 (WIN). To this purpose, in a parallel set of heroin-trained rats, we evaluated whether WIN (12.5 µg/kg/inf) SA substituted for heroin (30 µg/kg/inf) SA after different periods of extinction (substitution study). We found that, contrary to immediate (1 day)
or delayed (90 days) drug substitution, rats readily self-administer WIN when access is given after 7, 14 or 21 days of extinction from heroin, displaying a rate of responding positively correlated with the extinction period. Notably, in these animals, cannabinoid intake is enhanced by RIMO and blocked by NX, demonstrating that blockade of opioid and cannabinoid receptors has different outcomes on drug-seeking reinstatement and drug-substitution in heroin abstinent rats. In particular, the finding that, after certain drug-free periods, cannabinoids may be readily self-administered by rats with a previous history of heroin SA indicates that the length of the extinction is a crucial modulator of drug-seeking incubation, suggesting that cannabinoid availability following heroin abstinence may represent a stimulus condition strong enough to elicit a reliable and persistent responding for the drug. If the same phenomenon is found in humans, it might reflect a form of plasticity that contributes to the inability of heroin addicts to remain drug-free.

INTERACTIONS BETWEEN µ OPIOID RECEPTOR AGONISTS AND Δ⁹-TETRAHYDROCANNABINOL IN RHESUS MONKEYS: SELECTIVE ENHANCEMENT OF ANTINOCICEPTION.

C. P. France, L.R. Gerak, J-X. Li. Department of Pharmacology, University of Texas Health Science Center at San Antonio

While opioids continue to be the drugs of choice for treating pain, they are not effective in many patients and their use is limited by abuse and dependence liability. These studies explore the possibility that antinociceptive effectiveness can be increased by combining drugs with similar actions that are mediated through different mechanisms (opioids with cannabinoids) and that this increased effectiveness occurs without increasing, and possibly decreasing, abuse and dependence liability. Drug discrimination, antinociception, and self-administration procedures were used in different groups of monkeys (n=4-5) to examine interactions between µ opioid receptor agonists and Δ⁹-tetrahydrocannabinol (THC). In non-dependent monkeys, THC attenuates the discriminative stimulus effects while enhancing the antinociceptive effects of morphine; the same doses of THC fail to modify the discriminative stimulus effects of naltrexone or morphine in morphine-dependent monkeys discriminating 0.0178 mg/kg naltrexone. THC also fails to modify the discriminative stimulus midazolam in monkeys discriminating 0.32 mg/kg midazolam and morphine fails to modify the discriminative stimulus effects of THC in monkeys discriminating 0.1 mg/kg THC. IV self administration of heroin is decreased by THC, regardless of whether THC is administered non-contingently prior to the session or contingently during the session (i.e., in an i.v. drug cocktail with heroin). Enhancement of the antinociceptive effects and not the discriminative stimulus or positive reinforcing effects of µ opioid receptor agonists by THC suggests that combinations of cannabinoids and opioids might be especially useful for treating pain and that these drug combinations might have reduced abuse and dependence liability as compared to opioids alone. Supported by USPHS grants DA05018, DA09157 and DA17918 (Senior Scientist Award to CPF).
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Poster Abstracts

Poster session 1 – Monday July 12

Novel opioid peptide agonists and antagonists

1. NOVEL OPIOID RECEPTOR LIGANDS FROM NATURAL PRODUCTS
K.M. Smith (1), C.M. Dersch (2), R.B. Rothman (2) and T.E. Prisinzano (1). (1) Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas (2) Clinical Psychopharmacology Section, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD

The opium alkaloids are among the most potent analgesics used today and are selective agonists at mu opioid receptors. Administration is associated with side effects such as respiratory depression and constipation. Chronic use leads to tolerance and dependence. A way to circumvent these problems is to find scaffolds with novel interactions at mu receptors. One approach to this goal is to explore natural products.

Dioclea grandiflora is a vine native to Brazil. The natural products dioclein and dioflorin are two minor constituents of this plant. Both produce antinociception in rodent models, where the activity of dioflorin is comparable to morphine. The effects of dioclein are attenuated by naloxone. This indicates that the mechanism of action involves opioid receptors, but this has not been investigated in vitro, and no structure-activity relationship studies have been published. We report our efforts towards the design, synthesis, and evaluation of dioclein and dioflorin analogs. Supported by DA018151, GM008545.

2. Ac-D-Trp-PheNH2 AND Ac-D-Trp-Phe-GlyNH2 - A NEW CLASS OF UNUSUAL OPIOID PEPTIDES

In the last few years we discovered and investigated novel cyclic opioid peptides, [Tyr-Xaa-D-Trp-Phe-Yaa]n, based on the sequence of EM-1. These peptides, deprived of a protonable amino group, bind and activate the MOR receptor mainly through the side chains of the amino acids 2 and 3, as revealed by molecular docking analysis. As a consequence, we synthesized and tested short di- and tri-peptides containing the sequence D-Trp-Phe, aiming to determine the minimal requisites necessary for the pharmacological effect. This study lead to the new linear peptides Ac-D-Trp-PheNH2 and Ac-D-Trp-Phe-GlyNH2, which revealed a good affinity for MOR, and agonist efficacy in vivo. Substitution of D-Trp with other amino acids gave inactive compounds or moderate antagonism. Conformational analysis and docking suggested that the short peptides adopt a bioactive conformation characterized by a beta-turn. We thank “Fondazione del Monte di Bologna e Ravenna”, and Stepbio s.r.l., Bologna.

3. BU08028, THE FIRST HIGH AFFINITY UNIVERSAL OPIOID RECEPTOR FAMILY LIGAND
W. E. Polgar (1), T. V. Khroyan (1), L Toll (1), G. Cami-Kobeci (2), SM Husbands (2). (1) SRI International, Menlo Park, CA, USA, (2) University of Bath, Bath UK.

Buprenorphine appears to have certain in vivo properties, including partial agonist activity and attenuation of alcohol consumption, caused by activation of NOP receptors. This is despite moderate affinity and very low efficacy at NOP receptors. We hypothesized that increasing NOP receptor affinity and efficacy would improve the profile as a drug abuse medication and reduce addiction liability. Novel compounds were designed based on overlap of the buprenorphine structure and a NOP receptor pharmacophore. Using this strategy, we identified several compounds with universally high affinity, i.e., less than 10 nM at mu, delta, kappa, and NOP receptors. Among these, BU08028 had the highest NOP receptor efficacy in the [35S]GTPgS assay, though still having only partial agonist activity, with efficacy similar to buprenorphine at mu receptors. In vivo, BU08028 is a very potent analgesic, however, it seems to have significant mu character, as indicated by induction of a Staub tail and a significant conditioned place preference. Results will be discussed with respect to the involvement of NOP receptors in the actions of mixed NOP/mu compounds.

4. PHYLOGENETIC DIVERSITY OF THE C-TERMINALLY EXPRESSED HEPTAPEPTIDE UNIT IN PROENKEPHALIN A
E. Bojnik (1), F. Babos (2), A. Magyar (2), A. Borsodi (1), B. Sandor(1). (1) Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences 6726 Szeged, Temesvari krt 62, Hungary, (2) Research Group of Peptide Chemistry, Hungarian Academy of Sciences and Eötvös Lorand University, Budapest, Hungary

The heptapeptide Met-enkephalin-Arg^{2}-Phe^{5} (HsYGGFMRF or MEFR) is a potent opioid cleft
from the sequence of proenkephalin A (PENK), the common precursor of Met- (ME) and Leu-enkephalin (LE). Our bioinformatic analysis exposed chemical biodiversity at the heptapeptide region of PENK among 56 animals. Moreover, with alignment outcome, it became clear that the C-terminal heptapeptide domain of the PENK was far more conserved, compared to the octapeptide region, which was also noticed in our previous studies. Nevertheless, four novel orthologous sequences were found, such as YGGFMGY (Zebrafish), YGGFMRY (Newt), YGGFMKF (Hedgehog) and YGGFMRI (Mudpuppy). Each novel heptapeptides, together with the human ME and MERF, were chemically synthesized and subjected to functionality studies, using receptor binding and G-protein activation assays. Equilibrium binding affinities changed from good to modest measured by various [3H]opioid radioligands in rat brain membranes, while unlabelled homologous ligands exhibited the highest affinities. The relative affinities of the heptapeptides reveal rather mu-receptor preference over the delta-receptors. [35]GTPγS assay has demonstrated that these novel heptapeptides are also potent in stimulating the regulatory G-proteins. As a conclusion, we have put forward our recent results of the bioinformatic studies that revealed novel endogenous heptapeptide structures within PENK sequence of various animal species. All of the newly identified orthologues were able to bind and to activate mammalian opioid receptors and G-proteins, with quite good affinities and potencies. Supported by OTKA-NKTH CK-78566 grant, Budapest, Hungary

5. TOWARD A NATURAL, GENOM-BASED OPIOID PEPTIDE LIBRARY

S. Benyhe and E. Bojnik, Inst Biochem, Biol Res Ctr, Hungarian Acad Sci, Szeged, Hungary

Synthetic chemical peptide libraries, utilizing the combinatorial chemistry approach, have become powerful tools in identifying selective biomolecules. Apart from this progress, Darwinian natural selection, the impulsive force of the evolution, has also generated crowds of various bioactive compounds. The entire set of genomic data, particularly those sequences encoding for precursor polypeptides, represent remarkable chemical biodiversity at the level of mature oligopeptides, moreover provide comparative and comprehensive structural information for neuropeptide families. By screening public protein databases up to 40 novel endogenous opioids have been identified. After bioinformatic analyses, many of them were synthesized and studied also by biochemical means[1-3]. Here we show our latest collection of opioid ortholog and paralog sequences found in around 60 animal species. Structures, endopeptidase recognition motifs, sequence frequency and similarity, evolutionary distances and phylogenetic trees are also given. One significance of these mutationally polymorph sequences is that they altogether compose a natural “combinatorial” library emerged by the evolution. The various peptides evolved by gene mutations offer template sequences for structure-activity relationship studies. As the mass of genome sequencing data grows rapidly, an increasing impact of the phylogenetic and bioinformatic studies on experimental biology is expected. 1) Bojnik et al., Neuroscience, 158:867-874;2009. 2) ibid, Neuroscience, 165:542-552;2010. 3) ibid, Current Annual Meeting, INRC, Malmö, Sweden (accompanying study). Supported by the Hungarian OTKA-NKTH CK-78566 grant in the year of biodiversity by UN (2010).

6. DESIGN AND SYNTHESIS OF SMALL SUBSTANCE P (1-7) MIMETICS

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Substance P (SP), which is a neurotransmitter and neuromodulator at the G-protein coupled NK1 receptor, plays a well known role in pain transmission [1]. The N-terminal fragment of SP, the heptapeptide SP_{1-7}, (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-OH), is a bioactive metabolite that has been shown to oppose several effects of SP [2]. Specific binding sites for SP_{1-7} that differ from the NK1 receptor have been identified in both mouse and rat [3, 4]. In the aspiration to characterise the binding site for SP_{1-7}, and for studies aiming to reveal the role of the heptapeptide in complex animal models, drug-like low molecular weight SP_{1-7} mimetics as research tools are highly desirable. Recently, we reported the discovery of small peptides as potent ligands of the specific binding site for SP_{1-7} [5, 6]. In this poster we report the synthesis of a set of dipeptide compounds, including both peptide and non-peptide features, evaluated in a binding assay displacing [3H]-SP_{1-7}, as tracer. Furthermore, the investigation of different C-terminal functional groups and their influence on binding affinities are studied. References [1] Zubrzycka, M., Janecka, A. Endocr Regul. 34, 195 (2000) [2] Hallberg, M., Nyberg, F. Curr Protein Pept Sci. 4, 31 (2003) [3] Igwe, O J. et al. J Neurosci. 10, 3653 (1990) [4] Botros, M. et al. Peptides 27, 753 (2006) [5] Fransson, R. et al. Neuropeptides 42, 31 (2008) [6] Fransson, R. et al. J. Med. Chem. 53, 2383 (2010)
7. CHARACTERISATION OF 5'-AMINO AND AMIDINO-ALKYL NALTRINDOLE DERIVATIVES AT THE KAPPA-OPIOID RECEPTOR

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There has been a long-standing interest in the unusual pharmacokinetics of kappa-opioid receptor (KOP) antagonists. 5’-(2-Aminomethyl) naltrindole (5-AMN) and 5’-(2-Methylamidino) butyl naltrindole (5-MABN), previously reported by our group and others as selective KOP antagonists in vitro, have not been fully characterised in vivo. Compound affinities were assessed using competitive 3H-diprenophrine binding. Antagonist activity was determined against the KOP agonists U50,488 and U69,593 in the guinea pig ileum and in CD-1 male mice, using the tail withdrawal test. These two naltrindole derivatives differed in their pharmacokinetic profile in vivo. 5-MABN was only active as a KOP antagonist at 3 days post-treatment, whereas 5-AMN displayed activity after 24 hours. 5-AMN and 5-MABN displayed comparable potency to norBNI as KOP antagonists in vivo. We are currently investigating longer chain 5’-amino and -amidino-alkyl, and phenylpiperidine derived antagonists. Supported by the University of Bath, The Royal Society (SJB), NIDA DA07315 (SMH).

8. PYRIDINYL ISOSTERES OF N-(2-[1,1′-BIPHENYL]-4-YL-ETHYL)-3-(CYCLOPROPYLMETHYL)-1,2,3,4,5,6-HEXAHYDRO-6,11-DIMETHYL-2,6-METHANO-3-BENZAZOCINE-8-CARBOXYLIC ACID, A HIGH AFFINITY LIGAND FOR OPIOID RECEPTORS

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We recently reported the high binding affinity of N-(2-[1,1′-biphenyl]-4-ethyl)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocine-8-carboxamide for opioid receptors. This lead compound is developed based on the well-known opioid cyclozocline where its prototypic (of opioids) phenolic-OH is replaced by a carboxamido group. The first generation of such agents, 8-carboxamidocyclozocline displays unexpectedly high affinity for μ and κ opioid receptors (comparable to cyclozocline) and has a much longer (15 h vs. 2h) duration of action in a mouse antinociception model. We now report derivatives of this lead compound where each CH of the biphenyl group was individually replaced by N. Compared to the lead compound, several of the new derivatives displayed considerably higher affinity for the μ receptor (Ki = 0.065 nM). (Supported by NIDA grants R01-DA012180 and K05-DA00360)

9. DIPRENORPHINE IS A PARTIAL AGONIST AT THE MU, DELTA, AND KAPPA OPIOID RECEPTORS

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Historically, diprenorphine has been labelled as a high affinity, nonselective opioid antagonist. Previously, we have shown that other opioid “agonists” such as nalmefene and naltrexone have partial agonist properties as measured using [35S]GTPgS binding assays. We characterized diprenorphine and etorphine in the [35S]GTPgS binding assay using CHO cells stably transfected with either the human m, d, or k opioid receptor. Diprenorphine stimulated [35S]GTPgS binding to m, d, and k opioid receptors with E_max values of 14, 47, and 62%, respectively. The EC_50 values for diprenorphine at m, d, and k receptors were 3.2, 0.64, and 0.45 nM, respectively. Diprenorphine inhibited agonist-stimulated [35S]GTPgS binding as measured using selective agonists DAMGO (m), SNC-80 (d), and U50,488 (k). Imax values were 83, 34, and 28% at m, d, and k receptors, respectively. In contrast, etorphine had no antagonist properties and was a full agonist with E_max values of 160, 100, and 110% at the m, d, and k receptors, respectively. These data show that diprenorphine is a partial agonist and etorphine is a full agonist at the m, d, and k opioid receptors.

10. EL2 LOOP INTERACTIONS FOR BINDING, SELECTIVITY AND ACTIVATION OF THE NOP RECEPTOR: STUDIES WITH SMALL-MOLECULE/PEPTIDE CHIMERIC LIGANDS.

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The minimum sequence of heptadecapeptide Nociceptin/Orphanin FQ (N/OFQ) required to bind and activate the nociceptin receptor NOP is N/OFQ(1-13), and includes four basic amino acid residues in positions 8-13, considered the ‘address’ domain of the peptide. These basic residues interact with the EL2 loop of the NOP receptor, confer selectivity for NOP over other opioid
receptors and have also been shown to be crucial for receptor activation. On the other hand, small-molecule ligands for the NOP receptor that fully activate the receptor are presumed to bind in the transmembrane region of NOP, and not the EL2 loop. We synthesized chimeric molecules, containing small-molecule ligands linked to hexapeptides containing basic amino acids mimicking N/OFQ (8-13), to explore the contribution of the EL2 loop interactions for selectivity and agonist activity. Our results show that binding affinity at NOP increases by an order of magnitude by addition of the peptide fragments to the small molecule and the position of the positively charged amino acids in the chimera plays a key role in the selectivity for NOP over other opioid receptors.

11. BIPHASIC MODULATION OF MU-OPIOID RECEPTOR (MOR) TRANSCRIPTION BY NOCICEPTIN IN NEUROBLASTOMA CELLS CO-EXPRESSING MOR AND NOP RECEPTORS

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Nociceptin (NC), the endogenous NOP receptor (NOPr) ligand, counteracts several effects of opioids and is involved in the development of morphine tolerance and in the modulation of neuropathic pain in animal models. In this study we found that NOP receptor activation by nociceptin-amide [NC-NH$_2$], a NC derivative more resistant to peptidases, caused a biphasic, concentration-dependent modulation of MOR expression in SH-SY5Y cells: 10-100nM NC-NH$_2$ determined an initial up-regulation of MOR mRNA levels followed by a significant down-regulation after 18h-24h exposure. Ongoing transcription is required for NC-NH$_2$-induced modulation of MOR expression, whereas the translational inhibitor cycloheximide prevented the translational inhibitor cycloheximide prevented any involvement of the positively charged amino acids in the chimera plays a key role in the selectivity for NOP over other opioid receptors.

12. BIVALENT LIGANDS FOR THE CHARACTERIZATION OF OPIOID RECEPTOR HETERODIMERS

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Opioid receptors are implicated in both pain transmission and drinking behavior. However, drugs targeting these receptors often have serious side effects, such as tolerance and dependence for opiates, or highly variable efficacy in the case of naltrexone. These problems reflect the fact that despite decades of research, several persistent mysteries remain in opioid receptor pharmacology. For example, while the MOR plays a well-established role in modulating analgesia and reward, the role of the DOR is less clear. While there is only one DOR gene, pharmacologically there are at least two distinct subtypes of the DOR, DOR1 and DOR2. These subtypes have opposing effects on analgesia and drinking. Several lines of evidence suggest that receptor heterodimerization might affect the pharmacological subtypes in vivo. We have synthesized a panel of tuned-affinity bivalent ligands designed to selectively target opioid receptor heterodimers. We will use these ligands to probe the role of receptor heterodimers in analgesia and drinking in vitro and in vivo. J. Harvey is supported by the AP Gianini Foundation and PBBR Foundation.

13. $^{18}$F-BETA-ENDORPHIN: NOVEL PEPTIDIC RADIOTRACER FOR OPIOID RECEPTOR PET IMAGING


Most radiotracers developed for PET imaging of neuropeptide receptors have been analogs of small molecule ligands (e.g. $^{11}$C-carfentanil and $^{18}$F-cyclofoxy for opioid receptors). Our initial goal is to develop a peptidic radiotracer, coupling $^{18}$F selectively to beta-endorphin. Using a differential deprotection synthetic scheme we have synthesized K$_{19}$-aminooxy-beta-endorphin. p-$^{18}$F-Benyaldehyde, synthesized according to published techniques, is coupled to this analog with aniline catalysis in 15 minutes, generating p-$^{18}$F-benzyloxime-K$_{19}$-beta-endorphin. Starting from 480 MBq of aqueous K$^{18}$F, 6 MBq of $^{18}$F-beta-Endorphin can be recovered, with >95% radiochemical purity. “Cold” $^{18}$F-beta-endorphin was determined in vitro binding assays to retain high affinity binding to the mu opioid receptor (K 8.1 nM, $^{3}$H-DAMGO). Support: NIH-NIDA Grants P60-DA05130 (M.J.K.), R03-DA029130 (B.R., E.B.).
A vast number of opioid peptides are derived from processing of precursors from three separate genes. The prodynorphin gene generates dynorphin A, the predominant product and the natural ligand for the kappa, opioid receptor. However, other products of this gene have also been identified, including dynorphin B and α-neocendorphin. Studies of kappa receptors have utilized 3H-U69,593, a highly selective drug with poor affinity for both mu and delta opioid receptors. However, evidence suggests that 3H-U69,593, may be labeling more than one class of kappa receptor in brain tissue. Further exploration of these binding sites requires the development of novel radioligands. In an effort to further the characterization of these potential kappa receptor subtypes, we have developed 125I-labeled opioid peptides with high affinity and established receptor binding assays for them. Funded by grants to GWP (DA0641, DA07242, DA02615 and DA00220) and a training grant to JEP (DA07274) from the National Institute on Drug Abuse.

