

INRC ABSTRACT INDEX

Special Lectures	Page
Plenary – Eric Nestler	2
Founders – Charles Chavkin	2
Symposia	
EPIGENETICS OF DRUG ABUSE GENES	2
HUMAN BRAIN IMAGING OF OPIOID RECEPTORS	4
NEW PERSPECTIVES ON BUPRENORPHINE	5
THERAPEUTIC POTENTIAL OF NOCICEPTIN RECEPTOR LIGANDS	7
SEX DIFFERENCES IN PAIN AND OPIOID ANALGESIA	9
GENETIC MOUSE MODELS FOR THE OPIOID SYSTEM	10
DELTA OPIOID RECEPTORS – NOVEL COMPOUNDS AND USES	12
NOVEL THERAPEUTIC APPLICATIONS OF KAPPA OPIOID RECEPTOR LIGANDS	14
MOR REGULATORY PROTEINS	16
YOUNG INVESTIGATOR SYMPOSIUM: OPIOID MODULATION OF NEURAL CIRCUITS	18
ORAL SESSION THURSDAY, JUNE 23	20
POSTERS (ALPHABETICAL ORDER)	23

PLENARY FRIDAY, JUNE 24

Transcriptional and Epigenetic Mechanisms of Drug Addiction

Eric J. Nestler, M.D., Ph.D. Director, Brain Institute; Mount Sinai School of Medicine, New York, NY USA

Synopsis:

Eric Nestler will discuss the role played by changes in gene expression, and related changes in chromatin remodeling, in the brain's reward circuits in mediating the long-lasting alterations induced by chronic exposure to drugs of abuse that underlie aspects of drug addiction. Particular attention will be given to two transcription factors of interest, CREB and DeltaFosB, and to their numerous target genes and downstream functional consequences, as important mediators of drug action.

Abstract:

Drug addiction can be viewed as a stable form of drug-induced neural plasticity, whereby long-lasting changes in gene expression mediate some of the stable behavioral abnormalities that define an addicted state. Our laboratory has focused on two main transcriptional pathways in addiction. Chronic exposure to cocaine or opiates causes the prolonged activation of the transcription factor CREB within the brain's reward circuits and several other brain regions, and this adaptation mediates aspects of drug tolerance and dependence. In contrast, induction of another transcription factor, DeltaFosB, in brain reward regions by virtually all drugs of abuse exerts the opposite effect and contributes to sensitized responses to drug exposure. Studies are underway to explore the detailed molecular mechanisms by which CREB and DeltaFosB regulate target genes and thereby contribute to the complex state of addiction. One way to approach such molecular mechanisms of drug action in vivo is through the study of chromatin remodeling, that is, changes in the acetylation or methylation of histones that bind to certain drug-regulated gene promoters, or changes in methylation of the promoters themselves, as revealed by chromatin immunoprecipitation (ChIP). We are utilizing ChIP to examine chromatin changes at specific candidate genes for CREB and DeltaFosB, as well as genome-wide measures to gain a more global view of target genes for these transcription factors. Prominent among these targets are those that regulate synaptic function and plasticity as well as the morphology of drug-regulated neurons. We have also demonstrated drug regulation of some of the enzymes that catalyze chromatin modifications, which indicates that chromatin remodeling mechanisms are themselves important targets of drug action. These findings establish chromatin remodeling as an important regulatory mechanism underlying drug-induced neural and behavioral plasticity, and provide fundamentally new insight into how CREB and

DeltaFosB, and several other drug-regulated transcription factors, contribute to addiction by regulating the expression of specific target genes in the brain's reward circuitry. These advances can now be mined to develop improved diagnostic tests and treatments for addictive disorders.

FOUNDERS LECTURE SATURDAY, JUNE 25

Dynorphins and the Kappa Opioid Receptor System – Past and Future

Charles Chavkin, Ph.D., Department of Pharmacology, University of Washington School of Medicine, Seattle, WA 98195-7280, USA

I am grateful to the INRC Executive Committee for selecting me as the Founders' lecturer for the 2011 conference. This is a tremendous honor for me because the INRC has been my scientific home since I first attended this conference while a graduate student in 1979. Looking over the list of previous lecturers, I am proud to be included in this distinguished group of scientists. The charge to the speaker is to answer the question, "how did we get here and where should we be going next?" Having participated in many of the important debates over the years within the INRC, I will be happy to present my personal views on the events leading to our current understandings of opioid receptor signaling, receptor desensitization and tolerance mechanisms, and dynorphins' structure and function. Each of us have individual views on where we should be going next, and mine is that we need to use our growing understanding of the opioid peptide system's cellular and molecular actions to better understand complex motivated behaviors. The opioid peptide systems have a central role in the stress response, and I believe that we need to better understand their roles in both healthy and pathological responses to stress. Hopefully these insights will yield novel therapeutics for the treatment of the adverse effects of stress that include the mood disorders of anxiety and depression and also include stress-induced increases in drug addiction risk. The rational design of novel therapeutics based on basic molecular pharmacological insights has been a long-standing goal of the opioid field, and this goal seems increasingly to be within our grasp.

SYMPOSIA

EPIGENETICS OF DRUG ABUSE GENES

DNA methylation: a dynamic and stable regulator of memory

C.A. Miller, Department of Metabolism & Aging, Department of Neuroscience, The Scripps Research Institute, Jupiter, FL, USA

A new line of neuroscience research suggests that epigenetics may be the site of nature and nurture integration by providing the environment with a

mechanism to directly influence the read-out of our genome. Epigenetic mechanisms in the brain are a series of post-translational chromatin and DNA modifications driven by external input. Given the critical hub of epigenetics, neuroscientists have come to suspect its fundamental influence on how our minds change in response to our unique environment and, in turn, how these changes can then impact our future interactions with the environment. We are particularly interested in the role that associative memory plays in driving relapse to drug use, as well as the epigenetic influences on the long-term maintenance of this behavior. Because neuroepigenetics was such a young field at the time we began, we first investigated the mechanisms of simple associative fear memories. Our approach was particularly focused on an epigenetic transcriptional silencing mechanism that has been studied extensively as a lifelong molecular information storage mechanism put in place during development, DNA methylation. We found that learning is associated with hippocampal upregulation of the enzymes responsible for methylation (DNA methyltransferase; DNMT), as well as a rapid increase in the methylation of memory-associated genes. Specifically, a memory suppressing phosphatase, PP1, is transcriptionally silenced through methylation, while a memory promoting gene, *reelin*, is activated. Further, formation of the associative memory is blocked by intra-hippocampal administration of a DNMT inhibitor. Interestingly, these hippocampal changes return to baseline less than a day after learning. This shifted our focus to the cortex, where many types of memories are thought to reside in the long-term. We found that persistent, gene-specific hypermethylation is induced in the cortex by a single, hippocampus-dependent associative learning experience. Further, pharmacologic inhibition of methylation one month after learning disrupts long-term memory maintenance. We are currently taking this new knowledge of neuronal DNA methylation's roles in memory and applying it to animal models of relapse to drug-seeking. Funding provided by NIDA (4R00DA024761-03).

The role of chromatin modifying enzymes in the acquisition and extinction of context-drug associated memory

M. Malvaez, S.C. McQuown, G.A. Rogge, M.A. Wood, Dept. of Neurobiology and Behavior, Center for the Neurobiology of Learning & Memory, Univ. of California Irvine, CA, USA

Repeated use of drugs of abuse causes persistent alterations in gene expression responsible for the long-term behavioral and structural changes in central reward pathways. Recently, it has been suggested that epigenetic mechanisms are

responsible, in part, for these drug-induced changes in gene expression. Epigenetic regulation of gene expression may provide transient and potentially stable conditions, which in turn may ultimately participate in the molecular mechanisms required for neuronal changes subserving long-lasting changes in drug-seeking behavior. Our research is focused on understanding the role of chromatin modifying enzymes in the acquisition and extinction of context-drug associated memory formation. In particular, we examine how the histone acetyltransferase CREB-binding protein (CBP) and the histone deacetylase 3 (HDAC3) are pivotally involved in regulating histone acetylation required for transcription underlying context-cocaine associated memory formation using the conditioned place preference (CPP) paradigm. One exciting result of this research is that HDAC inhibition after establishing a CPP significantly facilitates extinction of drug-seeking behavior in a manner that is refractive to reinstatement. Thus, understanding chromatin modifying mechanisms that establish and maintain drug-dependent plasticity changes may lead to a better understanding of substance abuse disorders as well as novel approaches for treatment. Supported by NIDA (DA025992) and NIMH (MH081004) grants to M.A.W., an NRSA fellowship (DA029368) to M.M., and Repligen Corporation.

Epigenetics of opioid receptor genes – nutrients, drugs and behavior

L.-N.Wei, Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455, USA

The three opioid receptor genes, MOR, DOR and KOR, are differentially regulated but share a highly conserved genomic structure and promoter feature. Classical studies established various combinations of transcription factors in regulating these genes in different cellular contexts. Recent studies uncovered fundamentally important roles for chromatin remodeling in the manifestation of these genes' plasticity, which underlines distinct behavior of the three opioid receptor genes in response to different transcription factors' action and in various biological contexts. Diets, drugs and behavior all can potentially modulate these genes' chromatin remodeling processes, thereby altering their chromatin conformation that is principally responsible for the gene's activity. This paper will present findings supporting epigenetic regulation of opioid receptor genes by various environmental factors, and discuss studies that have begun to examine the molecular mechanisms.

Acknowledgment: This work is supported by NIH grants DA11190, DA11806, DK54733, DK60521 and K02-DA13926, and the Distinguished McKnight University Professorship to LNW.

Chromatin plasticity in addicted brain: prodynorphin upregulation in human alcoholics

G. Bakalkin, M.M.H. Taqi, H. Watanabe, O. Kononenko, T. Yakovleva and I. Bazov, Dept. Pharmaceutical Biosciences, Uppsala University, Sweden.

Genetic, epigenetic and environmental factors may influence the risk for neuropsychiatric disease through their effects on gene transcription. We hypothesize that these effects may be integrated through changes in chromatin states involving methylation of CpG dinucleotides that overlap with single-nucleotide polymorphisms (SNPs) associated with a disorder. We addressed this hypothesis by analyzing methylation of prodynorphin (*PDYN*) CpG-SNPs, reported to be associated with alcohol dependence, in the brain of human alcoholics. Analysis of postmortem human brain specimens demonstrated that *PDYN* expression is activated in discrete brain loci including the dl-PFC in alcoholics. This activation may contribute to cognitive dysfunctions relevant for “preoccupation / anticipation” stages of addiction and disrupted inhibitory control. Three of five *PDYN* SNPs associated with alcohol dependence were found to overlap with CpG dinucleotides. Methylation of these three CpG-SNPs was analyzed by pyrosequencing in the dl-PFC and motor cortex (MC; no expression changes) from 14 alcohol dependent and 14 control subjects. In the dl-PFC but not in the MC of alcoholics, methylation levels of one of these three CpG-SNPs, the C, non-risk variant of 3'-untranslated region (3'-UTR) SNP (rs2235749; C>T) were increased ($P < 0.001$). This methylation positively correlated with *PDYN* mRNA and dynorphins ($P < 0.05$). A DNA-binding factor that differentially targeted the T, risk allele and methylated and unmethylated C allele of this SNP was identified. This factor may be involved in *PDYN* transcription through binding to the methylated 3'-UTR SNP C or T allele. The findings suggest a causal link between alcoholism-associated *PDYN* 3'-UTR CpG-SNP methylation, activation of *PDYN* transcription, and vulnerability to develop alcohol dependence in subjects with the non-risk SNP variant. Methylation of CpG-SNPs associated with a disease under environmental influences may be a general phenomenon affecting gene expression and contributing to disease susceptibility. Supported by the Swedish Council for Working Life and Social Research, and the Swedish Science Research Council.

HUMAN BRAIN IMAGING OF OPIOID RECEPTORS

Imaging opioid effects on brain systems

Lino Becerra, Center for Pain and the Brain, Harvard Medical School, Boston, USA

Imaging has provided opportunities to evaluate drug effects on brain function and structure. Opioids, classically used as analgesics are also drugs of abuse. In this session we will discuss two aspects of opioid actions on brain function. The first will discuss different opioid agonist and antagonist pHMRI results, showing that specific features of opioid subtypes may be evaluated using functional and pHMRI. The second will discuss potential long-term effects of opioids on brain structure and function. Acknowledgements: Louis Herlands Fund for Pain. Imaging Consortium for Drug Development.