A growing amount of evidence suggests that kappa opioid (KOP) receptors are involved in the abuse related effects of stimulants. KOP receptor agonists have been shown to modulate the activity of dopamine neurons and decrease self-administration of cocaine in non-human primates, while KOP receptor antagonists have the potential to be utilized as opioid abuse therapies and in the treatment of stress-induced reinstatement (a model of drug relapse). With this in mind, we are exploring salvinorin A (SVA), the first non-nitrogenous natural product having high affinity and efficacy at KOP receptors. However, SVA contains a furan ring which has been shown to be hepatotoxic in other natural products such as teucrin A and aflatoxin B1. Chemical modifications were made to SVA to explore the role of the furan ring in binding and to reduce potential hepatotoxicity. We report our efforts towards the synthesis and evaluation of novel analogs. Supported by DA018151, GM008545.

Various findings have implied that kappa opioid (KOP) receptors are involved with the alteration of the effects that psychostimulants have on the central nervous system (CNS). The neoclerodane diterpene salvinorin A is isolated from the Mexican sage Salvia divinorum (Lamiaceae) and is a selective KOP agonist. In addition, salvinorin A is a potent hallucinogen and, despite its dysphoric effects, it has gained an increase in popularity. Currently, there is a need for a more in depth understanding of the interactions of salvinorin A at the KOP receptor. Here we report our efforts toward the synthesis of salvinorin A analogues using olefination, cycloaddition, and palladium mediated chemistry. These analogues were prepared in order to explore their potential as drug abuse therapies by targeting KOP receptors. While several molecular models have been proposed, there is no X-Ray crystal structure available of the KOP receptor, thus validation through structure-activity relationships continues to be necessary. Supported by DA018151.

Salvinorin A (SA), a highly selective kappa opioid ligand, is the active drug in Salvia divinorum (SD; estimated 1.8M US users). We studied 6M and 2F SD-experienced subjects using ascending-dose (100-4000 µg SA sublingual), placebo-controlled design. Outcome measures included Likert-scale questionnaires and VAS items. A sensitive and specific LC/MS/MS plasma assay for SA and Salvinorin B (SB, the major metabolite of SA) was developed, and plasma obtained at 20 and 40 min post dose. Sublingual SA was minimally psychoactive at doses between 1000-2000 µg (VAS Intoxmax 6.0±14.6; T max 15 min) and effects were brief (less than 1 hour). SB was detectable in plasma at 20 min with a SB level of 0.921 ng/mL. SA levels, although detectable, were below the reliable limit of quantification, suggesting SA is rapidly metabolized to SB in vivo. We conclude that SA is psychoactive in humans, and plasma levels of SA and SB can be measured using LC/MS/MS. Effects

14. EXPLORATION OF NOVEL RADIOIODINE-LABELING TECHNIQUES FOR OPIOID PEPTIDES

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15. PREPARATION AND EVALUATION OF SALVINORIN A ANALOGS WITH REDUCED HEPATOTOXICITY

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16. DESIGN AND SYNTHESIS OF SALVINORIN A ANALOGUES AS PROBES TO FURTHER EXPLORE THE FURAN RING BINDING POCKET


Salvinorin A (SA), a highly selective kappa opioid agonist, is the active drug in Salvia divinorum (SD; 100-4000 µg SA sublingual), placebo-controlled design. Outcome measures included Likert-scale questionnaires and VAS items. A sensitive and specific LC/MS/MS plasma assay for SA and Salvinorin B (SB, the major metabolite of SA) was developed, and plasma obtained at 20 and 40 min post dose. Sublingual SA was minimally psychoactive at doses between 1000-2000 µg (VAS Intoxmax 6.0±14.6; T max 15 min) and effects were brief (less than 1 hour). SB was detectable in plasma at 20 min with a SB level of 0.921 ng/mL. SA levels, although detectable, were below the reliable limit of quantification, suggesting SA is rapidly metabolized to SB in vivo. We conclude that SA is psychoactive in humans, and plasma levels of SA and SB can be measured using LC/MS/MS. Effects
produced with these doses and routes were lower than those normally experienced by participants when smoking SD. Effects may be limited by low sublingual bioavailability. Supported by a private donation.

18. PERIPHERAL INHIBITION OF OPIOID PEPTIDE DEGRADATION

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During inflammation, circulating immune cells are recruited to the damaged tissue where they release opioid peptides. Secreted opioids bind to their receptors on peripheral terminals of sensory neurons resulting in decreased neuronal excitability and pain. Two metallopeptidases, neutral endopeptidase and aminopeptidase N, have shown to be involved in the degradation of enkephalins. Our first goal is to verify if immune cells containing opioid peptides also co-express their degrading enzymes. Secondly, we examine whether blocking NEP and APN results in increased opioid peptide concentrations in inflamed tissue. In a rat model of hindpaw inflammation, using immunofluorescence, both enzymes were detected on opioid peptide-containing immune cells. Also, using in vivo microdialysis, increased extracellular enkephalin concentrations were measured in the inflamed tissue following peptidase inhibitor application. Blocking enzymatic degradation of endogenous opioids offers a promising strategy for pain control without adverse centrally-mediated side effects. Supported by a grant of the Deutsche Forschungsgemeinschaft.

19. METABOLIC STABILITY OF OPIOID PEPTIDES ANALOGUES IN HUMAN PLASMA

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Discovery of opioid peptides created a hope for development of new type of analgesics. However, evolution has selected natural peptides for action at particular site(s), in most of the cases at the site of release. Therefore, low permeabilities of biological barriers and/or low enzymatic stability are optimal properties of endogenous peptides. In contrary, the optimal “classical” drug acting at central nervous system has to be very resistant to metabolism and has to have ability to permeate biological barriers (gut-blood, blood-brain barriers). In the last thirty years the transformation of endogenous opioid peptides into peptidomimetics with properties similar to properties of exogenous drugs (morphine, fentanyl) has been a real challenge for peptide medicinal chemistry. Our scientific team proposed a different approach (from “highly permeable with high selectivity” to “controlled permeability with broad selectivity”) in which “natural” properties of peptides could be an advantage [1]. Based on this idea, several new opioid analogues have been projected and pharmacologically tested. The different biological barriers permeabilities could select particular peptides for different type of pain (central, peripheral, acute, chronic, cancer, inflammatory, et cetera) and different method of applications (site directed, peripheral, central, et cetera). In all applications, greater resistance to metabolic degradation than endogenous peptide is needed. This communication reports the effectiveness of introduction of various structural modifications in opioid peptide analogues on their metabolic stability in human serum. This study has been partially supported by 6FP EC STREP grant “Normolife. [1] A.W. Lipkowski, et al. Polish J. Chem. 68, 907-912 (1994).

20. METABOLIC STABILITY OF STRUCTURALLY MODIFIED ARODYN ANALOGS IN BIOLOGICAL MATRICES

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It is important to determine the fate of peptides for in vivo use in biological matrices that affect their stability after systemic administration. Arodyn, a kappa opioid receptor (KOR) selective peptide antagonist, shows potential for the treatment of cocaine addiction (Carey et al., Eur. J. Pharmacol. 2007, 569, 84). While, the plasma half life of arodyn is 103 min, it disappears from rat blood in < 5 min. It disappears from rat brain homogenate (RBH) and rat brain slices within 2-5 min due to enzymatic degradation. The sites of enzymatic cleavage appear to lie mainly at the N-termini of the basic residues. N-terminal modified Arg or Lys increased the stability in rat brain slices up to 30-60 min. Preliminary data shows that [N^α-Arg] arodyn can be detected in rat brain slices up to 30 min, but disappears from RBH within 10 min. Here we report the metabolism of arodyn and selected analogs in different biological matrices (rat plasma, whole blood, brain slices, and brain homogenate). A detailed analysis of the stability of KOR peptide antagonists is useful for further structural optimization to attain systemically useful peptides. Supported by R01 DA029324.
21. THE SELECTIVE MU-OPIOID RECEPTOR (MOR) ANTAGONIST, ADC5510, REDUCES L-DOPA INDUCED DYSKINESIA (LID) IN THE MPTP MACAQUE MODEL OF PARKINSON'S DISEASE (PD).


While L-DOPA therapy remains the standard to lessen the motor symptoms of PD, disabling dyskinesias often develop. We tested the efficacy of a MOR-selective antagonist (ADC5510) in reducing LID in the MPTP macaque PD model. Parkinsonian macaques with LID were challenged acutely with L-DOPA + vehicle, ADC5510 or the nonselective antagonist naltrexone (NTX). Total activity, parkinsonism & dyskinesia was monitored & rated in a blinded manner for 6h. NTX had no significant effect on LID. ADC5510 reduced LID without decreasing the anti-parkinsonian actions of L-DOPA. The effect of ADC5510 on LID was U-shaped- very efficacious at 1 & 3 mpk (72% & 40% reductions), less effective at 10 mpk. As ADC5510 reduced LID without affecting the anti-parkinsonian actions of L-DOPA, the effect of ADC5510 on LID was considered higher potency than morphine. They significantly reduced mechanical hypersensitivity in rats with carrageenan-induced inflammatory pain. Introduction of a 14-phenylpropoxy group in 6-glycine-substituted morphinans results in interesting alterations in opioid activity by influencing the pharmacological properties of compounds interacting with opioid receptors and such opioids may emerge as novel analgesics.

22. IN VITRO AND IN VIVO PHARMACOLOGICAL PROFILE OF 6-GLYCINE SUBSTITUTED 14-PHENYLPROPOXYMORPHINANS, HIGH AFFINITY AND POTENT OPIOID ANTIMUCINEPTIVE AGENTS


A series of 14-phenylpropoxymorphinans containing a glycine residue in position 6 were synthesized and their pharmacological profile was investigated. Binding assays showed that all compounds displayed high affinities at mu opioid receptors and also at delta and kappa receptors. The 14-phenylpropoxymorphinans produced dose-dependent antinociceptive effects in the rat tail-flick test after subcutaneous (s.c.) administration with considerably higher potency than morphine. The activation of kappa-opioid receptor has been suggested to suppress the development of psychological dependence of mu-opioid receptor agonists. In the present study, the psychological dependence liability and its related locomotor-enhancing effect of amidino-TAPA were evaluated. Amidino-TAPA injected s.c. produced extremely potent and long-lasting antinociception than morphine in ddY mice. Unlike morphine, amidino-TAPA injected s.c. did not induce remarkable locomotor-enhancing effect and rewarding effect at antinociceptive dose even at the highest doses in ddY mice. Instead, amidino-TAPA produced potent locomotor-enhancing effect and rewarding effect at antinociceptive dose in prodynorphin-knockout mice. The present results suggest that amidino-TAPA is a potent analgesics lacking psychological dependence liability by releasing the endogenous kappa-opioid peptides. Supported by KAKENHI 18613015, 19603011 and 21600013 in Japan.

23. LACK OF THE REWARDING EFFECT AND LOCOMOTOR-ENHANCING EFFECT OF MU-OPIOID RECEPTOR AGONIST AMIDINO-TAPA

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We recently developed new mu-opioid receptor agonist amidino-TAPA, which has a distinct antinociceptive profile from morphine that is the release of endogenous kappa-opioid peptides. The activation of kappa-opioid receptor has been suggested to suppress the development of psychological dependence of mu-opioid receptor agonists. In the present study, the psychological dependence liability and its related locomotor-enhancing effect of amidino-TAPA were evaluated. Amidino-TAPA injected s.c. produced extremely potent and long-lasting antinociception than morphine in ddY mice. Unlike morphine, amidino-TAPA injected s.c. did not induce remarkable locomotor-enhancing effect and rewarding effect at antinociceptive dose even at the highest doses in ddY mice. However, amidino-TAPA produced potent locomotor-enhancing effect and rewarding effect at antinociceptive dose in prodynorphin-knockout mice. The present results suggest that amidino-TAPA is a potent analgesics lacking psychological dependence liability by releasing the endogenous kappa-opioid peptides. Supported by KAKENHI 18613015, 19603011 and 21600013 in Japan.

24. EFFECTS OF ZYKLOPHIN, A DYNORPHIN ANALOG, ON MOUSE STRIATAL DOPAMINE LEVELS


Zyklophin, a cyclic dynorphin A (1-11) amide analog, shows nanomolar affinity and high selectivity for kappa opioid receptors and antagonizes l-opioid receptors in vitro (Patkar et al., J Med Chem 2005) and in vivo following systemic subcutaneous administration (Aldrich et al., Proc Natl Acad Sci USA 2009). To determine the effect of zyklophin on striatal dopamine levels,
microdialysis was conducted in freely moving C57BL/6J mice with guide cannulae in the striatum. Zyklophin (0.3, 1, 3 mg/kg sc) did not significantly affect striatal dopamine levels, whereas the kappa agonist U50488 (1, 3, 10 mg/kg sc) significantly decreased striatal dopamine levels in a dose dependent manner. When zyklophin was injected 1 hr before U50488 was given, it blocked decreases in striatal dopamine levels induced by U50488. This effect was gone 24 hrs after zyklophin injection. Zyklophin blocks the effect of kappa opioid agonist U50488 on dopamine and its effect is not long lasting. This study provides in vivo evidence that zyklophin has potential as a therapeutic agent in treatment of drug abuse. IH-NIDA: P60 DA05130 (MJK), R01 DA023924 (JVA).

25. ANALGESIC ACTIVITY OF FRAGMENTS OF ATYPICAL OPIOID PEPTIDES
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We demonstrated that the Tyr-Pro sequence present in majority (63%) of atypical opioid peptides represents the shortest fragment with analgesic activity. Dipeptide Tyr-Pro administered i.p. at doses from 0.1 to 10.0 mg / kg exhibited naloxone-reversible analgesic activity in somatic and visceral pain tests (tail immersion, tail clip, acetic acid writhing, formalin pain). Tyr-Pro displayed a weak depressive action on electrically stimulated contraction of the mouse vas deferens preparations, but did not influence contraction of guinea-pig ileum. Pharmacological activity of atypical opioid peptides did not depend on the position of the Tyr-Pro sequence in molecule, while peptides with the C-and N-ends of this sequence extended by natural amino acid residues retained analgesic activity. Analysis of 22 peptide analogues, identified a new group of compounds possessing analgesic activity, with consensus sequence A-Tyr-Pro (D-Pro, dehydro-Pro, D-dehydro-Pro, Hyp)-X, where A, B - the natural amino acids, and X - methyl or amide. Analogic activity of these peptides does not correlate with their activity in the guinea-pig ileum and mouse vas deferens assays. In this group, peptides Tyr-Pro-[D,L]-Ser-NH2 and Tyr-D-Pro-Ser-NH2 displayed the highest analgesic activity.

Transcription and epigenetics of the opioid genes

26. mRNA LEVELS OF THE ENDOGENOUS OPIOID LIGANDS AND RECEPTORS IN C57BL/6J AND 129P3/J MICE: STRAIN AND HEROIN EFFECTS.
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We have shown strain and dose differences in heroin-induced behavior and reward in C57BL/6J (C57) and 129P3/J (129) mice (Schlussman et al. 2008). Using Real Time PCR we examined the effects of five doses of heroin on the levels of the endogenous opioids and their receptors in the mesocorticolimbic and nigrostriatal pathways in these same mice. Compared to C57 animals, 129 mice had higher mRNA levels of KOP-r in the nucleus accumbens (NA) and of DOP-r in the NA and a region containing both the substantia nigra and ventral tegmental area (SN/VTA). In the frontal cortex of 129 mice, lower levels of both KOP-r and DOP-r mRNAs were observed. pDyn mRNA was also lower in the caudate putamen of 129 mice. Strain differences were not found in the levels of MOP-r, or pEnk or POMC in any region examined. Heroin dose-dependent changes in the levels of MOP-r, KOP-r and DOP-r mRNAs were observed in the SN/VTA. Additionally, DOP-r mRNA was dose-dependently elevated in the hypothalamus. Additional heroin-induced effects were not observed. Supported by NIH DA05130 and the Arcadia Charitable Trust.

27. ELEVATION OF MOR EXPRESSION IN SH-SY5Y BY CONDITIONED MEDIUM FROM LPS TREATED TPA DIFFERENTIATED HL-60 CELLS
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Lipopolysaccharide (LPS) has been shown to increase reactive oxygen species (ROS) levels and induce the secretion of pro-inflammatory cytokines by macrophage. Pro-inflammatory cytokines have been shown to modulate the expression of mu-opioid receptors (MOR). In this study, HL-60 cells were differentiated with 12-O-tetradecanoylphorbol-13-acetate (TPA) into macrophage like cells, TPA-HL-60. Laser scanning confocal microscopy was used to show LPS-induced intracellular accumulation of ROS in TPA-
28. 14-3-3 ZETA PROTEIN REGULATES CELL SURFACE EXPRESSION OF THE HUMAN KAPPA OPIOID RECEPTOR (hKOPR) IN NEURO2A CELLS

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14-3-3 proteins are a family of conserved regulatory molecules in eukaryotic cells, where they participate in fundamental biological processes such as signal transduction, metabolism, and membrane protein transport. Using proteomic analyses, we found that 14-3-3 zeta co-immunoprecipitated with hKOPR from extract of Neuro2A cells stably expressing FLAG-hKOPR (N2A-FLAG-hKOPR cells). We further confirmed that hKOPR associated with endogenous 14-3-3 zeta and transfected HA-14-3-3 zeta in N2A-FLAG-hKOPR cells by co-immunoprecipitation. In addition, the KOPR C-tail interacted directly with 14-3-3 zeta in rat brain extracts by pull-down assay. 14-3-3 zeta siRNA decreased the amount of 14-3-3 zeta by 70% in N2A-FLAG-hKOPR cells and reduced cell surface expression of hKOPR by about 25% by ligand binding and immunoblotting. Expression of the 14-3-3 scavenger protein pGpLI-R18 also decreased cell surface expression of hKOPR. Pulse chase study showed that 14-3-3 zeta siRNA decreased the amount of mature hKOPR exported to surface from ER and Golgi, but did not change the rate of export. These results indicate that 14-3-3 zeta affects forward transport in the secretory pathway. Expression of the dominant negative mutant 14-3-3 zeta (R56A/R60A), which interrupts the interaction of 14-3-3 zeta with Raf1 and inhibits Raf1 activation, did not reduce surface expression of hKOPR, suggesting that Raf1 activation is not involved in 14-3-3 zeta-induced enhancement in hKOPR expression. The sites of hKOPR involved in interaction with 14-3-3 zeta are being studied. Supported by NIH grant DA17302.

29. ALCOHOL EXPOSURES INDUCE SELECTIVE ALTERATIONS ON ENDOGENOUS OPIOID SYSTEM GENES REGULATION IN RAT AMYGDALA: POSSIBLE EPIGENETIC MECHANISMS

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We investigated molecular mechanisms of adaptive transformations occurring at cellular level in brain opioid systems after alcohol exposure. Sprague Dawley rats received intragastric administration resembling human drinking with several hours of alcohol exposure: water or 1.5 g/kg alcohol (20% in water) three times daily for 1 (acute) or 5 (repeated) days. Amygdale were dissected 30 minutes (acute and one repeated group), 20 hours (Early Withdrawal) or 3 days (Late Withdrawal) after the last administration. Real-time RT-PCR was used to assess mRNAs of interest abundances and amounts of specific immunoprecipitated DNA fragments at genes promoter. Prodynorphin and pronociceptin increased gene expression was observed following 1 day of ethanol, during the reinforcement period, and during early withdrawal, possibly for the negative dysphoric state. A potential epigenetic mechanism of gene regulation was observed in peptides promoter regions with changes of histone 3 lysine 27 trimethylation and lysine 9 acetylation, associated with gene repression and activation respectively. Our findings could help to the understanding of how alcohol differentially affects the opioid system and provide evidences of a linkage between gene expression alterations and epigenetic modulation. Acknowledgement: PRIN 2007 (2007R93XF_004).