Mu-opioid receptors and cocaine addiction

D.A. Gorelick, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA

Mu-opioid receptors (mOR) are expressed on neurons in several brain regions considered to play a role in cocaine use and craving, and are up-regulated by binge administration of cocaine to rodents. We conducted a series of studies, in collaboration with the Johns Hopkins PET Center, evaluating regional brain mOR binding potential (BP) in healthy adults with current cocaine abuse or dependence, no other current psychiatric disorder (except nicotine dependence), and minimal recent use of other drugs (except cigarettes). The PET radioligand was [^{11}C]carfentanil, a selective mOR agonist. The initial study of 10 men found significantly increased (10-50%) mOR BP, compared to 7 non-addicted controls, in frontal, temporal, and anterior cingulate cortex and striatum after 1-4 days of abstinence. Increased BP in most regions was positively correlated with cocaine craving, and declined towards normal in most subjects after 28 days of abstinence. A second study in 17 non-treatment-seeking cocaine users and 16 healthy controls found increased mOR BP in frontal, anterior cingulate, and lateral temporal cortex after 1 days of abstinence, which correlated with cocaine craving and amount of cocaine use in the 2 weeks prior to admission. Binding remained elevated after 1 week in the frontal cortex, and after 12 weeks in the anterior cingulate and anterior frontal cortex. A shorter interval before relapse to cocaine use (after discharge from the secure research ward) was associated with increased mOR BP in frontal and temporal cortex and with lesser decrease in BP between 1 and 12 weeks. A third study in 25 outpatients receiving psychosocial treatment for cocaine addiction found significant associations between increased mOR BP in medial and middle frontal gyri and greater cocaine use and shorter duration of cocaine abstinence during the 12 weeks

of treatment. These findings suggest that brain mOR play an important role in human cocaine addiction and may offer a therapeutic target for developing new treatments. Supported by the IRP, NIH, NIDA and NIH grants R01-DA 09479, DA-11774, & DA-12274.

Development and clinical use of a PET radioligand for the kappa receptor

D. Martinez¹, F. Liu¹, Y. Huang², D.R. Hwang¹, R. Narendran³, R. Carson² and M. Slifstein¹, ¹Division on Substance Abuse, Columbia University/New York State Psychiatric Institute; ²Positron Emission Tomography Center; Yale University School of Medicine; ³Department of Radiology, University of Pittsburgh, USA

Both pre-clinical and postmortem human studies investigating kappa receptor binding in cocaine abuse shown that the kappa receptor plays an important role in addiction. Thus, we developed a radiotracer to image this receptor in humans. The initial work performed in baboons showed that this radiotracer was able to cross the blood brain barrier, and had a good ratio of specific to non-specific binding. In addition, the uptake kinetics showed that significant washout occurred within the time frame of the PET experiment. PET blocking studies with naltrexone showed that the cerebellum could be used as a reference region. Subsequent to this, biodistribution studies were performed in human volunteers, in order to measure the organ exposure, which showed that the radiotracer could be used in clinical studies that required multiple scans. Based on these findings, brain imaging studies were performed in human volunteers. To date, studies in control subjects show that kinetics of the radiotracer vary significantly from the baboon studies, such that long scan times are required. In addition, there is no observable reference region in human subjects, such that scans with naltrexone are needed to obtain the non-specific distribution volume. Thus, while clinical studies performed with this radiotracer remain feasible, these issues must be taken into consideration when developing a PET imaging study with this radiotracer. Supported by the National Institute on Drug Abuse.

Endogenous opioid system modulation of motivation circuitry

J.K. Zubieta^{1,2}, T.F. Love², M. Peciña², C.S. Stohler³, ¹Department of Psychiatry and ²Molecular and Behavioral Neuroscience Institute, University of Michigan, and ³School of Dentistry, University of Maryland, USA

The endogenous opioid system, together with dopaminergic circuits, is emerging as a principal site of action of most drugs of abuse, including alcohol, opiates, psychostimulants and marijuana. Within the

3 receptor types involved in opioid neurotransmission, the μ -opioid receptor has been the best studied in humans. Using external imaging with positron emission tomography and selective radiotracers, studies in healthy humans have shown that there is substantial interindividual variation in the function of this neurotransmitter system, both in the *in vivo* availability of the receptors, as well as in the release of opioid peptides (e.g., β -endorphin, enkephalins, endomorphins) interacting with the μ receptor. In response to a stressful challenge, variations in the concentration of receptors and in the magnitude of neurotransmitter release have been linked to the capacity to regulate the stressful experience. These variations have been linked to specific genetic polymorphisms (e.g., COMT val158met) enriched in substance abusing samples, suggesting that they may underlie variations in the propensity to use drugs and the development of addictions. For example, and in healthy subjects, trait impulsiveness was highly associated with resting and stress-induced μ -opioid system functional measures in the medial and orbitofrontal cortex, anterior cingulate, thalamus, nucleus accumbens and amygdala, accounting for up to 50% of the variance in that personality trait. Patient groups that present high levels of comorbidity with the addictions, such as borderline personality disorder, also present similar alterations in the function of this neurotransmitter system even in the absence of a frank diagnosis of drug dependence. Last, variations in the function of μ -opioid receptors also appear to impact on other neurotransmitter systems, such as the dopaminergic. A common genetic polymorphism in the μ -opioid receptor gene was associated with greater dopaminergic responses to nicotine in tobacco smokers. These data suggest that variation in this neurotransmitter system is implicated in both risk for the addictions and variation in the neural effects to substances of abuse. Supported by grants R01 DA016423, R01 DA027494, R21 DA027066, and R21 MH 069612

NEW PERSPECTIVES ON BUPRENORPHINE

The unique pharmacology of buprenorphine

J. Traynor, Department of Pharmacology and Substance Abuse Research Center, University of Michigan, Ann Arbor, MI 48109, USA