30. SHIFT IN EPIGENETIC MECHANISM IN HUMAN ALCOHOLICS: DNA DEMETHYLATION IN A SINGLE NUCLEOSOME MAY UNDERLIE PRODYNORPHIN UPREGULATION

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We previously demonstrated that PDYN expression is elevated in the prefrontal cortex (PFC) of human alcoholics that may be relevant for alcohol dependence. Here we investigated epigenetic mechanism of PDYN activation. The 1.7 kb promoter area was analyzed in the PFC of alcoholics and controls (n=14 / group) using pyrosequencing and ChIP-qPCR. The short (~100 nt) CpG-rich promoter region (DMR) apparently located within a single nucleosome was found to
be differentially methylated between groups (35%; p<0.001). The DMR contains canonical E-box that interacted in EMSA and ChIP-qPCR with USF2, a dominant E-box binding factor in the PFC. USF2 was colocalized with PDYN protein in cortical neurons by immunostaining. PDYN mRNA, CpG methylation and USF2 correlated with each other. Directions of these correlations differed between controls and alcoholics suggesting shift in epigenetic mechanism. Thus, the DMR may function as “epigenetic switch” affected by alcohol; DNA demethylation may allow recruitment of USF2 to the promoter resulting in PDYN activation.

31. MOLECULAR MECHANISMS UNDERLYING THE REDUCED OPIOID REWARD ASSOCIATED WITH ALTERATION OF MICRO-RNA EXPRESSION AND EPIGENETIC MODULATION UNDER THE NEUROPATHIC PAIN


It has been reported that the ascending anatomical dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens is mostly related to the reinforcing effects of opioids. We previously demonstrated that the dysfunction of dopaminergic neuron could be responsible for the suppression of opioid dependence under the neuropathic pain by sciatic nerve ligation. To a further understanding of the machinery of this phenomenon, we performed a multiplex analysis for profiling of the expression of microRNA and candidate genes along with epigenetic modification in the VTA of nerve-ligated mice. In the present study, we found that the expression of some microRNAs was altered in this region of mice with neuropathic pain. The present findings further explain the molecular mechanism that underlies the dysfunction of dopaminergic neuron linked to the reduced opioid reward under the neuropathic pain-like state by sciatic nerve ligation.

32. SITE- AND TISSUE-SPECIFIC METHYLATION OF CpG SITES FORMED BY PRODYNORPHIN SNPs ASSOCIATED WITH ALCOHOLISM: ANALYSIS IN HUMAN BRAIN


How epigenetic and genetic factors interact to affect gene expression, and how that translates into disease predisposition is not well known. Here, we investigated methylation of SNPs in PDYN promoter (rs1997794) and 3´-UTR (rs2235749), both forming CpG site and associated with alcoholism (Xuei et al., 2006), and effects of these SNPs on PDYN expression in brain of human alcoholics. Promoter SNP/CpG was not methylated; its T-allele compared to risk C-allele showed PDYN expression lower in controls and similar in alcoholics, and formed AP1-like site targeted by FOSB/JUND in EMSA. Methylation of 3´-UTR SNP/CpG was high, correlated with PDYN expression and was increased in alcoholics. The risk T-allele of this SNP forms T-box targeted in EMSA by 60-65 kDa DNA-binding factor that showed intermediate and low affinity for methylated and unmethylated C-allele. Thus, both SNPs may impact PDYN expression. Epigenetic and environmental factors contributing to alcoholism may converge on the 3´-UTR SNP through modification of its CpG variant by methylation.

33. METHYLATION OF CpG SITES FORMED BY PRODYNORPHIN SNPs ASSOCIATED WITH ALCOHOLISM: ANALYSIS IN HUMAN BRAIN


Previously, TSSs for 5´-truncated mRNAs, promoter activity, TF binding sites, and SNP associated with alcoholism, were described for the dynorphin-encoding PDYN exon 4 region (Bakalkin at al., 1995, 1997; Nikoshkov et al., 2005; Xuei et al., 2006). Here, we analyzed CpG methylation of this region using pyrosequencing. Methylation patterns were conserved across human individuals, while strongly differed between human tissues and cultured tumor cells; brain and peripheral tissues; and brain areas. Thus, CpG#3 showed 18 ± 9% methylation in cell lines, and 90 ± 7.1% in tissues, while CpG#7 methylation was 1.5-fold higher in PFC compared to other tissues. Methylation was completely abolished in DNMT1/3b deficient cells. Notably, CpGs with robust methylation differences flanked enkephalin-encoding sequences. Canonical full-length PDYN mRNAs and CpG methylation did not correlate. We propose a novel epigenetic function for the enkephalin-encoding sequences in regulation of DNA conformation / chromatin remodeling, possibly not involved in transcription of canonical PDYN mRNA.
34. DIFFERENTIAL METHYLATION OF CPG-RICH REGIONS OF PDYN GENE IN HUMAN POSTMORTEM BRAIN TISSUES AND PBMCs


Epigenetic factors such as DNA methylation play an important role in modulation of gene expression. We analyzed DNA methylation patterns of three CpG-rich regions of PDYN, a CpG island and a cluster ‘A’ in the proximal promoter, and a cluster ‘B’ in coding exon 4, by bisulfite sequencing of DNA from caudate and anterior cingulate cortex of 34 human postmortem brains and 20 matched PBMCs. We found remarkably similar patterns of methylation across CpG sites in these tissues. There were tissue-specific differences in methylation levels of the CpG island: levels in PBMCs > caudate > anterior cingulate cortex, but there was higher PDYN expression in caudate than in anterior cingulate cortex. In contrast, cluster A near the transcription start site was hypomethylated. This DNA methylation profile of the PDYN gene is typical for primary responsive genes with regulatory elements for both basal and tissue-specific transcription. Support: NIH-NIMH R01-MH79880 (MJK); NIH-NIDA P60-DA05130 (MJK), NIH-NIMH R24-MH59724 (SM).

35. POSSIBLE INVOLVEMENT OF INCREASED TRANSCRIPTION OF CHEMOKINE RECEPTORS WITH HISTONE MODIFICATIONS IN THE METHAMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION


A growing body of evidence suggests that the behavioral sensitization, which is expressed as a progressive enhancement of the behavioral activating effects of the drug when repeated injections, may be accompanied by long-lasting neural plasticity. In this study, to further understand mechanisms that underlie methamphetamine-induced behavioral sensitization, we investigated changes in epigenetic modification at 14 chemokine and cytokine genes in the limbic forebrain including the nucleus accumbens of mice that were intermittently treated with methamphetamine. To the best of our knowledge, the present data are the first to indicate that chronic treatment with methamphetamine induces a dramatic increase in the expression of the CCR2 gene along with epigenetic modifications in the limbic forebrain including the nucleus accumbens. This phenomenon may be, at least in part, responsible for the development of sensitization to methamphetamine.

36. DISTINCT PHYSIOLOGICAL ROLE OF SPINAL MMOR-1 SPLICE VARIANTS

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We recently reported that mu-opioid receptor agonist amidino-TAPA causes the release of endogenous opioid peptides to produce spinal antinociception. In the present study, involvement of spinal mMOR-1 splice variants on the release of endogenous opioid peptides by amidino-TAPA was investigated. In naive mice, the antinociception induced by i.t. injected amidino-TAPA was attenuated by i.t. pretreatment with antisera against the endogenous opioid peptides dynorphin A (A/Dyn A), dynorphin B (A/Dyn B), alpha-neo-endorphin (A/NeoE), and [Leu]^5-enkephalin (A/L-Enk). However, in the condition MOR-1J was desensitized, the inhibiting effect of A/Dyn A against amidino-TAPA-induced antinociception was disappeared. In contrast, the inhibiting effect of A/Dyn B and A/NeoE was disappeared in the condition MOR-1K was desensitized. Moreover, the inhibiting effect of A/Dyn A and A/L-Enk was disappeared in the condition MOR-1L was desensitized. The present results suggest that the release of endogenous opioid peptides by amidino-TAPA in spinal cord is mediated through the activation of MOR-1J, MOR-1K or MOR-1L. Supported by KAKENHI 18613015, 19603011 and 21600013 in Japan.

37. EPIGENETIC MECHANISMS INVOLVED IN THE INDUCTION OF MU OPIOID RECEPTORS IN T CELLS BY INTERLEUKIN-4

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The expression of the mu opioid receptor (MOR) gene in resting T cells is repressed. However, it is induced by interleukin (IL)-4. We investigated mechanisms of this induction with a special focus on epigenetic mechanisms. Using chip analysis we showed that STAT6, the transcription factor responsible for the induction of the MOR gene in response to IL-4, binds to the MOR promoter three hours after stimulation of Jurkat T cells with the cytokine. This is followed by MOR mRNA production starting later than three hours after IL-4 stimulation. Chip
experiments furthermore indicated that alterations in the chromatin architecture of the MOR gene, which are indicative for transcriptional active euchromatin, occurred within the first three hours after IL-4 stimulation. Thus, transient association of the MOR promoter with phosphorylated (S10) plus acetylated (K14) histone H3, trimethylated (K4) histone H3 and acetylated (K16) histone H4 were found. In addition, demethylation of MOR promoter DNA in response to IL-4 is suggested, because the methyl-binding protein MeCP2-association with the MOR promoter was reduced in response to IL-4.

38. INCREASED EXPRESSION OF SPINAL CHEMOKINE RECEPTORS VIA EPIGENETIC MODULATION UNDER LONG-LASTING NEUROPATHIC PAIN

Neuropathic pain is the most difficult pain to manage in the pain clinic. Although several animal models of chronic pain have been created, it remains a controversial as to what events are critical for its development and maintenance. One important development in our understanding of the cellular and molecular processes that produce neuropathic pain concerns the role of the immune system. Although inflammatory and neuropathic pain syndromes are often considered distinct entities, emerging evidence suggests that pro-inflammatory substances produced in association with the innate immune response are clearly implicated in the actual development of neuropathic pain. To gain further insight into these issues, we investigated the possible alteration in cytokine/chemokine gene-expression along with epigenetic modifications in the spinal cord of mice with neuropathic pain induced by nerve ligation. Our studies may provide new insight into the molecular mechanisms at the DNA level related to spinal plasticity under a neuropathic pain.

39. DYNAMIC ASSOCIATION OF P300 WITH THE PROMOTER OF THE G PROTEIN-COUPLED RAT DELTA OPIOID RECEPTOR GENE DURING NGF-INDUCED NEURONAL DIFFERENTIATION
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The G protein-coupled delta opioid receptor DOR plays a role in neuronal differentiation and survival. Nerve growth factor (NGF) is critical for the development and maintenance of the central and peripheral nerve systems. Our previous studies have shown that sustained activation of NGF/P3K/Akt/NF-kB signaling is essential for NGF-induced dor gene expression during neuronal differentiation and that the acetylation at histone 3 lysine 9 temporally correlates with the dor gene transcription. In this study, we cloned the rat dor gene promoter and identified an NGF-responsive region. We further identified p300, a known NF-kB binding partner with intrinsic histone acetyltransferase activity, to be dynamically associated with the dor gene. We also found that assembling of RNA polymerase II (Pol II) at the promoter took place before NGF stimulation. Taken together, these results implicate that preassembly of the Pol II preinitiation complex, sustained activation of P3K/Akt/NF-kB signaling, and dynamic p300 association at the promoters sequentially is one of the mechanisms of induction of the late phase genes during NGF-induced neuronal differentiation.

40. JAK/STAT SIGNALING MEDIATES POMC TRANSCRIPTION IN LYMPHOCYTES
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Cytokines such as IL-1beta and leptin have been shown to increase transcription of the ACTH and beta-endorphin precursor proopiomelanocortin (POMC) mRNA expression in the pituitary and hypothalamus, respectively. Here we investigated the transcriptional regulation of opioid peptide expression in rat lymphocytes. In vitro, IL-4 but not IL-1beta treatments dose-dependently elevated POMC (exon 2-3) mRNA levels in naïve cells. IL-4-induced POMC mRNA expression was fully blocked by the pan JAK inhibitor pyridon 6. To interfere with the binding of transcriptions factors to the POMC promoter, cells were transfected with decoy oligonucleotides. This resulted in decreased IL-4-induced POMC mRNA levels when competing with STAT1/3 but not with STAT6 binding sites. Western Blot analysis confirmed that STAT1 and 3 were phosphorylated in response to IL-4 treatment. Radioimmunoassays showed that IL-4 treatment increased beta-endorphin production in concanavalin A-activated cells and this elevation was fully inhibited in the presence of pyridon 6. Together, our findings indicate that the JAK/STAT pathway is crucial for the IL-4-induced POMC gene expression and beta-endorphin synthesis in lymphocytes. Acknowledgement: Supported by DFG grants KFO 100 and GRK 1258.
41. MORPHINE INHIBITS THE INDUCTION OF CANNABINOID RECEPTOR TYPE 1 BY MODULATION OF NF-KAPPA-B SIGNALING

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Opioid-cannabinoid interactions are well known. Here we demonstrate that morphine (1 µM) strongly inhibited the tumor necrosis factor (TNF; 100 U/ml)-triggered induction of the cannabinoid receptor type 1 (CB1) gene in primary rat neurons and in SH SY5Y human neuroblastoma cells, which is mediated by NFkappaB. Characterizing the mechanisms of this effect, we found that the TNF-induced NFkappaB signaling was inhibited by morphine via an induction of the expression of the NFkappaB-inhibitor, IkappaB. Interestingly, this mu opioid receptor-mediated induction of IkappaB was itself dependent on NFkappaB. In fact, we show that morphine transiently induced NFkappaB, resulting in an increased expression of IkappaB. The increase in IkappaB in turn resulted in the inhibition of subsequent NFkappaB signaling, and thus in the inhibition of the TNF-induced, NFkappaB-dependent induction of CB1. These results suggest multiple interactions between the opioid and cannabinoid systems, which are mediated by modulation of NFkappaB signaling.

42. ORPHANIN FQ/NOCICEPTIN ACTIVATES NUCLEAR FACTOR KAPPA B IN NEURONAL CELLS

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Endogenous neuropeptide orphanin FQ/nociceptin (OFQ/N) and its receptor ORL1, play a modulatory role throughout the body including nociceptive sensitivity, motor function, spatial learning and the immune system. ORL1 is a Gi protein coupled receptor (GPCR) that modulates expression and release of inflammatory mediators from immune cells and in the CNS. Inhibitory GPCRs have been shown to activate the immune system regulator, NFkB, whose family consists of several subunits. When activated, NFkB translocates to the nucleus and can modify transcription. To determine if OFQ/N modulates NFkB activity, SH-SY5Y human neuroblastoma cells were treated with OFQ/N and assessed for changes in nuclear accumulation, DNA binding and protein expression. OFQ/N increases the nuclear accumulation of NFkB at 30 min and increases the DNA binding of NFkB by 1 hr as determined by electromobility shift assay. Additionally, immunoblot analysis of SH-SY5Y cell lysates indicates that NFkB p50 protein expression is up-regulated by 2 hr. This suggests that OFQ/N may modulate immune system function by activating NFkB. These studies were supported by DA017380 and OCAST HR08-152.

43. EFFECT OF CHRONIC MORPHINE TREATMENT ON EXPRESSION OF THE ALTERNATIVELY SPliced VARIANT MRNAS FROM THE MU OPIOID RECEPTOR (OPRM1) GENE IN SELECTED MOUSE BRAIN REGIONS

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The mu opioid receptor, OPRM1, gene undergoes extensive alternative pre-mRNA splicing. The functional significance of these splice variants has been suggested by differences in their region-specific expressions, agonist-induced G protein coupling and receptor internalization. Our recent knockout study further suggested the functional importance of exon 11-associated variants in mediating actions of a subset of mu agonists including morphine-6β-glucuronide and heroin. In the current studies, we examined the effect of chronic morphine treatment on expression of the variant mRNAs in selected mouse brain regions using our established real-time RT-PCR assays. The results showed that chronic morphine treatment significantly altered the expression of most splice variants’ mRNAs in the selected brain regions, providing a new insight of understanding the actions of morphine in animals and humans. Supported by DA013997 and DA02615.

44. THE DELTA-OPIOID RECEPTOR DIVERSITY IN HUMAN SKIN

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Besides the regulation of pain, the peripheral opioid receptor (OR) system is able to control the homeostasis of neuronal and non-neuronal interactions by affecting functions like cell differentiation, proliferation, apoptosis, and migration after tissue injury or during inflammation. There is evidence that the OR system is functional active in different skin structures including keratinocytes, melanocytes, fibroblasts, peripheral nerve fibres, hair follicles, sweat glands, immune cells and around blood vessels. It is known that humans react differently to opioids and that pharmacokinetics and pharmacodynamics can vary between individual patients. Polymorphisms in the human delta- (DOR) and mu- (MOR)
opiod receptor gene are numerous (>400 in both oprm1 and oprd1 gene region). In addition to single nucleotide polymorphisms (SNPs) 13 different transcript variants have been described for oprm1, which makes functional research on MOR in human primary model systems even more challenging. The influence of ORs in skin is substantially affected by their expression level. Our most recent results show for the first time that cultured human primary keratinocytes have different expression levels of both MOR and DOR among different individual cell donors. A variation of relative DOR mRNA quantity up to factor 10 could be detected. The influence on keratinocyte function was observed in preliminary experiments in-vitro and different functional tests are ongoing to further verify a correlation of DOR expression level and a corresponding phenotype. Additionally, in analysis of the nucleotide sequence of the oprd1 gene region we have uncovered a high variant pattern of different SNPs among individual donors. We are investigating if there is any functional impact of these polymorphisms in primary cultured keratinocytes and to what extent. Lately, a SNP in the oprd1 promoter region was suggested to influence transcription factor binding and therefore to influence the expression level of DOR (Zhang et al., 2010). These findings underline the importance of the characterization of the oprd1 SNPs. In order to develop successful strategies for clinical treatments using OR ligands, we need to understand the correlation between genotype and phenotype and our study is an important attempt to identify the reasons for interindividual differences.

**Ligand-directed Signaling and Functional Selectivity**

**45. MORPHINE TOLERANCE PRODUCED BY ARRESTIN-DEPENDENT IMPAIRMENT OF MOR RESENSITIZATION**

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Beta-arrestin-2 (Barr-2) dependent processes attenuate MOR signaling during persistent agonist stimulation and appear to be involved in opioid tolerance. We used patch clamp recording in locus coeruleus (LC) neurons from Barr-2 knockout (k.o.) and wild-type (w.t.) mice. Recovery of MOR from desensitization occurred at the cell surface and did not require Barr-2 dependent receptor endocytosis in untreated mice. Disruption of Barr-2 dependent endocytosis in neurons from Barr-2 k.o. mice and direct inhibition of GRK2 or dynamin in neurons from w.t. mice accelerated MOR resensitization. After chronic morphine, MOR tolerance developed in neurons from w.t. but not Barr-2 k.o. mice. Recovery of MOR function from a brief desensitizing stimulus was impaired by chronic morphine in w.t. mice but in neurons from morphine treated Barr-2 k.o. mice it was rapid and similar to untreated mice. Impairment of MOR resensitization by chronic morphine was reversed in w.t. neurons by inhibition of GRK2 or dynamin. This establishes that chronic morphine modifies GRK2-Barr-2-dynamin-dependent MOR trafficking to impair recovery from desensitization, thereby causing tolerance in LC neurons.

**46. WITHDRAWAL FROM CHRONIC MORPHINE, BUT NOT COCAINE INDUCE MARKED UPREGULATION OF MGLUR5 BINDING IN THE MOUSE BRAIN**

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Evidence shows that the mGluR5 receptor plays an important role in opiate and cocaine addiction. To determine whether chronic morphine and/ or cocaine or acute or chronic withdrawal from those drugs alter mGluR5 density, we carried out quantitative autoradiographic mapping of the mGluR5 receptor labelled with [3H]MPEP in brains of mice treated with chronic morphine or cocaine and of mice acutely and chronically withdrawn from those drugs. Chronic morphine caused a small overall increase in mGluR5 binding which persisted during acute withdrawal in many brain regions. A 2-3 fold increase in [3H]MPEP binding was found in almost all the brain regions of chronically morphine withdrawn mice compared to controls. In contrast,
chronic cocaine or acute or chronic withdrawal had no significant effect on mGluR5 binding in any of the regions analysed, suggesting that the alterations in mGluR5 density are drug dependent. These data suggest that chronic withdrawal from opioids, triggers alterations in the mGluR5 system which might play an important role in the mechanism of opioid craving after opioid abstinence. Funded by EC (GENADDICT:LSHM-CT-2004-005166).

47. AMOUNT OF MU-OPIOID RECEPTORS EFFECTING OPIOID-INDUCED BEHAVIOR

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To understand the relation between the number of mu opioid receptors and receptor-induced effects we investigated mu receptor knock-out mice together with wild type and heterozygous mice. Binding studies revealed heterozygous mice showing exactly half the amount of mu receptors (24.5 vs.49 fmol/mg) in frontal cortex compared to wild type. Behavioral studies showed half-reduced effects in heterozygous mice on the measurement of analgesia with the hot plate, electric pain threshold determination, morphine-induced hypothermia and place preference, locomotion and depression-related immobility on tail suspension, in comparison to wild type mice. Our results indicate a direct correlation of these effects with the number of mu receptors. In contrast we found a non linear relation between the number of mu receptors and diverse qualities of emotional behavior such as fear, startle response and central excitability. Our experiments indicate the mu receptor to play a modulating role on the named qualities and central functions being influenced by opioidergic effects with different intensities. Acknowledgement of funding source: Sponsorship of Young Academics, Medical School Magdeburg

48. WITHDRAWAL FROM CHRONIC MORPHINE INDUCES MARKED UPREGULATION OF V1A VASOPRESSIN RECEPTOR IN THE MOUSE BRAIN

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New evidence shows that the vasopressin system plays an important role in opiate addiction. To determine whether chronic morphine or acute or chronic withdrawal from morphine alters vasopressin receptor density, we carried out quantitative autoradiographic mapping of the vasopressin receptors in brains of mice treated with chronic morphine and of mice acutely and chronically withdrawn from morphine. In order to discriminate between V1a and V1b, we displaced [3H]AVP binding with the selective V1b vasopressin receptor antagonist SSR149,514. A significant 2-3 fold increase in SSR149,514 resistant [3H]AVP binding was observed in the prelimbic cortex, the cingulate cortex, the piriform nucleus, the olfactory tubercle and the nucleus accumbens of chronically morphine withdrawn mice. No SSR149,514 sensitive [3H]AVP binding sites was observed suggesting a lack of V1b receptors in the brain regions analysed. These data suggest chronic withdrawal from opioids, triggers alterations in the V1a system which might play an important role in the mechanism of opioid craving after abstinence. Funded by EC (GENADDICT:LSHM-CT-2004-005166).

49. CHANGES IN THE ADENYLYL CYCLASE PATHWAY WITHIN GABAERGIC NEURONS IN THE PAG IS ASSOCIATED WITH MORPHINE TOLERANCE


In vitro studies have shown that chronic morphine pretreatment causes upregulation of adenylyl cyclase and an increase in GABA release within the periaqueductal gray (PAG). If the adenylyl cyclase pathway is important for tolerance, then activation of adenylyl cyclase should produce morphine tolerance and enhance GABA release. Repeated microinjections of an adenylyl cyclase activator (NKH 477) caused a rightward shift in the morphine dose-response curve resembling morphine tolerance. Similar results were found following pretreatment with a cAMP analog (RP-cAMPS). In order to measure changes in GABA release, the GABAA antagonist, bicuculline, was administered following repeated morphine or NKH 477 microinjections. Both pretreatments caused a rightward shift in the bicuculline dose-response curve, suggesting greater intrinsic synaptic release of GABA. The results of these studies indicate that morphine tolerance causes an increase in GABA release via upregulation of adenylyl cyclase that attenuates morphine-induced antinociception.

50. HIGH POTENCY INHIBITION OF 5-HT_3 RECEPTORS BY MORPHINE

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There is great interest in ligand directed signalling focused on efforts to identify agonists that differentially recruit pathways downstream of opioid receptor activation. However, alkaloid agonists, such as morphine and methadone, are notoriously promiscuous. Potent “off target” effects of alkaloids likely contribute to their specific
signalling profiles in vivo. When applied prior to 5-hydroxytryptamine (5-HT) morphine exhibits a potent non-competitive inhibition of recombinant 5-HT3 receptors (Kb=0.06 uM). Methadone has a much lower potency as an antagonist (Kb=2.4 uM). 5-HT3 receptors are responsible for rapid excitation by 5-HT and participate in pain and reward, making them worthy of consideration in the ligand-specific effects of morphine in vivo. When applied with 5-HT, morphine became a low potency competitive antagonist (Kb=12 uM). We hypothesize that potent inhibition by pre-applied morphine involves binding to the 5-HT site leading to desensitization. We are testing this using rapid morphine and 5-HT application techniques. Reports of morphine induced hyperalgesia in opioid receptor knockout mice highlight the potential importance of off target effects. Funded by DA05010

51. CONDITIONAL KNOCKOUT OF P38-ALPHA MAPK IN SEROTONERGIC NEURONS BLOCKS KAPPA OPIOID DEPENDENT BEHAVIORS.


Prior work has shown that the dynorphin-kappa opioid receptor (KOR) and p38 MAPK activity are key mediators of the aversive response to stress, and KOR antagonists are effective in blocking stress-induced reinstatement to drug seeking. To understand the mechanism of KOR-dependent p38 activation and neuronal circuitry affected, we developed conditional knockout (CKO) mice for p38alpha MAPK. Injection of AAV1-cre (but not control vector) into dorsal raphe of CKO mice selectively disrupted p38 transcription, blocked KOR-mediated CPA, and social defeat stress (SDS)-induced reinstatement of cocaine CPP. Serotonergic selective CKO mice (SERT-cre and PET1-cre) also identified a role for p38alpha in mediating these KOR-dependent behaviors. P38 regulation of serotonin transporter (SERT) function was previously suggested, and we confirm that KOR activation by U50,488 increased SERT activity. Together these results suggest that activation of the p38alpha MAPK signaling cascade in the dorsal raphe and its projection fields is required for KOR-dependent stress-induced behaviors, including aversion and reinstatement. Supported by DA25970 and DA25182.

52. REPEATED MORPHINE EXPOSURE PROMOTES THE INSERTION OF GLUR2-LACKING AMPA RECEPTORS AT HIPPOCAMPAL SYNAPSES: AN ELECTROPHYSIOLOGICAL STUDY IN VITRO

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Addictive processes are thought to involve persistent neurobiological changes that facilitate compulsive drug-seeking behavior and propensity to relapse. Recent studies show that long-term neuroadaptation in the hippocampus may underlie opiate-induced behavioral modifications. Glutamatergic systems, including AMPA receptors (AMPARs) are thought to be involved in opiate-induced neuronal and behavioral plasticity. In the present study we employed a series of electrophysiological recording techniques to investigate the functional impact of repeated morphine treatment on AMPAR-mediated synaptic transmission at CA1 hippocampal synapses. Twelve hours following the last injection of morphine we observed a significant increase in field EPSP magnitude. In addition, the postsynaptic AMPARs are switched to Ca2+-permeable, affecting the magnitude of long-term depression. We propose that morphine-induced insertion of GluR2-lacking AMPARs at hippocampal synapses may play an important role in the neuroadaptations induced by repeated morphine administration. This work was supported by the NIDA-DA025036 to J.A.M.

53. REGULATION OF AMPA RECEPTOR TRAFFICKING BY MORPHINE IN THE HIPPOCAMPUS

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Brain regions involved in learning and memory, such as the hippocampus, have also been implicated in opiate dependence. We have previously found that the administration of four escalating doses of morphine produced locomotor sensitization as well as altered AMPA receptor (AMPAR) composition and distribution in the hippocampus, suggesting a potential role of AMPAR trafficking in this behavioral effect. GRIP1, PICK1, and stargazin have been shown to regulate the trafficking of AMPAR. In this study, we investigated the effects of morphine on these molecules in both the total homogenate and the postsynaptic density (PSD) within the hippocampus. We found that 12 hours after morphine administration the phosphorylation levels of GluR1 and GluR2 were increased at the PSD, whereas levels of GluR1, 2, and 3 remained unchanged. Interestingly, we also observed that levels of GRIP1 were dramatically increased.
However, no alteration of PICK1 or stargazin was detected. When examining the changes in the total homogenate, we found that morphine increased the expression of all the proteins studied. These data suggest that morphine may regulate the synaptic expression and distribution of AMPARs by altering their interactions with GRIP, PICK and stargazin. We are currently performing additional experiments to test this hypothesis. This work is supported by RO1 DA25036 to JAM.

54. ENDOGENOUS OREXINS CONTRIBUTE TO STRESS-INDUCED ANALGESIA THROUGH ENDOCANNABINOID SIGNALING IN THE VENTROLATERAL PERIAQUEUDCTAL GRAY

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Stress-induced analgesia (SIA) is attributed to that stress activates a neural circuit involving the periaqueductal gray (PAG). Previously, we have shown that orexin A induced antinociception in the ventrolateral PAG (vLPAG) via OX1R-mediated PLC-DAGL cascade to generate 2-arachidonoylglycerol, an endocannabinoid which retrogradely inhibits GABA release via CB1R. Orexin-containing neurons are localized in the lateral hypothalamus but project widely through the brain. After 30 min-restraint, the mouse expressed SIA in a manner blocked by i.p. injection of AM251 and SB334867, the CB1R and OX1R antagonists, respectively. Both antagonists (i.p.) had no effect in non-restrained mice. Intra-vLPAG microinjection of AM251 and SB334867 also blocked SIA. Restrained mice expressed more c-Fos-containing orexin neurons in the lateral hypothalamus than non-restrained mice. These results suggest that hypothalamic orexin neurons are activated during stress, the released orexins contribute to SIA through exciting the vLPAG via OX1R-mediated endocannabinoid retrograde signaling. Supported by grants NSC-98-2320-B-002-011-MY3, NHRI-EX97-9506NI, and NTU-97HM00275.

56. ABILITY OF OPIOID LIGANDS TO DESENSITIZE ENDOGENOUS MU OPIOID RECEPTOR IN LOCUS COERULEUS NEURONS


We have recently shown that overall there is a very good correlation between the opioid agonist relative efficacy to activate G proteins and their ability to induce mu opioid receptor (MOPr) interaction with arrestin-3. Here we investigate, using electrophysiological methods in rat brain slices, the extent of MOPr desensitization in mature locus coeruleus neurons induced by different opioid agonists. The maximum level of MOPr desensitization produced by the agonists followed the tendency: DAMGO > etorphine > nornepipedrine > leu-enkephaline = M6G = morphine > oxycodone. This correlates well with the relative ability of these agonists to induce arrestin-3 translocation and with the agonist efficacy in G-protein activation. These data are compatible with the view that in the absence of protein kinase C activation, the ability of agonists to induce MOPr desensitization is related to their agonist efficacy and is likely to be through a GRK/arrestin-dependent mechanism. Funding: Wellcome Trust & NIDA.
57. CO-ACTIVATION OF DELTA-OPIOID AND ALPHA-2-ADRENERGIC RECEPTORS ACTIVATES PKC-_EPSILON TO ENABLE SPINAL ANALGESIC SYNERGY


Co-activation of delta opioid (DOPs) and alpha-2A-adrenergic receptors (A2ARs) produces spinal analgesic synergy. Because PKC has been shown to mediate this interaction, we sought to determine the isofrom responsible. We tested the ability of DOP agonist deltorphin II (DELT), A2AR agonist clonidine (CLON) or the combination to activate different PKC isoforms in DRG neurons and evaluated the antinociceptive potency of i.t. administered DELT, CLON or the combination in PKC epsilon knockout mice. We conclude that (1) only the PKC epsilon isoform was activated by co-administration of DELT/CLON, not by the separate agonists, and (2) PKC epsilon knockout mice lose DELT/CLON synergy without altering the effect of separate agonist administration, as confirmed by isobolographic analysis. Supported by US NIH grant R01 DA 015438 to GLW.

58. CORTICAL DELTA-OPIOID RECEPTORS AND Na⁺-K⁺ HOMEOSTASIS IN HYPOXIC/ISCHEMIC STRESS

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Hypoxic/ischemic stress causes neuronal injury in the cortex, leading to serious neurological disorders. There is, however, no promising strategy against such injury at present. Our recent studies show that activation of delta-opioid receptors (DOR) protects the cortex against cerebral ischemia or hypoxic stress and the DOR protection is associated with increased stabilization of Na⁺-K⁺ homeostasis. In mouse cortical slices, hypoxic stress or simulated ischemia (oxygen and glucose deprivation) caused cellular Na⁺ overloading and K⁺ leakage. DOR activation significantly attenuated the hypoxic/ischemic disruption of Na⁺-K⁺ homeostasis, which could be blocked by naltrindole, a DOR antagonist. Tetrodotoxin, a Na⁺ channel blocker, reduced the stress-induced Na⁺ influx and accordingly decreased K⁺ leakage. In the oocytes with co-expression of DOR and Na⁺ channels, DOR expression and/or activation reduced Na⁺ currents through Na⁺ channels. The exploration of signal pathways showed that the DOR protection relied on a PKC-dependent and PKA-independent pathway. We conclude that in hypoxic/ischemic condition, DOR may stabilize Na⁺ channel function, thus maintaining Na⁺-K⁺ homeostasis and neuronal viability in the cortex. Supported by the grants of NIH-HD34852, NIH-AT004422 and AHA-0755993T.

59. P-TYPE CALCIUM CHANNELS IN PURKINJE NEURONS OF RAT ARE MODULATED BY µ-OPIOID RECEPTOR IN G-PROTEIN-INDEPENDENT MANNER

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Using conventional whole-cell patch clamp techniques we have studied the effect of µ-opioids on P-type calcium channels in acutely isolated Purkinje neurons from rat cerebellum. Selective µ-opioid agonists DAMGO and endomorphin-1 produced a small, but consistent facilitation of current through P-type calcium channels (10±1%, n=27, p<0.001). This effect was rapid and fully reversible. The EC50 for the effect of DAMGO was 1.3±0.4 nM and the saturating concentration was 100 nM. Intracellular GTPγS or GDPβS (0.5 mM) did not eliminate facilitatory action of DAMGO indicating the lack of G-proteins involvement. Intracellular application of H7, non-specific inhibitor of PKA and PKC, (10 µM) was also of no effect. DAMGO–induced facilitation of P-current was abolished by naloxone (100 nM) and CTOP (100 nM). Thus, µ-opioids can modulate P-type calcium channels via G-protein-independent mechanism which involves activation of µ-opioid receptor.

60. AUTORADIOGRAPHIC STUDIES ON MU- AND DELTA RECEPTOR IN THE RAT BRAIN AFTER TREATMENT WITH GROWTH HORMONE

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Long-term use of opiates can cause memory loss and impaired cognitive functions in both humans and animals. Opioids have also been demonstrated to inhibit neuronal cell growth and induce apoptosis, effects that may lead to above-written effects. On the contrary, growth hormone (GH) stimulates cell growth and counteracts apoptosis in neuronal cells and it improves cognitive functions e.g. memory and mental alertness. Recently, we reported that recombinant human GH may reverse opioid induced apoptosis in cells derived from
prenatal mouse hippocampus. The mechanisms underlying the beneficial effect of GH on cognition is poorly understood. Therefore we investigated if repeated administration of GH affects the mu-(MOP) and delta opioid peptide (DOP) receptor functionality in the male rat brain. Rats were given s.c. GH (saline, 0.7 IU/kg or 0.07 IU/kg) twice daily for 7 days. Following treatment, \[^{[35]}S\]GTP\_S autoradiography was performed on sagittal sections, using the selective agonists DAMGO and DPDPDE. Results suggest that GH treatment did not significantly affect DOP receptor activity. However, alterations in the MOP receptor functionality were seen in certain regions e.g. the central amygdaloidal nucleus and laterodorsal thalamus, regions that are involved in declarative and spatial learning and memory. Supported by SMRC, grant 9459.

61. BETA-ARRESTIN 1 REGULATION OF THE ACTIN CYTOSKELETON AFFECTS DELTA RECEPTOR FUNCTION AT THE CELLULAR AND BEHAVIORAL LEVELS


Beta-arrestins, scaffolding and signal transduction molecules, are involved in diverse aspects of G-protein coupled receptor signaling. The non-visual beta-arrestins also regulate actin polymerization enabling the appropriate chemotactic response in lymphocytes. We have found that arrestin regulation of the actin cytoskeleton is not limited to controlling chemotaxis but controls the function of the delta opioid receptor in neurons. Spatio-temporal regulation of cofilin, an actin severing protein, by beta-arrestin 1, modulates delta receptor function. In the absence of this control, neurons lacking beta-arrestin 1 show increased coupling to voltage-dependent Ca\^2+ channels. This regulation occurs within the Golgi network and is not a result of altered export of the delta receptor but rather the interaction of proteins such as cSrc and PKCalpha with the receptor. At the behavioral level, mice lacking beta-arrestin 1 show an enhanced and prolonged response to SNC80, a delta agonist, in the open-field test of exploration demonstrating the importance of this effect. Funded by DA005010

62. NOVEL DYNAMIC COMPLEXES BETWEEN THE DELTA-OPIOID RECEPTOR, STAT5B AND SELECTIVE G PROTEIN SUBUNITWS


Previous work from our laboratory has shown that the conserved YXXL motif within the C-terminal tail of the mu-opioid receptor (MOP) serves as a docking site for STAT5A binding, with the latter being phosphorylated upon MOP stimulation (Mazarakou and Georgoussi, 2005). Given that the delta-opioid receptor (DOP) contains the same structural motif within its C-terminal tail (DOP-CT), we wondered whether STAT5A/B interact in a similar manner with the DOP. Co-immunoprecipitation and pull-down assays, have shown that STAT5B interacts directly with the conserved juxtamembrane region of the DOP-CT. Agonist exposure of HEK293 cells, stably expressing the flag-DOP, led to a G protein-dependent STAT5B phosphorylation and transcriptional activation mediated by c-Src kinase. Additional studies have shown that the DOP serves as a platform for the formation of a multi-component signaling complex, consisting of STAT5B, c-Src, Gbetagamma and selective Galpha protein subunits. Collectively, our results uncover a novel signaling pathway through which DOP might be involved in the transcriptional regulation of STAT5B-dependent genes. This work was supported by the EU grant «Normolife» (LSHC-CT2006-037733). References: Mazarakou, G. and Georgoussi, Z. (2005) STAT5A interacts with and is phosphorylated upon activation of the mu-opioid receptor. J. Neurochem., 93, 918-931.

63. CHARACTERIZATION OF OPIOID RECEPTORS IN HUMAN SKBR-3 MAMMARY CARCINOMA CELLS

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Besides their analgesic property, recent data indicate that opioids may also interfere with cell proliferation and survival. SKBR-3 cells serve as a model system for antibody based tumor therapy strategies targeting HER2, a receptor tyrosine kinase over-expressed in about 30% of human breast cancer patients. Here we report that SKBR-3 cells carry a single population of kappa-opioid receptors, as revealed by RT-PCR and radioligand binding studies. Exposure of the cells to U50, 488 and morphine results in stimulation of intracellular cAMP production, which in both cases is blocked by NorBNI. Kappa-opioid receptors fail to transactivate HER1-mediated mitogen-activated protein kinase pathways. In contrast, PI3K- and AKT-dependent cleavage of procaspase-3 is stimulated. Activation of kappa-opioid receptors enhances apoptosis induced by a monoclonal anti-HER2 antibody, indicating that in SKBR-3 cells kappa-opioid receptors are coupled to inhibition of HER2- rather than to stimulation of HER1-associated signal transduction pathways. These results imply that concomitant opioid treatment might enhance the efficiency of anti-HER2 directed antineoplastic therapy.
64. INVOLVEMENT OF UBIQUITINATION IN OPIOID-INDUCED DOWNREGULATION OF RGS4

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Regulator of G protein signaling protein 4 (RGS4) is a negative modulator of mu-opioid receptor (MOR) signaling. Chronic treatment of rats with morphine leads to a change in RGS4 levels in several brain regions. Here we have used SH-SY5Y cells, in which we have shown RGS4 is abundantly expressed, to study the mechanism of opioid agonist-induced downregulation of RGS4. Overnight treatment with either the MOR (DAMGO) or DOR (DPDPE) agonist decreased RGS4 protein by ~50%, but had no effect on the level of RGS4 mRNA. The decrease in RGS4 protein was prevented by the opioid antagonist naloxone and by pre-treatment with pertussis toxin confirming a receptor-mediated event through a Galphai/o-mediated signaling pathway. The agonist-induced downregulation of RGS4 protein was reversed following 2h exposure of the cells to naloxone. It was also blocked by treatment with the proteasome inhibitor leupeptin. Involvement of the ubiquitin-proteasome inhibitor MG132, but not the lysosome protease inhibitor norBNI, was blocked by pretreatment with Gö6976.