Since its introduction into clinical medicine in the 1970's in the U.K., buprenorphine has been much studied for its unique pharmacology; properties that have lead to its successful introduction into the opiate abuse medication armamentarium, but properties that still remain to be fully explained. Important assets of buprenorphine include its low rate of dissociation from the μ -opioid (MOP) receptor and its profile as a MOP receptor agonist and kappa opioid receptor antagonist; it also has very low delta opioid receptor

efficacy. Of special interest has been the bell-shaped dose-response relationship that is observed in many behavioral assays, including antinociception. This means that high doses show less robust effects and a shorter duration of agonist action than lower doses. Whether a bell-shaped dose-response curve is seen for buprenorphine in rodent models of antinociception is dependent on both the dose and timing of drug administration. The phenomenon has most recently been explained by extensive data supporting an antinociceptive action via MOP receptors at low doses and a physiological antagonism by an action at nociceptin/orphanin FQ (NOP) receptors at higher doses. However, this contradicts the fact that in vitro assays buprenorphine has low affinity and low efficacy at NOP receptors. Thus, for example across all brain regions of the rat when assayed using [³⁵S]GTPgammaS autoradiography buprenorphine acts only as an antagonist. In addition, earlier findings showed that both the ascending and descending arms of the buprenorphine dose-effect curve are sensitive to naloxone antagonism, suggesting an interaction at classical opioid receptors, and structurally dissimilar opiates that also give a bell-shaped dose-effect curve, such as methoclocinnamox, have even lower affinity for, and efficacy at, NOP receptors. These apparent contradictions suggest we still have a lot to learn about the pharmacology of buprenorphine. Supported by NIDA grant DA04087.

New ligands from an old friend

S.M. Husbards, Department of Pharmacy and Pharmacology, University of Bath, Bath, UK
The use of buprenorphine in the treatment of opiate abuse and dependence by detoxification, substitution and maintenance, is the most noteworthy recent addition to the repertoire of methods available for the treatment of substance abuse disorders. In addition to its activity as a mu opioid (MOP) receptor partial agonist, buprenorphine is a kappa/delta (KOP/DOP) receptor antagonist and more recently profiled as a partial agonist at the nociceptin/orphanin FQ (NOP) receptor. It has been postulated that buprenorphine-like ligands with higher NOP receptor activity might have efficacy as non-addicting analgesics and potential drug abuse medications, while buprenorphine-like compounds with lower, or no, MOP receptor efficacy may have utility as relapse prevention agents in the treatment of drug abuse. Control over efficacy at four different receptors is difficult to manage, but notable successes have been achieved within the orvinol series. In the search for MOP/NOP receptor partial agonists, ligands with affinities from 8 nM – 133 nM at NOP receptors were generated (buprenorphine K_{iNOP} 77 nM). Of the compounds with appreciable NOP receptor affinity, efficacy at this receptor ranged from very low (5% of

nociceptin) to moderate (58% of nociceptin) with buprenorphine being intermediate in this range (21% of nociceptin). One compound, BU08028, was found to have comparable affinity at opioid and NOP receptors (all between 1.6 – 8.5 nM) and very similar activity to buprenorphine in the [³⁵S]GTPgammaS assay, but with higher efficacy (48% of nociceptin) at NOP receptors. In the search for NOP partial agonists with antagonist activity at MOP and KOP receptors, ligands have been developed with the desired profile in vitro. The further evaluation of these ligands, including initial in vivo evaluations, will be presented and the recent developments in our understanding of structure-activity relationships in this remarkable series discussed. This work was supported by NIDA grants DA020469 & DA007315 (SMH) and DA023281 (L. Toll).

Buprenorphine: a novel receptor target and mechanism of action

S. Grinnell, S. Majumdar, Y.-X. Pan and G.W. Pasternak, Molecular Pharmacology and Chemistry Program and the Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Buprenorphine is a potent analgesic whose use is becoming increasingly widespread, due in part to a number of advantages over traditional mu opioids. However, a number of features of this agent have raised questions regarding its mechanism of action. It is often considered a partial mu agonist. Yet, it also has high affinity for other classes as well. Interestingly, it has a methyl-c-propyl substituent on the nitrogen, similar to naltrexone, but still remains an analgesic. Furthermore, many investigators have suggested that its actions are less easily reversed by the potent opioid antagonist naloxone. These observations led us to examine its mechanism of action. In our hands, buprenorphine is a potent analgesic. However, in a MOR-1 knockout model in which exon 11 and its associated splice variants are eliminated, buprenorphine shows no analgesic actions at doses many fold higher than its normal ED₅₀. This contrasts with morphine and methadone which retain full analgesic activity in these same exon 11 knockout mice. Although there are several exon 11-associated variants of the mu opioid receptor Oprm1 that predict full length, 7 transmembrane receptors, most predict truncated variants containing only 6 TM domains. Evidence from our group suggests that these truncated variants offer a unique target in the design of opioid anaesthetics and our current results suggest that much of the analgesic actions of buprenorphine can be attributed to these new targets, explaining much of the overall pharmacology. Supported by grants from the National Institute on Drug Abuse (DA02615,

DA06241, DA07242) to GWP and a core grant from the NCI (CA08748) to MSKCC.

Abuse liability of buprenorphine in humans under various states of opioid physical dependence

S.D. Comer, M.A. Sullivan, S.K. Vosburg, J.M. Manubay, Z.D. Cooper, and J.D. Jones, NYSPI and Columbia University, New York, NY, USA

The abuse potential of buprenorphine (bup) as well as buprenorphine/naloxone (bup/nx) is unclear given the unique pharmacology of bup. Therefore, we conducted a series of studies to assess the abuse potential of bup and bup/nx under various states of opioid physical dependence. Heroin-dependent volunteers, who lived in the hospital for the duration of the studies, were given the opportunity to work for either drug or money using a progressive ratio self-administration procedure. None of the participants were interested in treatment for their drug use and were paid for their participation. The volunteers were detoxified from heroin, maintained on morphine, or maintained on sublingual bup. During a sample session, participants received \$20 and a dose of the test drug. During a subsequent choice session, participants could work for the test drug or money they had sampled by making finger press responses. In recently detoxified individuals, bup was self-administered as much as methadone and ratings of drug liking were similar for bup and methadone. When bup was compared to bup/nx in recently detoxified individuals, both drugs were self-administered at the same levels. However, ratings of liking for bup/nx were not different from saline. Instead, participants reported that they self-administered bup/nx because it alleviated mild withdrawal. In morphine-maintained participants, bup alone increased both positive and negative subjective effects, but it was not self-administered at any dose that was tested. In bup-maintained individuals, self-administration of bup/nx was lower than bup alone and heroin. Drug liking and desire to take the drug again also were lower for bup/nx. Consistent with its partial agonist profile, the abuse liability of bup varied depending on the state of opioid physical dependence. The addition of naloxone further reduced the abuse liability of bup under the various experimental conditions. Supported by NIDA (DA09236, DA10909), Schering-Plough, and Reckitt Benckiser.