Together, these results indicate that the JNK1 isofrom mediates ligand-directed inactivation of the KOR, whereas the JNK2 isofrom mediates inactivation of MOR and activation of JNK may require PKC. Supported by DA11672.

65. ROLE OF JNK ISOFORMS IN LIGAND-DIRECTED MU AND KAPPA OPIOID RECEPTOR INACTIVATION

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We previously showed that inactivation of the mu and kappa opioid receptors by specific ligands is dependent on the JNK signaling pathway and is distinct from the common GRK/arrestin inactivation mechanism. In the present study we used transgenic mice to identify the JNK- isoforms mediating receptor inactivation. Mice lacking the JNK1 isoform do not show the long-lasting antagonistic effects of norBNI on KOR, but do show normal inactivation of MOR by morphine. In contrast, JNK2/-/- mice do not develop acute analgesic tolerance to morphine, but still show long-lasting effects of norBNI. Previous studies have suggested a role for PKC in tolerance to morphine, but not to other MOR agonists. To determine the relationship between PKC and JNK-mediated effects, we used the small molecule PKC inhibitor Gö6976 in HEK293 cells expressing rMOR-GFP or rKOR-GFP prior to treatment with morphine or norBNI, respectively. The increased phospho-JNK-ir caused by both morphine and norBNI was blocked by pretreatment with Gö6976.

Together, these results indicate that the JNK1 isofrom mediates ligand-directed inactivation of the KOR, whereas the JNK2 isofrom mediates inactivation of MOR and activation of JNK may require PKC. Supported by DA11672.

66. MOR FUNCTION IS ALTERED IN A GALPHA[O] NULL MOUSE

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Galphao is expressed at significantly higher levels in brain than other Galphai/o subtypes. To evaluate the role of Galphao in mu-opioid receptor (MOR) expression and function, we have compared membranes prepared from whole brain of Galphao knockout (-/-) and wild-type (wt) mice by MOR radioligand binding and agonist-stimulated GTPγS incorporation. In membranes from Galphao +/- mice, there was no change in total MOR (fmol/mg protein: wt, 234 ± 19; +/-, 203 ± 24), although there was a 40 ± 10% decrease in the number of high affinity MOR binding sites. Analysis of MOR radioligand binding in the presence of Na+ and GTPγS revealed that remaining high affinity MOR binding in Galphao +/- mice was still dependent on G protein (Na+/GTPγS shift: wt, 3.6-fold; +/-, 4.3-fold). Despite the presence of these G protein-dependent high affinity MOR binding sites, GTPγS incorporation stimulated by the MOR agonists DAMGO and morphine was 90% decreased in Galphao +/- mice, compared to wt controls. These data indicate that loss of Galphao does not affect total MOR expression, but rather alters the equilibrium between MOR affinity states. Supported by GM077667 (JAT) and DA04087 (JRT).

67. STUDY OF OPIOID ACTION IN MOPR PHOSPHORYLATION-DEFICIENT (PD) MICE

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Acute or chronic opioids produce major behavioral responses. Upon exposure to agonists, MOPr undergoes phosphorylation in cultured cells, which is related to desensitization and internalization. To assess contributions of in vivo MOPr phosphorylation to regulation of opioid induced behaviors, we have generated a knockin mouse with the putative key phosphorylation residue T349 in MOPr mutated to alanine. Our preliminary study revealed that the MOPr-PD mice displayed interesting phenotypes at both behavioral and cellular levels. MOPr-PD mice showed attenuated acute tolerance to morphine-induced analgesia and different withdrawal responses following chronic morphine, compared
with their wild type littermates. At cellular levels, MOPr internalization in the spinal cord following systemic etorphine was diminished in the MOPr-PD mice. The MOPr-PD mice can therefore serve as a unique animal model to validate and more importantly extend our understanding of regulation of MOPr functions by opioid drugs from cellular models to whole animals. Supported by NIH grants DA011925 to JBW and DA17302 to LYLC.

68. FUNCTIONAL LINK BETWEEN PHOSPHOLIPASE D2-SIGNALING AND OPIOID RECEPTOR ENDOCYTOSIS
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We previously demonstrated that opioid-induced activation of Phospholipase D2 (PLD2) enhances μ- and δ-opioid receptor (MOPr and DOPr) endocytosis/recycling and thus reduces the development of opioid receptor desensitization and tolerance. Here we show that PLD2-generated phosphatidic acid (PA) and its conversion to diacylglycerol (DAG) plays a key role in facilitating the endocytosis of opioid receptors. In fact, blocking PA-derived DAG synthesis by inhibiting PA phosphohydrolase activity significantly attenuated agonist-induced opioid receptor endocytosis. On the other hand, increasing the DAG level by inhibiting the reconversion of DAG into PA with the DAG kinase inhibitor R59949 or the addition of the synthetic cell-permeable DAG analog DOG, further increased the agonist-induced opioid receptor endocytosis. Further studies established a functional link between PA-derived DAG and the activation of p38 MAPK, which has been recently demonstrated to be required for the induction of MOPr endocytosis. Taken together, our results revealed that the regulation of opioid receptor endocytosis by PLD2 involves the conversion of its product PA to DAG resulting in an activation of the p38 MAPK pathway. This work was supported by the Deutsche Forschungsgemeinschaft [Grant KR1740/10-1] and the Forschungszentrum “Center for Behavioural Brain Sciences” Land Sachsen-Anhalt.

69. RELATIONSHIP OF MU-OPIOID RECEPTOR PHOSPHORYLATION TO RECEPTOR INTERACTION WITH ARRESTIN AND INTERNALIZATION
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A major question is whether different MOPr ligands induce different receptor conformations that can trigger different mechanisms of desensitization (e.g. GRK or PKC-dependent). These differences could also depend upon ligand efficacy at the receptor. In this work, a range of MOPr agonists were used and their activity at different desensitization and internalization-related paradigms was measured: phosphorylation of MOPr at Ser175 in the C-terminus, interaction between MOPr-YFP and arrestin 3-CFP (by means of a FRET-based approach) and MOPr internalization. The experiments revealed that overall the degree of ligand-induced pSer175 in the MOPr is a good predictor of the interaction between MOPr and arrestin-3, and that these two phenomena also predict the degree of ligand-induced internalization. In addition, the relative efficacies of the ligands as measured in signalling assays by ourselves and others correlate well with the measures of MOPr regulation determined in this study.

70. PHOSPHORYLATION OF THE C-TERMINUS OF THE MU-OPIOID RECEPTOR
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We have investigated the phosphorylation sites of the C-terminus of the rat MOPr. It is already known that Ser175 is phosphorylated in an agonist-dependent manner, probably by a GRK. Using in vitro phosphorylation of GST fusion proteins of the rat MOPr C-terminus, we have identified Ser163 and Thr370 as potential phosphorylation site for PKC and CaMKII, respectively. We have now also analysed the C-terminus of MOPr by mass spectrometry to determine residues that are phosphorylated in the intact receptor either basally or in response to agonist. HEK293 cells stably expressing HA-tagged MOPr were treated with morphine or DAMGO, then cells were harvested, homogenized and the supernatant from a low speed spin incubated overnight with anti-HA antibody covalently linked to agarose. The resulting immunoprecipitate was subjected to SDS-PAGE and the gel stained with colloidal Coomassie blue. The MOPr band was excised, digested with trypsin and the fragments analysed by LC-MS/MS. So far these studies have identified Ser163, Thr370 and Ser175 as being phosphorylated in the presence of DAMGO or morphine.
71. REASSESSING THE ROLE OF CAMKII IN OPIOID-INDUCED MU-OPIOID RECEPTOR DESENSITIZATION AND INTERNALIZATION.


Using in vitro phosphorylation of GST fusion proteins of MOPr we have identified Ser261 and Ser266 in the 3rd ICL loop, and Thr270 in the C-terminal tail as putative CaMKII phosphorylation sites. Whole cell recording, measuring the GIRK channel current was next used to investigate MOPr desensitization in AtT20 cells stably expressing MOPr. DAMGO and morphine each triggered pronounced desensitization in the continued presence of agonist. Pretreatment with the CaMKII inhibitors KN93 and AIP-II partially reversed the desensitization of MOPr induced by either agonist. Agonist-induced cell surface MOPr loss induced by DAMGO was also assessed using an ELISA assay with AtT20 cells. The DAMGO induced loss of surface receptors was markedly reduced when cells were pretreated with KN93, indicating that CaMKII inhibition reduced DAMGO-induced MOPr internalization. These results suggest that CaMKII contributes to agonist-induced MOPr desensitization and internalization, possibly by phosphorylating the receptor. We have now constructed a MOPr with putative CaMKII phosphorylation sites mutated; data with this construct will also be presented. Funded by Taiwan SAS and MRC.

72. SPINAL MU-Delta OPIOID RECEPTOR INTERACTIONS IN RODENTS


The role of delta opioid receptors in tolerance is not clear. We published that the selective delta-antagonist TIPpsi eliminated the tolerance to the intrathecal (i.t.) analgesic (tail flick) action of the mu agonist DAMGO in mice. In this work mu-delta interactions in opioid tolerance were further studied. Male NMRI mice and Wistar rats were treated subcutaneously (s.c) with morphine (MO) for three and four days, respectively. Afterwards the effect of DAMGO was measured alone and combined with delta ligands DPDPE or TIPpsi given i.t. Three to six-fold tolerance to MO was found to DAMGO in both species. In naïve mice the delta-ligands did not influence the analgesic effect of DAMGO. In rats both drugs produced a potentiation in a dose of 1nmol/rat. In tolerant mice and rats TIPpsi restored the potency of DAMGO. DPDPE produced further inhibitory action in tolerant mice but potentiation in rats. We hypothesize the contribution of mu-delta heterodimer formation in the mu opioid tolerance. Delta ligands affect the dimer formation differently in rats and mice. Supported by OTKA K-60999, ETT-374/2009, Bolyai Fellowship of the HAS and Semmelweis University.

73. CONSTITUTIVELY ACTIVE MU OPIOID RECEPTORS: A NOVEL THERAPEUTIC TARGET FOR PAIN?


Alternative strategies to mu-opioid agonist induced antinoiception would be beneficial for pain treatment given their addictive and dependence-liability. We have previously reported that in the absence of either beta- arrestin 2 or c-Src signaling, constitutive mu-receptor activity is upregulated. In this study we have found that constitutive mu-receptor mediated inhibitory coupling to Ca2+ channels in beta-arrestin 2 knockout neurons is reversed by naloxone or naltrexone, consistent with inverse agonist activity, but not by 6alpha- and 6beta-naloxol or 6beta-naltrexol. The tail withdrawal assay of thermal pain revealed that constitutive mu receptor activity accounts for the increased analgesic threshold in mice lacking beta-arrestin 2. The aversive properties of naloxone were unaffected in mice lacking beta-arrestin 2 suggesting that constitutive activity of the mu-receptor does not affect basal hedonic tone. These studies suggest that upregulation of mu-receptor constitutive activity interaction may provide a novel therapeutic target for maintaining sustained analgesia without major adaptations within reward circuitry. Funded by DA005010

74. DESENSITIZATION OF MOPRS AT NERVE TERMINALS


Many studies have examined agonist-induced desensitization of MOPrs, however, such studies have generally examined MOPrs located at cell bodies. Here we have studied agonist-induced desensitization of MOPrs at nerve terminals. In the mouse ventral tegmental area (VTA), MOPrs are on both the cell bodies and nerve terminals of GABAergic interneurons that innervate dopaminergic neurons. Actions of MOPrs at nerve terminals can be isolated by performing whole-cell patch-clamp recordings on dopaminergic neurons and recording miniature IPSCs. Both morphine (30microM) and DAMGO (10microM) inhibited miniIPSC frequency, an effect that was sustained during 10-minute applications, even in conditions where there was no receptor reserve. In contrast,
MOPRs at the cell bodies of these neurons rapidly desensitized as DAMGO-induced K⁺-currents declined by approx. 50% over 10 minutes. In the rat vas deferens, nerve-evoked twitch is inhibited by activation of MOPRs at nerve terminals. Both normorphine (30microM) and DAMGO (30microM) produced sustained inhibition of twitch during 25-minute applications. These findings suggest that MOPRs located at nerve terminals do not readily desensitize, or do so by different mechanisms to those located at cell bodies. Funded by MRC (UK).

75. REGULATION OF HUMAN MU-OPIOID RECEPTOR (HMOP) DYNAMICS IN THE MEMBRANE UPON ACTIVATION OF OTHER G PROTEIN-COUPL ed RECEPTORS


The dynamic properties of membrane receptors can vary depending on their confinement and interaction with other protein partners. Here, the dynamic properties of YFP-tagged hMOP receptors in the plasma membrane of SH-SY5Y neuroblastoma cells were analyzed by measuring fluorescence recovery after photobleaching (FRAP) at variable spot radius. Activation of endogenous alpha2 adrenoreceptors or Neuropeptide FF₂ receptors, known to form heteromers with MOP receptors, increased the mobile fraction of opioid receptors. This change in dynamics could contribute to the modulation of opioid responses observed in the presence of alpha2 adrenergic or NPFF agonists. In contrast, activation of endogenous Neuropeptide Y or muscarinic receptors, not known to interact with MOP receptors, had no effect. Bimolecular fluorescence complementation approaches were then used to label specifically receptor homo or heteromers and identify differences in their membrane dynamics. Funding source: French Agence Nationale de la Recherche, ANR-09-PRI-0008-01, N°: piribio09_445026.

76. CLINICALLY EMPLOYED “MU” OPIOID AGONISTS SELECTIVELY ACTIVATE MU-DELTA HETEROMERIC RECEPTORS IN HEK-293 CELLS AND RHEUSUS MONKEYS

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We have previously shown that several standard and clinical opioid ligands selectively activate heteromeric opioid receptors. Here we report that the clinically employed opioid analgesics, morphine, methadone, and fentanyl, selectively activate mu-delta heteromeric opioid receptors in HEK-293 cells using the intracellular calcium release assay. Under these conditions, the delta opioid receptor antagonist, naltrindole (NTI), antagonized morphine, fentanyl, and methadone in cells co-expressing mu and delta receptors, but not in cells singly expressing mu receptors. These results suggest that mu and delta receptors associate as allosterically coupled heteromers. Moreover, the antinociceptive effects of these compounds were also significantly antagonized by NTI in rhesus monkeys, strongly suggesting that mu-delta heteromers mediate antinociception in vivo. The far-reaching implications of these results will be discussed. Support: NIH DA01533, DA11460 and DA07027.

77. MU/DELTA OPIOID RECEPTOR HETEROMER TRAFFICKING IN RESPONSE TO PROLONGED MORPHINE TREATMENT


Delta opioid receptor (DOR) trafficking to neuronal plasma membranes was examined. This occurs following chronic pain and prolonged mu opioid receptor (MOR) activation. Primary DRG neuron cultures were used to determine whether DOR trafficks as homomers or associated with MOR as M/DOR heteromers. Trafficking was induced by prolonged morphine (MS) treatment. Immunolabeling of MOR and DOR showed increased co-localization in MS-treated neurons. M/DOR could be immunoprecipitated using a heteromer-selective antibody; immunoblotting shows a high-weight band labeled by both MOR and DOR antibodies. Immunolabeling of M/DOR was increased in MS-treated neurons. M/DOR could be immunoprecipitated using a heteromer-selective antibody; immunoblotting shows a high-weight band labeled by both MOR and DOR antibodies. Immunolabeling of M/DOR was increased in MS-treated neurons. Typically, MOR homomers recycle via endosomes while DOR homomers are degraded in lysosomes. Labeling of MOR and lysosomal-associated membrane protein 1 (LAMP1) showed increased co-localization in MS-treated neurons. These findings support the increased formation of M/DOR following prolonged MOR activation and their DOR-directed sorting to lysosomes. Funding: CIHR, NSERC, NIH-NIDA.
78. NOVEL PEPTIDE ENDOCANNABINOIDS REVEAL AGONIST DIRECTED SIGNALING BY CANNABINOID RECEPTORS


We recently identified hemoglobin-derived peptides as endogenous CB₁ cannabinoid receptor (CB₁R) agonists. These peptides, termed longer hemopressins, activate a signal transduction pathway distinct from that of classical CB₁R agonists. For example, lipidic endocannabinoids or classical CB₁R agonists activate Gₛₐ₅₃₄₅₆₇₈₉-mediated signaling whereas longer hemopressins activate additional Gₛ₄₅₆₇₈₉ independent signaling such as robust mobilization of intracellular Ca²⁺ levels (that is not seen with endogenous lipid agonists). To further explore this ligand directed signal specificity we used a combination of reverse phase protein array consisting of 60 different signaling molecules and graph-theory inspired network analysis. These analysis revealed that longer hemopressins activate a distinct signaling network characterized by stimulation of p70S6 kinase, tuberous sclerosis 2 (TSC2), GSK3 phosphorylation indicating that CB₁R employs agonist directed signaling to expand its functional repertoire. Supported by NIH grants DA01952; DA08863 and GM071558 to LAD.

79. GROWTH HORMONE AFFECTS THE GABAB RECEPTOR DENSITY IN RAT BRAIN

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Growth hormone (GH) is suggested to play an important role in cognition. Growth hormone deficiency (GHD) may cause cognitive impairments in humans, which can be alleviated by treatment with GH. The growth hormone receptor (GHR) is widely distributed within the CNS, including several brain areas known to be involved in cognition. GABA₉ receptors are metabotropic receptors and are also widely distributed in the brain. Interestingly, it has been demonstrated that GABA₉ receptor agonists impair learning in animal models. In the present study, we investigated the effects of repeated administration of GH on the levels of the GABA₉ receptor in the male rat brain. Sprague Dawley rats were treated with GH (0.07 or 0.7 IU/kg) or saline, twice daily, during 7 days. Autoradiography, with the selective antagonist ³H-CGP54626, was used to determine the distribution of the GABA₉ receptor in the brain after treatment with GH. The GABA₉ receptor functionality was measured with the [³⁵S]-GTPγS autoradiography assay, where the selective agonist Baclofen was used to stimulate the GABA₉ receptors. Our results demonstrate that GH affects the GABA₉ density in certain brain areas. Support: Swedish Medical Research Council (Grant 9459).
**Poster session 2 – Tuesday July 13**

**Opioids in neuropathic pain - Preclinical part**

1. INVOLVEMENT OF SPINAL CB2 RECEPTOR ON THE NEUROPATHIC PAIN IN DIABETIC MICE

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Diabetic neuropathy is one of the most common complications of diabetes. We previously reported that diabetic mice exhibit thermal allodynia and hyperalgesia. The present study was designed to evaluate the involvement of cannabinoid system on the neuropathic pain in streptozotocin-induced diabetic mice. We found that intrathecally (i.t.) administered cannabinoid receptor antagonist WIN55,212-2 produced antihyperalgesia in diabetic mice. This effect was reversed by pretreatment with CB2 receptor antagonist AM630, but not by CB1 receptor antagonist AM251. Furthermore, i.t. treatment of CB2 receptor agonist L-759,656 also induced antihyperalgesia in diabetic mice. Although the expression levels of CB1 receptor was not changed, the expression levels of CB2 receptor was increased in the spinal cord of diabetic mice as compared with non-diabetic mice. In addition, there were no changes in the mRNA levels of CB1 and CB2 receptors. These results suggest that CB2 receptor is involved in the thermal hyperalgesia in diabetic mice, and they provide the possibility that the CB2 agonist will be a new curative for treatment of diabetic painful neuropathy.

2. THE EFFECT OF PARECOXIB ON NEUROPATHIC PAIN IS RAT STRAIN DEPENDENT: ROLE OF THE CANNABINOID SYSTEM

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Ligation of the Nervus ischiadicus is an accepted model in neuropathic pain research. Here, we investigated the effect of the COX 2 inhibitor Parecoxib in Wistar and Sprague-Dawley rats. Parecoxib had an analgesic effect after an acute and subchronic administration in the Wistar strain but not in the Sprague-Dawley rats reflecting the differences in the Nervus ischadicus anatomy and neurotransmission in both strains. Binding studies of spinal cord tissue (L4-L6) revealed significant differences in cannabinoid receptor binding in both strains of rats after parecoxib treatment. Surprisingly, both parecoxib as well as its active metabolite valdecoxib demonstrate a specific affinity to the cannabinoid receptor measured in CB1 transfected HEK 293 cells and brain tissue with radioligand binding. Agonist affinity was shown by GTPgammaS assay, cAMP formation experiments and ex vivo modulation of glutamate and GABA release of rat brain tissue. In comparison to the specific cannabinoid agonist, WIN 55,212-2, the COX 2 inhibitor is about 1.000 less potent. The data suggest that analgesic effects of Parecoxib in Wistar rats are to some extent mediated by the cannabinoid system.

3. 14-METHOXYMETOPON INHIBITS THERMAL AND MECHANICAL HYPERSENSITIVITY IN A MOUSE MODEL OF CANCER PAIN


In advanced cancer pain, mu opioids such as morphine provide the mainstay for analgesic therapy, however, their clinical use is associated with high incidence of adverse effects. 14-Methoxymetopon is a selective mu opioid agonist and a potent analgesic agent being genuinely safer than the “golden standard pain-killer” morphine. We report the first pharmacological data on the antinociceptive effects of 14-methoxymetopon in a murine model of cancer pain. Cancer pain was induced in C57BL/6J mice by subcutaneous (s.c.) implantation of lung carcinoma cells in the plantar and dorsal side of the right hindpaw. On day 9 post-inoculation, 14-methoxymetopon produced dose-dependent and naloxone-reversible inhibition of thermal (Hargreaves test) and mechanical (von Frey test) hypersensitivity in the tumour side (right paw) after s.c. administration. In summary, systemic s.c. administration of the mu opioid receptor agonist 14-methoxymetopon induces potent analgesic effects in mice with cancer pain via opioid receptor-specific mechanisms.

4. OPIOID USE IN CANCER PAIN MANAGEMENT: COMMON FINDINGS IN CLINICAL STATUS AND BASIC SCIENCE


In the case of pain management for patients with advanced cancer in spite of the systemic treatment with morphine, physicians step forward to...
interventional pain treatment such as spinal analgesia with morphine. In agreement with this clinical setting, we demonstrated that the antinociceptive action induced by s.c. injection of morphine was suppressed in mice with severe pain. However, there were no significant differences between normal and severe pain groups in antinociception induced by i.t. administration of morphine. It is also noteworthy that either s.c. or i.t. administered morphine-6-glucuronide (M6G)-induced antinociception was significantly suppressed under the long-lasting pain. Taken together, we propose here that the reduced analgesic efficacy of the systemic treatment with morphine under the cancer pain and/or severe pain result from the decrease in the M6G-induced antinociceptive effects.

5. UP-REGULATION OF BRADYKININ RECEPTORS FUNCTION IN THE DORSAL ROOT GANGLIA UNDER THE ACUTE PACREATITIS PAIN-LIKE STATE

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Pain management in acute pancreatitis represents a major clinical challenge and influences the clinical outcome of the disease. To better understand the molecular mechanisms that underlie acute pancreatitis pain-like state, we investigated the change in functions of the dorsal root ganglia (DRG) of caerulein injected mice. We found that the firing frequency and the mRNA levels of bradykinin receptors (BKR) in DRG neurons of mice were markedly increased following repeated treatment with caerulein. Furthermore, BKR antagonists attenuated the acute pancreatitis pain-like state in caerulein-treated mice. The present findings suggest that the repeated injections of caerulein to mice caused functional changes of sensory neurons accompanied by the acceleration of bradykinin signalings and the excitation of the DRG neurons. These changes may contribute to the development of the acute pancreatitis pain-like state in mice.

6. ANTI-NOCICEPTIVE DRUG TOLERANCE AT THE MU OPIATE RECEPTOR

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Morphine and its derivatives acting at the mu opiate receptor (MOP-R) are still the main drugs of choice in treating pain. A compounding issue in pain management is the incremental loss of drug potency, better known as tolerance, a common side effect that develops during chronic treatments with opiates. We have studied aspects of tolerance development to two drugs – morphine, and methadone using the tail flick paradigm. We utilized several genetically modified mouse strains to try and tease apart the contribution of: a) agonist promoted receptor internalization; b) postendocytic receptor sorting; and c) receptor/b-arrestin interaction; to morphine and methadone induced tolerance at the MOP-R. Our data indicate that multiple mechanisms contribute towards the establishment of tolerance. Further, the concentration of the drug used during the treatment determines which of these mechanisms will the dominating the tolerance process. Funding from National Institute on Drug Abuse (NIDA) grants DA015232 and DA019958 and funds provided by the state of California through UCSF, all to J.L.W.; and EMBO long-term fellowship, SSMF stipend for postdoctoral studies, both to J.E.
8. EFFECTS OF DOPR AND MOPR AGONISTS ON CAPSAICIN- AND FORMALIN-INDUCED NOCICEPTIVE BEHAVIORS AND NEURONAL ACTIVATION

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In rodents, delta opioid receptors (DOPR) are expressed by subpopulations of spinal cord and dorsal root ganglia neurons, suggesting an implication in pain modulation. Indeed, several groups have shown analgesic effects of DOPR agonists in various pain models. Expression of DOPR by peptidergic afferences and its role in controlling thermal pain are still controversial. In this study, we compared the analgesic effect of DOPR and MOPR selective agonists on capsaicin-induced pain (substance P (SP) mediated) or formalin model (partially SP mediated). Rats received DOPR or MOPR agonists intrathecally 5 min before paw injection of capsaicin or formalin and were observed for 60 min to evaluate their pain behaviors. DOPR and MOPR agonists were both efficient at reducing pain behaviors, an effect blocked by selective antagonists NTI and CTOP. In parallel, number of spinal neurons expressing c-fos (i.e. activated neurons) was significantly reduced after treatment with opiates. These results indicate that DOPR and MOPR are able to reduce pain behaviors as well as c-fos activation induced by either a SP-dependent or independent mechanism. Supported by CIHR and NSERC

9. POSSIBLE INVOLVEMENT OF OPIOIDERGIC NERVE SYSTEMS ON ANTINOCICEPTIVE EFFECT OF DHA

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Docosahexaenoic acid (DHA) is one of the omega-3 polyunsaturated fatty acid (n-3 PUFAs), and possesses many physiological functions. Previously, there are some reports that n-3 PUFAs contribute to a pain relief. However, antinociceptive effect of DHA alone has not been reported. In this study, we examined the antinociceptive effect of DHA on the various pain stimuli in mice. DHA (5-25 mmol/kg, p.o.) dose-dependently exerted the antinociceptive effect against thermal stimulation, chemical stimulation and mechanical allodynia in comparison to the olive oil. These effects of DHA were abolished by naloxone (1 mg/kg, i.p.). On the other hand, DHA (75 nmol/mouse, i.c.v.) also exerted the antinociceptive effect. Furthermore, it was inhibited by treatment of antiseraum against β-endorphin 1 h before DHA (i.c.v.) administration. Interestingly, the content of β-endorphin in the whole brain seems to increase in DHA administrated mice. These findings suggest that DHA has antinociceptive effect on various pain stimuli, and activates opioidergic nerve systems via the increment of β-endorphin contents in the brain.

10. STUDIES OF POSSIBLE ANTI-ADDITIVE EFFECTS AND MECHANISMS OF LOW DOSE MEMANTINE IN CHRONIC MORPHINE TREATMENT RATS

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The opiate drugs such as morphine, meperidine, etc are the most effective analgesic drugs used in treatment of severe and chronic pain in clinically. However, chronic use these drugs inducing side effects such as tolerance, physical dependence (withdrawal symptoms) and addiction have restricted their clinical use. It is well known that addiction formation is related to the activation of the mesolimbic dopaminergic and glutaminergic pathway. In addition to these neuro-transmission systems, the role of glial cell and their secretary products, especially the proinflammatory cytokines, might related to the development of morphine tolerance, physical dependence and addiction. Studies had shown that inhibition of glial activation or antagonizing the activity of proinflammatory cytokines attenuated the development of morphine tolerance and withdrawal-induced hyperalgesia in rat. Recent studies had found that the dextro-morphians have the neuorprotective effect due to their anti-inflammatory effects. When dextro-morphian was given systemically at extremely low dose attenuated the conditioned place preference produced by levo-morphine given systemically. Memantine is also one of dextro-morphians, showing neuroprotective and anti-inflammatory effects in vitro and in vivo. In the present study, we use the low dose of memantine which has no NMDA receptor blocking effect, further investigated its effect and possible mechanism on morphine addiction. Conditioned place preference test was used to investigate the drug-seeking related behaviors. Our results showed that pre-treatment and post-treatment of low dose of memantine (1 and 0.2 mg/kg, s.c.) were able to attenuate the rewarding effect induced by morphine. We also determined the plasma and related brain area (prefrontal cortex, nucleus accumbens and hippocampus) cytokine level to elucidate the low dose memantine effects. A significant cytokine increasing in following acute or chronic morphine administration was demonstrated in the plasma (IL-1beat and IL-6) and certain brain
area (IL-1beta). This cytokine increasing by morphine could be attenuated by peripheral low dose of memantine pretreatment. Based on our results, it is speculated that the low dose of memantine effectively attenuate morphine-induced rewarding effect. Cytokine analysis revealed that the effect of low dose memantine could be through its action on the anti-inflammatory effect, which could be activated by morphine and attributed to the cause of rewarding. Taken together, these studies suggest a possibility that repeated opiate uses may induce neuro-inflammation (inflammation in the brain) and subsequently may induce a degree of neuron damage can be associated with the addictive and withdrawal behaviors. Use the anti-inflammatory agents like the dextro-morphians may serve as possible therapies to prevent the chronic opioid-induced addiction.

11. REMIFENTANIL EXPOSURE PRODUCES HYPERALGESIA IN RATS
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Infusion of the MOR agonist remifentanil is administered during surgery to promote anesthesia and analgesia. It has been reported that intraoperative remifentanil increases postoperative pain and morphine requirements, suggesting that hyperalgesia and tolerance develop during remifentanil exposure. The present study investigated the effects of remifentanil exposure on pain thresholds. Rats were implanted with indwelling intravenous catheters and infused with remifentanil (0, 32, 100 mcg/kg/h) for 1 h followed by an intraperitoneal injection of 0.6% acetic acid, and abdominal stretches were measured for 30 min. Remifentanil exposure produced a dose-dependent increase in the number and duration of acetic acid-induced abdominal stretches. In flag-tagged MOR expressing HEK cells, remifentanil exposure produced a rapid, dose-dependent loss of cell surface MOR receptors. In conclusion, these data demonstrate that exposure to the MOR agonist remifentanil can produce hyperalgesic states, under some conditions, which may be due to the loss of MORs from the cell surface and loss of endogenous MOR signaling. This study was supported by USPHS grant 04087.

12. MIXED MU/NOP AGONISTS, DIFFERENTIAL EFFECTS IN ACUTE AND CHRONIC PAIN
SR14150 and SR16835 are NOP receptor agonists with affinity for mu-opioid receptors, but are not rewarding in the CPP paradigm. SR14150 is approximately 20 fold selective for NOP, but it is a partial agonist at both NOP and mu receptors. SR16835 is only 10 fold selective for NOP receptors, but it is a full agonist at NOP with very low efficacy at mu. We had previously demonstrated that SR14150, but not SR16835, has mu-mediated antinociceptive activity in the mouse tail flick assay. These compounds were tested in mice in chronic pain subsequent to spinal nerve ligation surgery. In SNL mice, both SR14150 and SR16835 had anti-allodynic activity when measuring mechanical allodynia with von Frey hairs. This effect was completely blocked by the NOP receptor antagonist SB612111 but not by the opioid antagonist naltrexone. In the same mice, SR14150 but not SR16835 increased tail flick latency, and this was blocked by naltrexone. These results indicate that, in mice, circuitry mediating antinociceptive activity is different for acute and chronic pain and that systemically administered NOP agonists may not be effective as analgesics for acute pain but may be effective for chronic pain.

13. DYNORPHIN MEDIATES LATENT PAIN SENSITIZATION IN MICE UNDERGOING SURGERY WITH REMIFENTANIL ANAESTHESIA
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We have investigated the role of spinal dynorphin in latent pain sensitization after surgery. Mice received 80 µg/kg s.c. remifentanil during surgery (plantar incision). Mechanical thresholds (von Frey) were assessed 2, 20, and 21 days, postoperatively. On day 21, mice received a s.c. challenge of (−)-naloxone (1 mg/kg) or nor-BNI (5 mg/kg). Other groups received MK-801 (s.c. 0.15 mg/kg) or antiserum anti-dynorphin (A/S Dyn 200 µg intrathecally), prior to nor-BNI. We determined dynorphin (immunoassay) and kappa opioid receptor (KOR) mRNA (RT-PCR) in the spinal cord and dorsal root ganglia. (−)-Naloxone and nor-BNI precipitated significant hyperalgesia (-52.9±26.7% and -51.8±18.2%, respectively; p<0.01 versus control). Either MK-801 or A/S Dyn completely prevented nor-BNI precipitated hyperalgesia. No changes in dynorphin and KOR mRNA levels were observed in the spinal cord, but KOR mRNA was increased in the ganglia (day 14). Binding of spinal dynorphin to NMDA receptors could be implicated in opioid antagonist-precipitated hyperalgesia and latent pain sensitization. Supported by FIS PI060669, PS09/01270; MaratóTV3 (071110); Cátedra Dolor UAB-IMAS-Menarini. *FPU fellowship (AP20064718)
14. SPINALY ADMINISTERED ATYPICAL ANTIPLSYCHOTIC, RISPERIDONE BLOCKS BOTH SYSTEMICALLY ADMINISTERED OPIOID AND CANNABINOID ANALGESIA DIFFERENTIAL CONTRIBUTION OF SPINAL 5-HT7 AND 5-HT2A RECEPTORS IN TWO DIFFERENT ANALGESIC SYSTEM

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We have previously reported that descending serotonergic pathways and spinal 5-HT7 receptors play an important role in the analgesic effects of both opioids and cannabinoids. It has been suggested that spinal 5-HT2A receptors involving in pain processing. In this study, we investigated the role of spinal 5-HT2A and/or 5-HT7 receptors on the analgesic effects of both opioid and cannabinoids. Risperidone, an atypical antipsychotic which displaying competitive 5-HT2A and dopamine D2 receptor antagonism, elicits a novel mechanism of antagonism on 5-HT7 receptors, irreversibly binding to and inactivating them. Experimental approach: Antinociceptive effects were evaluated in the radiant heat tail-flick test in Balb-C mice. Mu opioid receptor agonist, morphine and a mixed CB1 and CB2 receptor agonist, WIN 55, 212-2 were given systemically (i.p). Risperidone, selective 5-HT2A antagonist, ketanserin or dopamin D2 receptor antagonist, chlorpromazine were given intrathecally (i.th.) at a dose of 10 microgram, 30 min after morphine and WIN 55, 212-2 administration. Morphine and WIN 55, 212-2 (3, 5 and 10 mg/kg, i.p.) produced dose dependent antinociception. I.th. administration of risperidone completely blocks both morphine and WIN 55, 212-2-induced analgesia. Suprisingly, ketanserin blocks WIN 55, 212-2, but not mophine-induced analgesia. Chlorpromazine did not alter WIN 55, 212-2 and morphine analgesia. Both systemically administered opioids and cannabinoids produce antinociception involving “risperidone sensitive” spinal 5-HT, receptors. However, cannabinoid-induced antinociception involve spinal 5-HT1A in addition to 5-HT, receptors suggesting different spinal receptor mechanism in opioid and cannabinoid-induced analgesia. We thank Turkish Brain Research Society for funding.

15. CAPSAICIN-SENSITIVE AFFERENTS ARE RESPONSIBLE FOR ENHANCED DOR-MEDIATED THERMAL BUT NOT MECHANICAL ANTI-ALLODYNIC EFFECTS IN NEUROPATHIC RATS

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Recent evidence has identified the delta opioid receptor (DOR) as a promising target for treating neuropathic (NP) pain. We have used a peripheral nerve injury of NP pain to assess the cellular and subcellular localization of DORs within the dorsal spinal cord. Immunohistochemical experiments showed similar cellular DOR expression between nerve injury and sham animals, although electron microscopy demonstrate that while DORs were primarily localized within intracellular compartments of post-synaptic profiles within the dorsal spinal cord of sham rats, nerve injury significantly increased the cell surface expression of DORs within lamina IV-V dendritic profiles. This result is supported by in internalization of Fluor-deltorphin demonstrating an increase in the localization of fluorescent agonist in the dorsal spinal cord of neuropathic compared to sham animals. Interestingly, this event was sensitive to capsaicin treatment where destruction of capsaicin sensitive afferents blocked the enhanced thermal anti-nociceptive effects but not the anti-allodynic effects of delta agonists. These data reveal the unique regulation of DOR following peripheral nerve injury and suggest that agonists at this receptor may be useful tools in treating NP pain.

16. INVOLVEMENT OF AXONAL mRNA TRANSPORT AND LOCAL PROTEIN SYNTHESIS OF OPIOID RECEPTORS AND TRPV1 CHANNEL IN NEUROPATHIC AND INFLAMMATORY PAIN

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Axons of sensory neurons can translate complex proteins which can influence axon responsiveness under specific painful stimuli. It is known that exogenous and endogenous opioids exert their analgesic effect through receptors present on peripheral sensory neurons. In inflamed or injured tissues, binding of an opioid to its receptor leads to antinociceptive and anti-inflammatory effects. We are evaluating transport of opioid receptors and TRPV1 channel mRNAs along the axon of sensory neurons under inflammatory and neuropathic pain. Our “in vitro” approach will use DRG neuron primary culture in compartmented chambers. In situ hybridization will detect mRNA transported along
the axons and immunostainings to show presence of local translation machinery. With our “ex vivo” approach, which consists of measuring and visualizing mRNA expression in the sciatic nerve of rats with inflammatory or neuropathic pain, we have been able to detect kappa-opioid receptor as well as TRPV1 mRNA in the sciatic nerve. We have also shown that tight sciatic nerve ligation leads to the accumulation of TRPV1 mRNA proximal to the ligature, suggesting that axonal transport of TRPV1 mRNA occurs in sciatic nerve.

17. THE ROLE OF NITRIC OXIDE IN THE ANTIINOCICEPTIVE EFFECTS AND EXPRESSION OF DOR DURING NEUROPATHIC PAIN

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The local administration of DOR agonists attenuates neuropathic pain but the precise mechanism implicated in this effect is not completely elucidated. We evaluated if nitric oxide released during neuropathic pain could affect the antinociceptive effects of DPDPE and modulate DOR gene expression. In WT mice, at 21 days after chronic constriction of sciatic nerve, the antinociceptive effects of DPDPE alone or combined with a NOS1, L-guanylate cyclase or a PKG inhibitor were evaluated. DOR expression in the spinal cord and dorsal root ganglia of WT and NOS1-KO mice was also assessed. DPDPE-induced peripheral antinociception was enhanced by NO-cGMP-PKG pathway blockers. Nerve injury decreased DOR gene expression in WT but not in NOS1-KO mice. Data reveal that NO-cGMP-PKG peripheral pathway inactivation improved the local antinociceptive effects of DPDPE and that nitric oxide synthesized by NOS1 is implicated in the regulation of DOR gene transcription during neuropathic pain. Supported by FIS, PS0900968 and Fundació Marató TV3, 070810.