THERAPEUTIC POTENTIAL OF NOCICEPTIN RECEPTOR LIGANDS

To mix or not to mix: modulation of opioid activity by nociceptin receptor ligands

N.T. Zaveri¹, L. Toll², T.V Khroyan², W.E. Polgar², C. Olsen², F. Jiang², ¹Astraea ²SRI International, Menlo Park, CA, USA

Nociceptin/orphaninFQ via its cognate receptor NOP, modulates several opioid-mediated actions, particularly in reward and nociceptive pathways. We have hypothesized that modulation of opioid activity by NOP ligands could lead to non-addicting analgesics and drug abuse medications. To investigate this hypothesis for therapeutic development, we designed bifunctional NOP/mu-opioid receptor ligands that have varying selectivity and functional efficacy at both these receptors. These compounds were evaluated in a mouse thermal antinociception assay, and in the mouse conditioned place preference paradigm (CPP) against morphine. Our results showed that a NOP/MOP agonist showed significant MOP-mediated analgesia, but NOP agonist efficacy, and preferably NOP selectivity, was required to attenuate the MOP-mediated reward in the same molecule or morphine-induced CPP, when co-administered with morphine. On the other hand, a NOP full agonist with low or negligible efficacy at MOP, attenuated morphine CPP and had no CPP on its own. Our recent studies with buprenorphine, a MOP partial agonist, which has low affinity and efficacy at NOP, showed that its NOP agonist activity can attenuate its MOP-mediated antinociceptive potency, particularly at higher doses, leading to its well-noted inverted U-shaped dose response curve for antinociception. However, buprenorphine induces a place preference in the CPP paradigm, indicating that its low NOP efficacy and selectivity does not attenuate its rewarding effects. It appears therefore, that a bifunctional NOP/MOP agonist profile with a higher balance of NOP selectivity and efficacy, may be suitable as a non-addicting analgesic, whereas full NOP agonist activity is required to attenuate the rewarding effects of opioids. The effect on other opioid-mediated actions such as locomotion and opioid tolerance is still under investigation, and will likely play a role in the therapeutic application of such multitargeted compounds. Supported by grants DA14026, DA027811(NZ) and DA023281(LT).

Therapeutic potential of NOP ligands as spinal analgesics

M.C. Ko, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, USA

Itch/pruritus is the most common side effect derived from spinal administration of mu opioid receptor (MOP) agonists. Given that intrathecal administration of morphine dose-dependently produces antinociception with simultaneous itch/scratching responses in monkeys, this non-human primate model provides a valuable tool to identify a viable target as spinal analgesics. The nociceptin/orphanin FQ (N/OFQ) receptor (NOP) is defined as the 4th member within the opioid receptor family. Although the actions of N/OFQ have much in common with those of classical opioids at the cellular level, the in

vivo pharmacological profiles of N/OFQ and NOP-related ligands are not fully known in primates. This presentation provides an overview of recent studies of NOP- and MOP-related ligands in rhesus monkeys. First, intrathecal N/OFQ over a wide dose range produced antinociception without hyperalgesia, scratching, sedation, and muscle relaxation. In contrast, intrathecal MOP agonists such as morphine and DAMGO produced antinociception with profound scratching. When N/OFQ was combined with morphine intrathecally, this combination produced greater antinociceptive effect. Second, Ro 64-6198, a nonpeptidic NOP agonist, produced antinociceptive effects that are independent of MOP. Like the MOP agonist alfentanil, systemic Ro 64-6198 produced morphine-comparable antinociception. Unlike alfentanil, Ro 64-6198 did not produce reinforcing, respiratory depressant, or pruritic effects. Intrathecal Ro 64-6198 also produced NOP-mediated antinociception. Third, intrathecal UFP-112, a chemical modification of N/OFQ, produced long-lasting antinociception against acute noxious stimulus and capsaicin-induced allodynia. Antinociceptive effects of UFP-112 were antagonized by the NOP antagonist, J-113397, but not by the MOP antagonist, naltrexone. In addition, intrathecal combination of inactive doses of UFP-112 and morphine significantly produced antinociception. Taken together, these findings strongly support the therapeutic potential of NOP agonists as spinal analgesics. Supported by U.S. Dept of Defense, Grant W81XWH-07-1-0162.

The Nociceptin/Orphanin FQ system, as a treatment target for addiction.

R. Ciccocioppo, School of Pharmacy, Pharmacology Unit, University of Camerino, Italy.

Nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the NOP receptor, previously referred to as opioid receptor-like1 (ORL1) receptor, is a 17 aminoacid neuropeptide structurally related to the opioid peptide dynorphin A. From a functional point of view, N/OFQ possesses antiopioid properties and, acting as a presynaptic neuron inhibitor, it is able to control dopaminergic, noradrenergic and glutamatergic neurotransmission in different brain sites. In addition, it has been shown that N/OFQ possesses marked anxiolytic and anti-stress properties presumably mediated by its ability to blunt extrahypothalamic corticotropin releasing factor (CRF) activity. Altogether, these findings point at the N/OFQ–NOP receptor as a system potentially involved in the regulation of reward and drug abuse processes. Indeed, several studies demonstrate that activation of this system results in reduction of the rewarding properties of ethanol, morphine and cocaine. Recent rodent data suggest also that central administration of N/OFQ reduces reinstatement of

alcohol-seeking behavior elicited by stress and by environmental conditioning factors. Buprenorphine has long been in clinical use for treatment of moderate-to-severe pain and its use for maintenance treatment of heroin dependence has been approved in several countries. This drug has long been known to be a partial agonist at μ -opioid receptors but has also antagonistic or agonistic properties at κ and δ opioid receptors. In an unexpected development, it has recently been realized that buprenorphine also is agonist/partial agonist at the NOP receptors. Consistent with these findings we found that in rats this drug reduces excessive alcohol drinking via activation of NOP receptors. Based on these data we suggest that NOP receptors may represent a suitable target for addiction treatment development. Support: NIH/NIAAA grant AA014351.

Discovery and development of nociceptin receptor agonists in alcohol dependence

S.P. Brothers^{1,2,3}, Y.T. Chen¹, H. Salah-Uddin^{1,2,3}, M. Cameron¹, A. Thorsell⁴, M. Roberto⁵, T. Bannister¹, M. Heilig⁴, C. Wahlestedt^{1,2,3}, ¹Molecular Therapeutics, The Scripps Research Institute - Scripps Florida, Jupiter, FL, USA, ²Department of Neuroscience, The Scripps Research Institute - Scripps Florida, Jupiter, FL, USA, ³Department of Psychiatry and Behavioral Sciences and the Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA (current affiliation), ⁴National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda MD, USA, ⁵Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA, USA

Alcohol dependence and abuse represents a considerable health and economic burden on society with available pharmacotherapies demonstrating insufficient efficacy. We designed novel, potent, and selective NOP agonists as tools for research on alcohol dependence with potential as clinically effective therapeutic agents. Currently available NOP small molecule agonists all have some μ or κ opioid receptor activity, limiting their usefulness as research tools. Our unpublished data show several promising novel molecules that are selective for the nociceptin receptor over the μ and κ opioid receptors. Our molecules have been tested for in vitro activity and pharmacokinetic parameters. While our compounds are not orally bioavailable, they do show a high brain penetrance, and long half life in vivo. Our compounds also act in the central amygdala to reduce an ethanol dependant increase in GABA transmission. Finally, in animal models of hangover anxiety, our compounds show promising results that suggest some potential for future clinical translation. These studies were supported by NIH/NIAAA grant 5R01AA017943-02.

SEX DIFFERENCES IN PAIN AND OPIOID ANALGESIA

Gender differences in pain

L. LeResche, Dept. of Oral Medicine, University of Washington, Seattle, WA, USA

Although age- and sex-specific prevalence patterns differ for different pain conditions, prevalence rates of most common chronic pain conditions are higher in women than in men. For example, in population-based studies of adults, female:male ratios for headache, neck, shoulder, knee and back pain average around 1.5:1; for orofacial pains, ratios are about 2:1; for migraine headache, 2.5:1; and for fibromyalgia the ratio is over 4:1. Women are also more likely than men to experience multiple pains simultaneously. Having multiple pain problems (as opposed to a single pain condition) is associated with higher levels of disability and psychological distress, as well as higher risk of onset for new pain conditions. Differences in pain prevalence in men and women could be due to biological sex differences in nociceptive or perceptual mechanisms or to gender differences in pain appraisal, pain behavior or social roles. The gonadal hormone estrogen clearly plays a role in some pain conditions in women (migraine headache, temporomandibular pain). For other pain problems, evidence of hormonal involvement is less clear. However, rates of many pain conditions increase as girls pass through puberty, whereas rates for adolescent boys are stable or rise less steeply than for girls. Pain-related behavior differs by gender; women are more likely than men to seek health care for pain, resulting in a high proportion of women in many pain treatment settings. The higher rate of treatment seeking may in part be due to the fact that pain is more often severe for women than for men. Women's higher pain intensity also seems to be a major factor influencing clinicians' treatment decisions, especially prescription of medications for acute pain – although evidence suggests that clinicians' gender stereotypes also play a role in these decisions, independent of the patient's pain level. Women, particularly elderly women, are more likely than men to be prescribed opioid medications for pain and to use opioids long term. Understanding both biological and social contributions to gender differences in pain may help optimize treatment for people of both sexes. Supported by R01AG034181.

Opioid analgesia and sex differences: An overview

E. Sarton, Department of Anesthesiology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Although a contribution of sex in opioid efficacy has garnered much attention, the confirmation and direction of any such difference remain elusive. We performed a systematic review of the available literature on sex differences in μ and mixed μ/κ opioid effect on acute and experimental pain. Fifty

unique studies (including three unpublished studies) were included in the analyses. Across the 25 clinical studies on μ -opioids there was no significant sex-analgesia association. Restricting the analysis to patient-controlled analgesia (PCA) studies (irrespective of the opioid) yielded greater analgesia in women (n=15, effect size 0.22, 95% c.i. 0.02-0.42, P=0.028). Further restricting the analysis to PCA morphine studies yielded an even greater effect in women (n=11, effect size=0.36, 95% c.i. 0.17-0.56, P=0.003). Meta-regression indicated that the longer the duration of PCA, the difference in effect between the sexes further increased. Across experimental pain studies on μ -opioids women had greater antinociception from opioids (n=11, effect size=0.35; 95% c.i. 0.01-0.69, P=0.047), which was predominantly due to 6 morphine studies. Female patients had greater μ/κ opioid analgesia (n=7, effect size 0.84; 95% c.i. 0.25-1.43, P=0.005), but no sex-analgesia association was present in experimental studies (n=7). Sex differences exist in morphine-induced analgesia in both experimental pain studies and clinical PCA studies, with greater morphine efficacy in women. The data on non-morphine μ and mixed μ/κ -opioids are less convincing and require further study.

Impact of age and sex in the antihyperalgesic actions of morphine: Role of periaqueductal gray

A. Z. Murphy, Neuroscience Institute, Georgia State University, Atlanta, GA, USA

Opioid-based narcotics are the most widely prescribed therapeutic agent for the alleviation of persistent pain; however, it is becoming increasingly clear that morphine is significantly less potent in females compared to males. Indeed, studies from our lab using a variety of pain assays, including somatic, visceral and orofacial pain, have consistently shown that females require approximately twice the amount of morphine as a male to produce comparable levels of pain relief. The midbrain periaqueductal gray (PAG), via its descending projections to the rostral ventromedial medulla and the dorsal horn of the spinal cord, is considered an essential neural substrate for opioid-based analgesia. The PAG contains a dense population of mu opioid receptor (MOR) expressing neurons, and we hypothesized that MOR expression in the PAG was sexually dimorphic, and that these sex differences in opioid receptor levels contribute to the observed sex differences in morphine potency. Using a variety of techniques, including immunohistochemistry, western binding and autoradiography, we found that males have significantly higher levels of MOR expression in the ventrolateral PAG compared to cycling females. Inflammatory hyperalgesia induced by intraplantar administration of Complete Freund's Adjuvant (CFA) was significantly reversed in males following direct

administration of morphine into the PAG. By contrast, the antihyperalgesic actions of morphine were significantly attenuated in proestrus and estrus females. Additional studies by our lab have shown that selective lesions of MOR-expressing neurons in the ventrolateral PAG significantly reduces the antihyperalgesic effects of systemic morphine in males only, and this reduction was positively correlated with the level of MOR expression in the ventrolateral PAG. Together, our studies suggest that sex differences in PAG MOR expression may provide the biological bases for the observed sexually dimorphic actions of morphine. Funded by NIH grant DA16272.