18. INTRATHECALLY INJECTED INHIBITORS OF Dipeptidyl Peptidase IV, Ile-Pro-Ile and Vildagliptin Are AntiHyperalgesic by Stimulating Endomorphin-2 Generation in Rat Spinal Cord Dorsal Horn


Based on a hypothesis, raised upon the demonstration of the de novo biosynthesis of endomorphin-2 (E2) by dipeptidyl peptidase IV (DPP4) in isolated rat dorsal root ganglia, we tested the antihyperalgesic effect of intrathecally (i.t.) injected DPP4 inhibitors Ile-Pro-Ile (Diprotin A) and vildagliptin in carrageenan-induced hyperalgesia in rats. Compared at the i.t. dose of 3 nmol/rat, vildagliptin was slightly more potent and longer-lasting antihyperalgesic than Ile-Pro-Ile as detected by the Randall-Selitto test upon the induction of hyperalgesia by intraplantarly injected carrageenan. The antihyperalgesic effect of both DPP4 inhibitors could be antagonized either by co-injected specific antiserum to E2 (20x dilution) or s.c. naloxone (1mg/kg) / naltrexone (0,5mg/kg) pretreatment. Neither E2 antiserum alone nor pretreatment with the opioid antagonists affected hyperalgesic baseline. It is concluded that i) anti-hyperalgesia was opioid receptor-mediated and ii) it could be attributed to the stimulation of E2 production in spinal cord dorsal horn. Although carrageenan-induced hyperalgesia is a maintained inflammatory rather than a specific osteoarthritic pain model, our results suggest that targeting DPP4 inhibitors for pain relief in chronic osteoarthritic processes may be promising. The work was supported by benchfee for KK from Semmelweis University Doctoral School.
Opioids in neuropathic pain - Clinical part

19. OPIOIDS MAY NOT BE THE DRUG OF CHOICE IN WOMEN

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Background: Scientific results indicate that opioids are not the best pain killer in women. Women do not have as good analgesic effect as men and have more adverse reactions. Aim: To see if this theory could be shown in the clinic by an eventual difference between the gender concerning opioids and antidepressants. Method: Assessment of which medication gave the best analgesic effect in 225 patients with complex chronic non-malignant pain (150 women and 75 men). The focus was primarily on opioids and antidepressants though other analgesics could be given as well. The patients were all treated in the same clinic by the same staff and got the same medical offers. Results: For the women satisfactory analgesic effect with a minimum of adverse effects were achieved by opioids in 47% (n=69) and by antidepressants in 45% (n=68). For the men the results were opioids 68% (n=51) and antidepressants 45% (n=34). Conclusion: The analgesic response to opioids is better in men than in women whereas the response to antidepressants is the same. It may be more difficult to relieve pain in women than in men.

20. THE REINFORCING EFFECTS OF OXYCODONE IN HEROIN USERS AND OPIOID-ABUSING CHRONIC PAIN PATIENTS

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While there is significant misuse of opioids among chronic pain patients, the reinforcing effects of opioid drugs have yet to be investigated within Chronic Pain Patients (CPP). The data presented here sought to compare oxycodone self-administration between heroin users and chronic pain patients. Twelve heroin users and 18 chronic pain patients were given the opportunity to work for several doses of oral oxycodone (Heroin: 0, 15, 45 mg | CPP: 0, 10, 20, 40, 60 mg). Among chronic pain patients, none of the active doses of oxycodone produced significant increases in progressive ratio break-point (BP) values versus placebo. In contrast, oxycodone produced dose-related increases in progressive ratio BP values in heroin users. The highest oxycodone dose produced a BP (942), significantly higher than that produced by the 15mg (133) and 0mg (271) doses. Unlike heroin users and more similar to non-

drug abusing individuals, oxycodone failed to serve as a reinforcer among pain patients, suggesting that the impetus behind their abuse may differ from those of other opioid abusing populations. Supported by NIDA Grants DA020448 to MAS and DA16759 to SDC.
**Opioids & stem cell research - Recent progress in the stem cell biology and adult neurogenesis**

21. ANALYSIS OF MOLECULAR MECHANISM UNDERLYING THE CONTROL OF NEURAL STEM CELL DIFFERENTIATION BY THE STIMULATION OF DOPAMINE RECEPTORS LOCATED ON EMBRYONIC STEM CELLS


In the present study, we investigated the effect of dopamine on ES cell differentiation, and neural or glial differentiation from neural stem cells. Here we profiled the expression of dopamine-related genes in undifferentiated ES cells and differentiated neural stem cells. The stimulation of dopamine D3 receptor (D3-R) on ES cells produced a significant increase in the levels of NeuroD mRNA levels in the neurosphere. This phenomenon could be regulated by activation of NeuroD transcription associated with the suppression of SOX2 expression and activation of beta-catenin-signaling at the NeuroD promoter through the stimulation of dopamine D3-R. Under these conditions, stimulation of dopamine D3-R failed to change the levels of active histone modifications, H3K4, and two repressive histone modifications, H3K9 and H3K27 at the NeuroD. These findings provide evidence that dopamine may play a role in neural differentiation from ES cells. (1372/1400).

22. OPIOID SYSTEM REGULATES NEURAL AND ENDOTHELIAL CELL DIFFERENTIATION FROM ES CELLS


The aim of present study was undertaken to examine the role of opioids in ES cells differentiation and pluripotency. Here, we profiled the expression of opioids and opioid receptors in undifferentiated ES cells, embryoid body and ES cells-derived differentiated neural stem cells. We also found that the stimulation of delta-opioid receptor (DOR) promoted neural differentiation from ES cells-derived neural stem cells. In contrast, stimulation of either mu-opioid receptor (MOR) or kappa-opioid receptor (KOR) had no such effect. Addition of KOR agonists to ES cells-derived Flk1+ vascular progenitors inhibited endothelial cell differentiation. These results suggest that opioids play a crucial role in ES cells differentiation at the developmental stage. At the presentation, we will discuss the functional role of opioids in ES cells pluripotency.

23. MORPHINE ACCELERATES THE NEUROPATHOGENESIS OF SIV INFECTION IN RHEUS MACAQUES

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Morphine modulates the immune system by enhancing expression of the TH1 cytokine IL-4 and suppressing the TH2 cytokine IFNγ. It also upregulates expression of CCR5, a major co receptor used by HIV for cell entry. However, it is unclear how these effects of morphine alter the outcome of infection. In this study we have used the SIV macaque model of HIV pathogenesis to investigate the effects of morphine on the immune responses, neuropathogenesis and disease progression in infected animals. Sixteen SIV infected rhesus macaques were divided into 2 groups; 4 were virus alone controls (V) and 12 others were administered morphine continually (M+V). Morphine group exhibited significantly higher mortality rates, succumbing to infection within 5 weeks post-infection. These rapid progressors exhibited high incidence of CNS complications. Viral loads in the plasma of most M+V animals were at least 10^5 copies/mL or higher. CSF viral load in the morphine group was at least a log higher than the V group. Immunohistochemical studies showed more SIV gp120 antigen in the basal ganglia of the morphine versus the V group. The SIV gp120-positive antigen also corresponded to regions staining positive for CD68 indicating that the majority of infected cells are macrophages or activated microglia. Ex vivo studies on CD14+ monocytes further corroborated the in vivo findings. Taken together these findings suggest that morphine plays a direct role of in the comorbidity of SIV encephalitis. Acknowledgement: This work was supported by grants from the National Institutes of Health (SB).
24. CNS EXPOSURE TO MORPHINE AND HIV-1 TAT DISRUPTS OLIGODENDROCYTE STRUCTURE AND SURVIVAL IN INDUCIBLE TRANSGENIC MICE


Although opiate abuse appears to exacerbate neuroAIDS, the cell targets and modes of action are unclear. We examined the effects of HIV Tat and morphine on the morphology and survival of oligodendrocytes (OL) using GFAP-driven, doxycycline-inducible Tat transgenic mice. Tat by itself induced caspase-3 activation in OLs, which was enhanced by morphine co-exposure. Co-exposure to Tat and morphine increased TUNEL+ OLs. Tat and morphine exposure caused a significant increase in Golgi-impregnated OLs with truncated, aberrant, and fragmented processes. Electron microscopy revealed increased OLs with vesiculated mitochondria and abnormal intracytoplasmic membranes with Tat induction; morphine co-exposure worsened Tat-induced myelin defects, associated axonopathies, and caused marked degeneration in some OLs, which displayed abnormally dense, marginalized nuclear heterochromatin or pyknotic nuclei. Thus, OLs appear to be targets of HIV Tat and Tat-induced OL degeneration is exacerbated by morphine. Support: NIH DA19398 & DA24461.

25. INVOLVEMENT OF PROTEIN DEGRADATION BY THE PROTEASOME IN MORPHINE PLACE PREFERENCE CONDITIONING


Reorganization of neuronal networks for long-term memory storage requires protein degradation for consolidation to take place. A major mechanism controlling protein turnover is the Ubiquitin Proteasome System (UPS) that plays a role in the development and remodelling of synaptic connections. Addictive behaviours can be considered as pathological forms of memory. During morphine conditioned place preference (morCPP), the brain reward circuitry is triggered, including the Nucleus Accumbens (NAc). We show here that inhibition of UPS in the NAc by lactacystin injections strongly impairs the acquisition of morCPP. Moreover, injection of proteasome inhibitors during the consolidation time-window also abolishes place preference. This behaviour is correlated with changes in the level of poly-ubiquitinated proteins in the NAc. Additional experiments using behavioural sensitization and auto-administration paradigms suggest that proteasome inhibitors affect learning of drug/context association rather than rewarding properties of morphine.

26. EFFECT OF DEXTROMETHORPHAN ON 3,4-METHYLENEDIOXY-METHAMPHETAMINE-INDUCED REWARDING AND BEHAVIORAL SENSITIZATION IN RATS


We previously reported that coadministration of dextromethorphan with morphine attenuates morphine rewarding effect and related dopamine releases at nucleus accumbens in rats. In the present study, we have used conditioned place preference (CPP) and locomotor activity tests to investigate whether DM is also effective on decreasing the rewarding and behavioral sensitization induced by chronic 3,4-methylenedioxymethamphetamine (MDMA) in male S.D. rats. We found that co-administration of DM (10 mg/kg, i.p.) with MDMA (5 mg/kg, s.c.) during 6 days conditioning in rats almost completely abolished the MDMA-induced rewarding effects and behavioral sensitization.
Post-treatment of DM (20 mg/kg, i.p., bid) for 4 days after conditioning also completely abolished the MDMA-induced drug seeking effects. [18F]-ADAM/micro-PET were done after behavioral tests and it was found that co-administration of DM (10 mg/kg; i.p.) with MDMA counteracted the effect of MDMA-induced loss of serotonin transporter. Supported by NHRI-EX97-9401NP, Taiwan, ROC.

27. THE KAPPA OPIOID RECEPTOR AGONIST SALVINORIN A HAS PROTRACTED EFFECTS ON COCAINE-POTENTIATED DOPAMINE RELEASE AND REWARD

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Dynorphin is thought to contribute to negative affective states associated with drug dependence. However, the interaction between kappa opioid receptors (KORs) and drugs of abuse on reward is not fully understood. Previously we showed that the KOR agonist salvinorin A (salvA) decreases motivated behavior and phasic dopamine (DA) release in the nucleus accumbens (NAc) core. Here, we tested the effects of a single injection of salvA on cocaine reward using intracranial self-stimulation (ICSS) and on cocaine-potentiated DA release in the NAc core using fast-scan cyclic voltammetry. We found that 0- and 24-hr post-salvA the reward potentiating effects of cocaine were decreased. However, cocaine-potentiated DA release was unaltered at 0 hr—but decreased at 24 hr—post-salvA. This suggests that distinct mechanisms underlie the depressive-like effects of KOR activation over time. We found that ERK-dependent phosphorylation of tyrosine hydroxylase was reduced in the NAc 24 hr after salvA, raising the possibility that activation of KORs has a protracted effect on DA synthesis. Supported by Morris D. Braun Foundation (EHC) and NARSAD Young Investigator Award (MFR)

28. ALTERED EXPRESSION OF OPIOID GENES DURING HEROIN WITHDRAWAL AND ASSOCIATED STRESS-INDUCED RELAPSE VULNERABILITY IN RATS

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The hypothesis that heroin addiction is characterized in part by a relative deficiency in the endogenous beta-endorphin system is supported by findings that chronic opiate exposure in rodents down-regulates pro-opiomelanocortin (POMC) gene expression in the hypothalamus (Hyp). We report here that there was decreased POMC mRNA in the Hyp of rats immediately after chronic (10-day) intermittent escalating-dose experimenter-administered heroin, during early (12-hour) spontaneous withdrawal, and after 9-day withdrawal from 7-day intravenous heroin self-administration (SA). Increased orexin mRNA was found in lateral Hyp of both groups of heroin withdrawn rats, implicating this peptide in response to withdrawal stress. We further examined whether individual vulnerability to relapse in stress-induced reinstatement of drug-seeking behavior after comparable heroin consumption was related to altered expression of preprodynorphin (ppDyn) gene. We divided heroin SA rats into high and low responders (HR and LR) to foot-shock stress-induced reinstatement during withdrawal. LR had higher ppDyn mRNA in nucleus accumbens core and shell than LR rats. Our results suggest that withdrawal from chronic heroin is associated with decreased Hyp POMC mRNA, and that increased accumbal ppDyn mRNA is involved in modulating vulnerability to stress-induced heroin relapse. Support: NIH NIDA DA-P60-05130 (MJK); CIHR NET (FL)

29. MU OPIOID RECEPTOR TRAFFICKING AND RESPONSIVENESS TO DRUGS OF ABUSE

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The utility of morphine for the treatment of chronic pain is hindered by the development of analgesic tolerance and physical dependence. Morphine is unusual in its failure to promote desensitization and endocytosis of the mu opioid receptor (MOR). Recently we generated a knock-in mouse that expresses a mutant form of the MOR, RMOR that undergoes morphine induced desensitization, endocytosis and recycling. Mice expressing this mutant receptor develop reduced tolerance and dependence to morphine. More recently, we have examined whether altering trafficking of the MOR in the RMOR mice affects reward and addictive like behaviors, as well as synaptic plasticity in brain regions important for the reinforcing properties of morphine. We will report some of these findings here. Work supported by NIDA grant DA019958 and funds provided by the State of California for medical research through UCSF.

30. PHARMACOKINETICS OF KRATOM TEA EXTRACTS IN SPRAGUE DAWLEY RATS

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Kratom (Mitragyna speciosa) has garnered particular interest as it has recently appeared on the United States Drug Enforcement Agency’s list of drugs and chemicals of concern. It is a tree native to Thailand, Malaysia, and areas of South East Asia. Traditionally, these plant extracts have been utilized as an opium substitute or to alleviate opiate withdrawal symptoms. Since most of the scientific literature implicates mitragynine in the opioid actions, we have elected to compare the pure isolate to that of an aqueous tea extract of Kratom in our pharmacokinetic studies utilizing male Sprague Dawley rats. Mitragynine and an equivalent amount of mitragynine in the Kratom tea extract were administered in saline to rats at a dose of 10 mg/kg I.V. and 20 mg/kg P.O. The pharmacokinetic parameters were calculated using WinNonlin software. The bioavailability of mitragynine, when given as tea extract, increased to 36% compared to the mitragynine free base having absolute bioavailability of 21%. The C\text{max} and AUC of the tea extract were found to be significantly higher when compared to the mitragynine free base alone (830 ng/mL and 245181 ng×hr/mL). Also, T\text{max} and clearance were observed to be 76.49 L/hr/kg and 3.5 hr. Mitragynine was found to be stable in simulated gastric and intestinal fluids during the entire incubation time (1 hr in gastric fluid and 3 hr in intestinal fluid). Both the mitragynine free base and Kratom extract showed inhibition of intestinal transit by 42.84% ± 2.12 and 21.6% ± 2.18 compared to control 70.68% ± 4.81 at a time point of 15 minutes. The renal clearance of the Kratom when given as tea extract I.V. and oral were 0.013 ± 0.002 and 0.025 ± 0.008 mL/min/kg and the renal clearance when mitragynine free base was administered I.V and orally were 0.021 ± 0.0052 and 0.07 ± 0.009 mL/min/kg. The significance of the pharmacokinetic parameters of the Kratom tea extract compared to the mitragynine free base will be presented. (This work was supported by the National Center for Research Resources P20RR021929)

31. BLOCKADE OF OPIOID WITHDRAWAL SYNDROME BY ORALLY ADMINISTERED LYOPHILIZED KRATOM (Mitragyna speciosa) TEA AND MITRAGYNYNE IN MICE


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Mitragyna speciosa Korth (Rubiaceae) is a Southeast Asian tree whose leaves are the origin of the Thai traditional drug “Kratom”. The extract of this plant possesses unique pharmacological actions that include a coca-like stimulant as well opium-like depressant actions. Traditionally, the plant extract has been used as an opium substitute, and it has been clinically used in Thailand to wean addicts off opiates. More recently, human case reports have appeared in the literature from use in the United States. However, there have been no detailed pharmacological studies in animals to determine if Kratom or the major alkaloid, mitragynine, is able to attenuate the effects of opioid withdrawal. The current study aimed at examining the ability of Kratom and mitragynine, compared to methadone, to attenuate opioid withdrawal in mice that have been habituated to morphine. A decoction of Kratom leaves was lyophilized to a light brown powder that was characterized based on mitragynine content and dosed to mice based on a survey of human users. Detailed analytical and pharmacokinetic studies provided information on equivalent dosing of the individual alkaloid, mitragynine, in order that the results could be directly compared to those given in the lyophilized Kratom groups. Lyophilized Kratom was dosed orally and significantly attenuated opioid withdrawal with regard to locomotor activity, jumping, paw tremors, teeth chattering but did not attenuate wet-dog shakes or loss in weight. Interestingly, mitragynine alone, administered orally, produced significant blockade of all withdrawal effects and did not affect weight. This data indicates that formulations of Kratom or mitragynine are worthy of further investigation as potential pharmacological treatments for opioid withdrawal. Support: grant number P20RR021929, National Center for Research Resources

32. PERCEPTIONS ABOUT NARCOTICS DRUGS AND ALCOHOL AMONG MEDICAL STUDENTS IN DEVELOPING COUNTRY

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Drug abuse is hazardous and known to be prevalent among young adults, warranting efforts to increase awareness about harmful effects and to change attitudes. This study was conducted to assess the perceptions of a randomized group of medical students, regarding four drugs namely marijuana, benzodiazepines and alcohol in the year 2009. In total, 174 self-reported questionnaires were received (87% response rate). The most commonly cited reasons for why some students take these drugs were peer pressure (96%), academic stress (90%) and curiosity (88%). The most commonly cited justifiable reason was to go to sleep (34%). According to 77%, living in the college male hostel predisposed one to using drugs. Sixty percent of students said that the drugs did not improve exam
performance, while 54% said they alleviated stress. Seventy-eight percent said they did not intend to ever take drugs in the future. Females and day-scholars were more willing to discourage a friend who took drugs. Morality (78%), religion (76%) and harmful effects of drugs (57%) were the most common deterrents against drug intake. Five suggestions to decrease drug abuse included better counseling facilities (78%) and more recreational facilities (60%). Efforts need to be made to increase student awareness regarding effects and side effects of drugs. Our findings suggest that educating students about the adverse effects as well as the moral and religious implications of drug abuse is more likely to have a positive impact than increased policing. Proper student-counseling facilities and healthier avenues for recreation are also required.