The importance of sex in pain; sexual dimorphic expression in spinal cord of mu-opioid and kappa-opioid receptor heterodimers.

AR. Gintzler, State Univ. NY, Downstate Medical Center, Brooklyn, NY, USA

Sexually dimorphic nociception and opioid antinociception has been extensively demonstrated. In particular, the nociceptive vs. antinociceptive consequences of kappa opioid receptor (KOR) activation is sexually dimorphic. Although it has been established for some time that KOR agonists have weaker analgesic activity and produce greater nociception in males vs. females, determinants of the balance between nociceptive and antinociceptive properties of KOR agonists remain largely unknown. My laboratory had demonstrated that the concomitant activation of spinal μ -opioid receptors (MOR) and KOR is necessary for spinal morphine antinociception in females, but not males. This sexual dimorphism can be explained by spinal cord expression of a MOR/KOR heterodimer that is vastly more prevalent in the spinal cord of females vs. males. Cross-linking experiments in combination with *in vivo* pharmacological analyses indicate that heterodimeric MOR/KOR utilizes spinal dynorphin 1-17 as a substrate and is likely to be the molecular transducer for the female-specific KOR component of spinal morphine antinociception. The existence of heterodimeric MOR/KOR provides a mechanism for activating spinal KOR-mediated antinociception without the concomitant pro-nociceptive functions that monomeric KOR also subserves. The presence of an ovarian sex steroid-dependent functional interaction of KOR with MOR, suggested by the dependence of MOR/KOR expression on stage of cycle, can explain sexually dimorphic analgesic mechanisms solicited by spinal morphine as well as male female differences in the balance between pro-nociceptive vs. antinociceptive responsiveness to KOR agonists. Supported by R01 DA027663.

GENETIC MOUSE MODELS FOR THE OPIOID SYSTEM

Functional characterization of the *OPRM1* A112G SNP in mice

S. D. Mague¹, J. R. Turner¹, G. Carlson², J. A. Blendy¹, Departments of ¹Pharmacology and ²Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

A single nucleotide polymorphism (SNP) in the human μ -opioid receptor gene (*OPRM1* A118G) has been widely studied for its association in a variety of drug addiction and pain sensitivity phenotypes; however, the extent of these adaptations and the mechanisms underlying these associations remain elusive. To clarify the functional mechanisms linking the A118G SNP to altered phenotypes, we derived a mouse model possessing the equivalent nucleotide substitution (A112G), which corresponds to the same amino acid replacement in the *Oprm1* gene. These mice have alterations in basal and morphine-evoked responses in a variety of behavioral tasks, including nociception, behavioral sensitization and conditioned place preference. Some of these behavioral differences may be explained by reductions in MOPR expression levels, however MOPRs are reduced in a sub-set, but not all, brain regions. Specifically, the levels of MOPRs in the hippocampus are not different between genotypes. The hippocampus is an ideal structure to evaluate circuit function. Therefore, to investigate if this SNP impacts a functional response in the absence of reduced receptor levels, we utilized voltage-sensitive dye imaging in hippocampal slices before and after MOPR stimulation with DAMGO. Utilizing several analytical methodologies, we found significant reductions in DAMGO-mediated responses in animals with the G112 allele. These data further support claims that this SNP results in a loss of receptor function. Supported by DA-027066.

The role of *OPRM1* variation for alcohol reward examined using a reverse translational approach

M. Heilig, A. Thorsell. Laboratory of Clinical and Translational Studies, NIAAA, Bethesda MD, USA

Purpose: Mu-opioid (*OPRM1*) receptors are key to rewarding properties of alcohol, and the target for the approved alcoholism medication naltrexone. Based on secondary analyses of clinical trials, A118G variation at the *OPRM1* locus has been suggested to moderate therapeutic efficacy of naltrexone, but this notion remains highly controversial. The purpose of the present set of studies was to examine the role of *OPRM1* A118G variation for alcohol related behaviors using a reverse-translational approach. Humanized mouse lines carrying the human 118A and 118G variants, respectively, were generated on a C76BL/6 background. Ligand affinity was determined using displacement of [3H]DAMGO in cloned CHO-cells. Distribution, binding density and

signaling were determined using autoradiography. A standard behavioral phenotyping battery was carried out. Alcohol-induced DA-release was examined using microdialysis, and alcohol consumption was assessed using two-bottle free-choice drinking. Both humanized receptor variants showed normal ligand affinity, distribution, binding density, and signaling, with no differences by genotype. In the basic behavioral phenotyping battery, 118GG mice were more bold/exploratory than 118AA mice. Similar to our human 11C-raclopride PET data, alcohol-induced DA-release was greater in male 118GG than male 118AA mice. Male, but not female 118GG mice consumed higher amounts of alcohol than 118AA mice of the corresponding sex, in particular at higher alcohol concentrations. The functional OPRM1 118G variant is sufficient to confer greater alcohol-induced DA-release and consumption. These findings are consistent with a role of this variant to predispose human carriers to endorphin-dependent alcoholism, but also to render patients more responsive to opioid antagonist treatment.

Direct visualization of delta opioid receptor internalization under physiological conditions

D. Massotte¹, L. Faget¹, E. Erbs¹, J. Le Merrer¹, G. Scherrer², A. Matifas¹, J.-L. Vonesch³, F. Noble⁴, B. L. Kieffer¹, ¹Dept of Neurobiology and Genetics, IGBMC, Illkirch-Graffenstaden, France, ²Dept of Physiology and Cellular Biophysics, Columbia University, New York, NY 10032, USA, ³Imaging Center, IGBMC, Illkirch-Graffenstaden, France, ⁴Neuropsychopharmacologie des addictions, Université Paris Descartes, Paris, France.