33. DECREASED PSYCHOSOCIAL STRESS RESPONSE IN MU OPIOID RECEPTOR KNOCKOUT MICE


There is substantial evidence that opioid systems are involved in stress responses and the function of opioid systems change in response to stressors. Therefore, to further examine the role of opioid systems in stress responses, we studied chronic social defeat stress (psychosocial stress) induced behavioral and neurochemical changes in mu opioid receptor knockout (MOR-KO) mice. In our study, chronic social defeat stress induced aversion to social contact in wild type (WT) mice, but this consequence of psychosocial stress was decreased in MOR-KO mice. This result may suggest mu opioid receptor is involved in the behavioral sequelae of psychosocial stress. Psychosocial stress significantly decreased expression of brain derived neurotrophic factor (BDNF) mRNA in hippocampus in WT mice, but did not change BDNF in MOR-KO mice. These results suggest that the mu opioid receptor is an important regulator of the expression of BDNF by psychological stress in hippocampus. Neural plasticity in limbic regions could be important in the acquisition and memory of emotionally significant events that alter subsequent interactions with other mice, leading to social aversion in WT mice, and differences in BDNF expression changes in response to psychosocial stress may underlie the reduced impact of psychosocial stress in MOR-KO mice. Supported by Grants-in-Aid from MECST and Health Sciences Research Grants from MHLW, Japan. NIDA-IRP, NIH/DHHS (US)

34. ROLE OF SUBSTANCE P IN THE FUNCTIONAL COMPETENCE OF DELTA OPIOID RECEPTORS

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As opposed to most GPCR, delta opioid receptors (DOPR) were shown to be preferentially expressed inside the cells. In rodents, we demonstrated that plasma membrane levels of DOPR are increased by CFA injection in the hindpaw, specifically in small and medium-sized dorsal root ganglia (DRG) neurons. This effect is accompanied by an enhanced analgesic effect of delta agonists. In Tac1 KO mice missing the precursor for substance P (SP), absence of interaction between SP and DOPR was shown to interfere with membrane targeting of DOPR and with DOPR-mediated analgesia. Because SP is absent in most medium-sized DRG neurons we raised the hypothesis that SP is not always mandatory for regulating DOPR. Our objective was therefore to study the regulation of DOPR in the CFA model of inflammation in relation with expression of SP. Using the plantar test and the tail flick test, we found no difference in regards to the analgesic efficacy of intrathecal deltorphin II between Tac1 KO and littermate mice. Our results suggest that SP is not essential for increasing the functional competence and perhaps the membrane targeting of DOPR, at least in a inflammatory pain animal model.

Supported by CIHR.
Opioid addiction – Clinical aspects

35. THE EFFECTS OF 6B-NALTREXOL IN OPIATE DEPENDENT SUBJECTS

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Complications of opioid use include abuse and constipation. 6-beta-naltrexol (6BN), a neutral opioid antagonist, may decrease abuse liability and reverse opioid-induced constipation. In this proof-of-concept, placebo-controlled, blinded study, 3F and 1M opioid-dependent subjects (on 19-70 mg/day methadone) received ascending doses of 0.05, 0.15, 0.50 and 1.0 mg 6BN. Measures included vital signs, Visual Analog Scales (VAS), the Subjective and Objective Opioid Withdrawal Scales (SOWS and OOWS), oral-cecal transit times, and laxation. PK profiles were obtained with the 1.0 mg dose. 3 subjects (all F) received the maximal 1.0 mg of 6BN. The one M reached stopping criteria at 0.5 mg. No subject would have been advanced beyond 1 mg due to abdominal distress. Although 6BN produced abdominal discomfort, no significant changes in VAS measures of “Opioid Withdrawal” or “Sickness”, total SOWS, HR, or BP occurred. Doses greater than 0.5 mg produced laxation within 20 minutes in 3/4 subjects. PK was consistent with prior data. 6BN did not produce substantial opioid withdrawal but did produce laxation. These features suggest that 6BN may be useful in opioid combination formulations. Supported by AIKO Biotechnology.

36. AN 18-YEAR FOLLOW-UP OF PATIENTS ADMITTED TO METHADONE TREATMENT FOR THE FIRST TIME


Earlier studies have shown that discharge from methadone maintenance treatment (MMT) is frequent. Few longitudinal studies have followed subjects before, during and between MMT periods with regard to different life domains. In this study 157 opiate (heroin) dependent subjects (27 % women, 49 % HIV-positive), admitted to Stockholm MMT 1989-1991 were followed 1985-2003 in 7 official registers and in medical records. Each year, about 70 % of living subjects were in MMT. At the end of the follow-up, 71 subjects had died (73 % HIV-positive). In Cox PH multivariate regression, HIV-positivity (HR 3.8), lodging (HR 1.9), prison sentence (HR 1.7) predicted mortality. The problem severity during the observation period differed among the groups still in 1st MMT (n=25), in 2nd MMT (n=24), discharged from 1st MMT (n=38), and from 2nd MMT (n=45) at follow-up, but all had a decrease in hospitalisations and convictions during MMT. Time in MMT ranged from 100 % to 48 %. This study was supported by grants from The Swedish National Drug Policy Coordinator at the Ministry of Health & Social Affairs and by internal funds. Published in Journal of Addictive Diseases, 28:39–52, 2009.

37. INVESTIGATION OF CORRELATION BETWEEN METHADONE DOSES, LENGTH OF SUBSTANCE ABUSE AND SCORES ON THE ZUNG’S INVENTORY OF ANXIETY AND DEPRESSION

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The General Hospital, the methadone center in Sombor, conducted a study of patients on methadone maintenance during February and March 2010. The study included 50 patients. Research objectives were to verify if there were any statistically significant correlations between doses of methadone and: (1) score in the anxiety and depression inventory by Zung (2) years of substance abuse among the subjects (3) score on the depression and anxiety inventory and years of substance abuse. The method was non-experimental. We used inventories of anxiety and depression (Zung), POMPIDOU questionnaire for methadone patients. For statistical processing SPSS12 program was used. We used descriptive statistical measures (arithmetic mean, frequency), Pearson correlation coefficient to verify if there is a statistically significant correlation. Pearson correlation coefficient does not indicate a statistically significant correlation between doses of methadone and score on the anxiety inventory. Pearson correlation coefficient does not indicate a statistically significant correlation between methadone doses and depression score on the inventory. The results confirm the fact that substance abuse and the state of depression and anxiety are separate categories of illnesses. The correlation between years of addiction and depression scores on the inventory and anxiety proved to be statistically significant. The longer the addiction is, the higher the scores on depression and anxiety inventories are. The correlation between the doses of methadone and years of dependence was statistically significant - the longer the addiction lasts, the greater the dose of methadone is. Subjects with high scores on the Zung inventory received antidepressant or anxiolitic therapy, combined with psychotherapy. Follow-up in 6 months.
38. SUBSTANCE ABUSE AND SOME RISK FACTORS IN IRANIAN HIGH SCHOOL STUDENTS
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Substance abuse in adolescence is one of the most prevalent social problems. Having accurate information on the prevalence of the use of various substances is essential in planning and evaluating preventive interventions. This study assessed the rate of substance abuse and some related risk factors among high school students in Iran. In this cross-sectional study, a self-administered questionnaire and Beck Depression Inventory and Zung Self-Assessment Scale for Anxiety were distributed among 600 students (300 girls and 300 boys), who were selected by cluster sampling in the 2008/2009 scholastic year. A total of 537 students (273 girls and 264 boys) returned the completed questionnaire. The prevalence of substance use and its kind, amount of use, last use, usage frequency, and the influence of different factors on substance abuse were evaluated with SPSS 11.5 software. Six ty cases (11.2%) of the students had at least one-time substance abuse (including alcohol, cannabis, opium, heroin, ecstasy). General prevalence of substance use was shisha (qalian) smoking (43%), cigarettes (21.8%), codeine or tramadol tablets (13.2%), alcohol (9.9%), doping (3.6%), cannabis (2.8%), ecstasy (2.6%), opium (1.9%), and heroin (0.2%). Frequency of substance abuse in males (18.9%) was significantly higher than in females (7.7%; p<0.0001). There were significant positive relationships between older age, poor educational situation, family history of smoking, and depression with substance abuse (in all cases, p<0.05). Although the prevalence of substance use increased with the severity of anxiety, this relation was not significant (p>0.05). According to the findings, substance use in students should be considered a serious issue. Improved substance use prevention programs targeting students are necessary.

39. WATER PIPE SMOKING: GATE DRUG TO NARCOTIC DRUGS IN IRANIAN ADOLESCENTS
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Water pipe is a traditional tool for tobacco smoking in Iran and Middle Eastern countries. Unfortunately, there is cultural tolerance for it compared to cigarette smoking and many families who are very resistant to their children’s cigarette smoking accept their water pipe smoking as hobby; so its use is increasing in recent years in Iran. There are lots of studies about the role of cigarette as a gate drug and in this study we aimed at assessing this role for water pipe. This study was a cross-sectional observation study, which consisted primarily of a survey. The survey tool was consisted of a demographic questionnaire and also Beck Depression Inventory and Zung self-assessment scale for anxiety. Study sample consisted of 537 high school students including 273 girls (50.8%) and 264 boys (49.2%). Age range was between 14 and 23. Among students 43% (N=231) and 21.8% (N=117) of participants have had history of water pipe and cigarette smoking, respectively. These were significantly lower in girls than boys (water pipe: 34.4% vs. 51.9%; cigarette: 10.3% vs. 33.7%). Although lifetime experience of water pipe smoking was more, regular (daily) cigarette smoking was more prevalent than water pipe smoking (12.8 and 7.4%, respectively). All daily users of cigarette had a history of water pipe smoking. Eleven students (2.1%) who had a history of opiate substances use (10 people opium and one heroin) were male. This entire group had a history of water pipe tobacco smoking. Forty out of 15 students who had cannabis use experience reported water pipe smoking. 71 students (13.2%) have a history of narcotic drugs including codeine-containing pain relieving tablets and tramadol. This rate was more among girls than boys (16.8 vs. 9.5%). 36 students were regular abusers of these medications and among this group 83.3% had a water pipe smoking history. Among those students with a history of water pipe smoking 139 out of 231 (60.7%) have reported poor educational status; this rate among those who have not a history of water pipe was 39.3%, which is significantly lower. Among those students with a history of alcohol use 78.4% had a history of water pipe smoking; this rate was 15.1 among those who never used alcohol. Clinical depression and moderate to severe anxiety were significantly more frequent among those students who were regular users of water pipe. Water pipe smoking is associated with more drug abuse in Iranian adolescents. It is also associated with poor educational level and more anxiety and depression.

40. USING PILL PHOTOS TAKEN WITH A CELL PHONE TO ASSESS ADHERENCE IN A CLINICAL TRIAL
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Medication non-adherence is an important factor in everyday healthcare and research methodology. Though many types of adherence measuring utilities have been applied, there is as yet no “gold standard” for how to best accomplish this goal. In this study we assessed three types of adherence measurement methods (capsule count, MEMS and a novel method- pictures taken by cellular telephones) and compared their usefulness and
accuracy. Overall adherence estimated by capsule count was 94.9% (± 13.5%), by MEMS 93.6% (± 15.0%), and by photos 76.9% (± 14.6%). Weekly photographs and MEMS agreed with weekly capsule counts with similar frequency (36% vs. 39%, respectively; OR 1.11, p= .79). When weekly measures disagreed with capsule count, MEMS overestimated adherence more than photographs (39% vs. 14%; OR 3.88; p<0.001) and photographs underestimated adherence more than MEMS (49% vs. 22%; OR 3.48; p<0.001). Comparing against capsule count, the novel method was found to be as useful as MEMS. Given the ubiquity of cellular telephones, and the relative ease of this adherence method, we believe it to be a useful and cost effective approach that should be utilized in the future. Supported by NIH DA018179.

41. CLINICAL COURSE OF DEVELOPMENT OF ALCOHOL AND OPIOID DEPENDENCE: IMPLICATIONS IN PREVENTIONS

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The prevalence of alcohol use in Indian society is high, still it is considered socially acceptable to the extent that it has not been included in the Indian Narcotic Drugs and Psychotropic Substances (NDPS) Act, 1985 unlike opium. The aim of this study was to compare the clinical course of development of alcohol and opioid dependence with a view to prevent development of dependence. Consecutively admitted patients with ICD-10 (DCR) diagnosis of alcohol or opioid dependence syndrome were recruited for the study and administered the alcohol or other drug (opioid) section of SSAGA-II respectively and data entered in corresponding tally sheet. Total sample size was 112 of which 81(72%) were alcohol dependent and 31 (28%) were opioid dependent. Subjects with alcohol dependence had significantly (p=0.05) lower ages of onset-18.72(+ 6.84) years of substance use as compared to opioid dependents- 20.73(+3.93) years. Those dependent on opioids also had a significantly (p<0.001) faster progression from onset of use to development of dependence as compared to alcohol dependents. The lower ages of onset of alcohol use and rapid development of alcohol dependence once the first criterion appears calls for a debate on legal measures to stop further alcohol use. The rapid progression of development of dependence from onset of use in opioid dependents prompt the renewal of efforts to strengthen regulative measures.

42. THE EFFECT OF NALTREXONE ON VOLUNTARY ETHANOL CONSUMPTION IN RATS IS DEPENDENT ON EARLY ENVIRONMENTAL FACTORS

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Individual differences in the effect of naltrexone (NTX) on ethanol have been described. Besides genetic factors, environmental factors may contribute to these differences. The aim of the present study was to study the consequences of the early environment on NTXs ability to reduce ethanol intake. The rodent maternal separation (MS) model was used to simulate different early environmental conditions. The rat pups were separated 0 min (MS0), 15 min (MS15) or 360 min (MS360) from their mother daily the first three postnatal weeks. At postnatal day (PND) 26, the male rats were given intermittent access to ethanol (5% and 20%) and water for six weeks. NTX (0.3 mg/kg and 3.0 mg/kg) and saline were then injected sc. The ethanol consumption was measured after 30 min, 2 h and 24 h. The effect of NTX was dependent on early rearing condition. NTX had no effect in MS15 rats but decreased the ethanol intake in the MS0 rats and in the MS360 rats. A dose-dependent decrease was seen in the MS360 rats. The findings show that early environmental factors influence the efficacy of NTX and may thus contribute to individual differences in the response to NTX.

43. THE INTERSECTION OF CHRONIC PAIN AND ALCOHOL: SPINAL NERVE LIGATION IN ALCOHOL PREFERENCES P RATS


The intersection between chronic pain and alcohol was examined using alcohol preferring P rats that have been subjected to spinal nerve ligation (SNL). The alcohol preferring P rats consume alcohol voluntarily when using the 2-bottle choice paradigm, an established animal model to study alcoholism. SNL surgery leads to a long lasting painful neuropathy that is manifested by mechanical allodynia and thermal hyperalgesia. Alcohol consumption alone was sufficient to induce a transient allodynic response, but did not exacerbate or attenuate the SNL-induced allodynia or hyperalgesia. Voluntary alcohol consumption caused a decrease in food consumed and slower
rate of body weight increase in SNL rats as compared to sham controls. Intraperitoneal administration of the NOP receptor agonist SR16835 attenuated the SNL-induced allodynia. Studies are underway to determine the effect of SR16835 on alcohol consumption. These studies will examine the intersection of alcohol abuse and chronic pain, explore the involvement of the NOP receptor system in these processes, and determine the effectiveness of NOP agonists for treatment of both disorders.

44. DUAL EFFICACY OF DELTA OPIOID RECEPTOR SELECTIVE LIGANDS FOR ETHANOL DRINKING AND ANXIETY

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Naltrexone is the key therapeutic used in the treatment of alcoholism, the clinical utility of which is limited by poor efficacy and adverse side effects. Here, we demonstrate that the therapeutic limitations of naltrexone may reflect both its poor selectivity and its ability to produce an opioid withdrawal syndrome as a consequence of changes in mu opioid receptor trafficking. In addition we demonstrate that some opioid receptor ligands can alleviate anxiety-like behaviors associated with ethanol withdrawal. Together these data provide insight into the limited actions of the clinically important drug naltrexone and identify a novel opioid target with improved specificity and efficacy for the development of new therapeutics for the treatment of alcoholism. Work supported in part by NIDA grant DA019958, DOD grant DAMD62-10-5-071, the ABMRF, the NIAAA Center Grant AA017072, and funds provided by the State of California for medical research through UCSF.

45. TARGETING THE DELTA OPIOID RECEPTOR EFFECTIVELY MODULATES ETHANOL CONSUMPTION AND SEEKING AND REPRESENTS A NOVEL TARGET FOR NEW THERAPIES FOR ALCOHOL USE DISORDERS

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Our studies have investigated the role of the delta opioid receptor (DOP-R) in ethanol consumption using the novel naltrexone-derived opioid antagonist, SoRI-9409, a compound with high selectivity for the DOP-R, in a number of rat models of ethanol consumption and seeking. SoRI-9409 more selectively and potently reduces voluntary ethanol intake with reduced adverse effects, compared to naltrexone. Multiple administrations of SoRI-9409 produce selective, long-lasting and permanent reductions of voluntary ethanol intake. SoRI-9409 effectively reduces motivation to respond for ethanol prior to and after a period of ethanol deprivation, and prevents stress-induced relapse behavior. Our studies indicate that DOP-R antagonists, such as SoRI-9409, appear to be promising candidates for further development as treatments for alcohol use disorders. This study was funded by the DOD (W81XWH-08-1-0016) and funding from the State of CA for medical research through UCSF to S.E.B.
Opioid and cannabinoid interaction (joint session with the ICRS)

46. CB2 AGONIST CO-ADMINISTRATION PREVENTS SUSTAINED MORPHINE-MEDIATED SPINAL GLIAL ACTIVATION AND PAIN SENSITIZATION

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Morphine withdrawal after prolonged treatment unmasks a paradoxical increase in pain sensitivity (opiate-induced hyperalgesia (OIH)). Activation of spinal microglia play an important role in regulation of pain sensitivity. Recently it was found that CB2-selective cannabinoid agonists efficiently antagonize glial activation in neuropathies. Thus, we hypothesized that co-administration of a CB2 agonist with morphine will attenuate morphine-induced OIH. Our data demonstrate that systemic co-administration (6 days) of morphine with a CB2-selective agonist (AM1241) significantly attenuates tactile allodynia and thermal hyperalgesia upon drug withdrawal (96 h). AM1241 co-administration also prevented sustained morphine-mediated augmentation of microglia and astrocyte marker immunoreactivity in the spinal cord of the rats. These data suggest that by preventing morphine-mediated CNS glial activation, CB2 agonist co-treatment increases the efficacy and duration of pain relief, reducing the likelihood of side effects in chronic pain treatment. Supported by grants from the NIH and NIDA.

47. ANANDAMIDE INDUCES FEVER DEPENDENT ON PROSTAGLANDINS, OPIOIDS AND IL-1

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We showed before that anandamide (AEA) induced fever by the activation of CB1 receptors. The present study investigated the mediators involved in this response. Changes in rectal temperature (Te) of male Wistar rats were measured in a 30min interval up to 6h. The non-selective cyclooxygenase (COX) inhibitor indomethacin 2.0mg/kg, i.p. and the selective COX-2 inhibitor celecoxib 5.0mg, p.o. reduced the fever induced by AEA 1.0microg, i.c.v. (62.2% and 40%, respectively). Pre-treatment with the selective CB1 antagonist, AM251 5microg, i.c.v. abolished the increased PGE2 concentration in the cerebrospinal (CSF) promoted by AEA. Moreover, AEA increased the concentration of beta-endorphin in the CSF and the non-selective opioid antagonist, naloxone 1.0mg/kg, s.c. abolished the fever induced by this cannabinoid. Pre-treatment of the animals with dexamethasone 0.5mg/kg, s.c. and IL-1ra 200 microg, i.c.v. reduced the fever induced by AEA (87% and 64%, respectively). The results suggest that AEA, through the activation of the CB1 receptors promotes fever dependent on the synthesis/release of prostaglandins, opioids and IL-1. [1] Fraga et al., BJP, 2009, 157:1494.

48. CROSS-REGULATION OF CANNABINOID AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR EXPRESSION AND SIGNALING

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Cell starvation has been shown to lead to enhanced cannabinoid-mediated cell differentiation and neurite outgrowth in neuronal cells. It is also known to modulate expression of platelet-derived growth factor receptors (PDGFRs), suggesting a possible interaction between the cannabinoid receptor CB1R and PDGFRs. In the present study, we directly examine this interaction by measuring the modulation of CB1R and PDGFR expression by quantitative PCR and Western blotting analyses in hepatic stellate cells and neuroblastoma cells that express both these receptors. We also examine the functional interaction between these receptors by studying the modulation of signaling and of downstream responses, such as PDGF-induced hepatic stellate cell migration and cannabinoid-induced neurite outgrowth in a neuroblastoma cell line. These studies suggest functional interactions between CB1R and PDGFRs that play an important role in regulating their activity in normal cell function and in pathology. Supported by NIH grants DA08863 to Devi LA and AA017067 to RR.
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