Drug addiction is a complex disorder involving gradual and long-term adaptations of the brain in response to repeated drug exposure. This entails modifications of neuronal connectivity, signaling and plasticity. In heroin addicts, re-exposure to environmental elements previously associated with heroin abuse induce intense drug craving. Therefore, numerous behavioral studies addressed the impact of environmental cues on drug seeking. We developed a protocol in which morphine was repeatedly administered in a given environment at a dose leading to physical dependence. This paradigm elicited context-induced withdrawal upon re-exposure of drug-free animals and induced activation of the hippocampus. Using knock-in mice expressing a functional fluorescent delta opioid receptor (DOR-eGFP), we then investigated delta receptor activation and subsequent internalization by fluorescence microscopy to address *in vivo* dynamics of the receptor under physiological conditions. The authors acknowledge NIDA support to the Center for Opioid Receptors and Drugs of Abuse (#DA 005010), ANR, CNRS, INSERM, the University of Strasbourg and the Alsace region.

Opioids induced cellular and behavioral changes in MOPr phosphorylation-deficient (PD) mice

J.B. Wang¹, E. Barbier¹, Y. Chiu², and L.Y. Liu-Chen², ¹Dept. of Pharmaceut. Sci. Univ. of Maryland Baltimore, Sch. of Pharmacy, ²Dept. of Pharmacol, Temple Univ Med Sch., Philadelphia, PA, USA

Acute or chronic opioid treatment produces 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 study revealed that the MOPr -phosphorylation deficient (PD) mice displayed interesting phenotypes at both behavioral and cellular levels. MOPr -PD mice showed attenuated acute tolerance to morphine and etorphine-induced analgesia and different withdrawal responses 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. 2D DIGE analysis of the brain tissue from the MOPr-PD mice will provide a further insight regarding the role of receptor phosphorylation for the actions of different opioids. Therefore, the MOPr-PD mice 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]

Dynorphins regulate the intensity of fear memory: from mice to men

A. Bilkei-Gorzo¹, S. Erk², K. Michel¹, B. Schürmann^{1,2}, H. Boecker², L. Scheef³, H. Walter², and A. Zimmer¹, ¹Institute of Molecular Psychiatry, ²Department of Psychiatry and ³Functional Neuroimaging Group, Department of Radiology, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

The formation of fear memories and their extinction are necessary for the adaptation to a changing environment. Here with a translational approach we investigated the role of dynorphins in the dynamic change in fear memories in mice and in humans. In mice, genetic deletion of the dynorphin encoding gene *Pdyn* in mice resulted in enhanced cue-dependent fear conditioning, as well as delayed extinction in contextual and cue conditioning/extinction paradigms. The pharmacological blockade of kappa opioid receptors produced a similar effect on fear extinction as the dynorphin deletion. The behavioral data are supported by the analysis of the induction of the immediate early gene *c-fos*, which demonstrated that

the absence of dynorphin results in reduced neuronal activity in key limbic structures during extinction. Translating these findings into the human domain, we could demonstrate that a polymorphism in the dynorphin encoding gene *Pdyn* impacts the activity of the amygdala, functional coupling between amygdala and the prefrontal cortex and the intensity of stress responses during extinction. Our findings establish a role of *Pdyn*/KOR signaling in fear extinction and suggest a biological mechanism for the success of trauma exposure therapy.

DELTA OPIOID RECEPTORS – NOVEL COMPOUNDS AND USES

Dual efficacy of DOR subtype selective ligands for ethanol consumption and its side effects of withdrawal-induced anxiety and hyperalgesia

J. L. Whistler and R. van Rijn, Ernest Gallo Clinic and Research Center at the University of California San Francisco, USA

A strong co-morbidity exists between alcoholism and anxiety disorders. Indeed, alcohol withdrawal-induced anxiety is a primary contributing factor for relapse, and anxiolytics are a common adjuvant therapy prescribed for treatment-seeking alcoholics. Treatment for anxiety disorders and alcoholism exist but are not universally effective. The delta opioid receptor (DOR) has been shown to play a role in both alcohol consumption and anxiety in preclinical animal models making it a very interesting clinical target. Although, there is only one gene that encodes the DOR, there are two distinct pharmacologically-defined subtypes of DOR, DOR1 and DOR2, *in vivo*. Importantly, we have found that DOR1- and DOR2-selective ligands have opposing effects on ethanol consumption. Specifically, DOR1 agonists and DOR2 antagonists decrease drinking while DOR2 agonists increase drinking and non-selective ligands produce no effect. If the DOR subtypes have opposing effects on anxiety and pain as well, targeting the “wrong” DOR subtype may be ineffective or may actually exacerbate withdrawal and relapse. Another key observation regarding the DOR is the dynamic regulation of its location in the cell. In naïve animals, many DORs are stored in large dense core vesicles beneath the plasma membrane. Importantly, functional DORs are translocated from intracellular compartments to the cell surface in response to multiple external stimuli, including chronic stress, inflammatory pain, morphine treatment and, as we and others have recently shown, after chronic alcohol exposure as well. However, the functional relevance of these “unmasked” DORs to anxiety, pain and ethanol consumption remains unknown. Here we will report the changes in responsiveness to DOR subtype-selective drugs that occur during chronic voluntary ethanol consumption. Supported by Department of Defense Grant

DAMD62-10-5-071 (JLW), NIAAA Center Grant AA017072-01, NIDA Grants DA015232, DA019958 (JLW), and the State of California funds for medical research on alcohol and substance abuse through the UCSF.

Inhibition of human multiple myeloma cell proliferation by naltrindole

J. Joshi, A. Terskiy, R.D. Howells Dept. Biochem. & Mol. Biol., UMDNJ-NJ Med. Sch., Newark, NJ, USA

The antiproliferative activity of naltrindole (Nti), a delta opioid receptor (DOR) antagonist, toward human multiple myeloma (MM) cells was evaluated. Nti inhibits the mixed lymphocyte reaction *in vitro*, and blocks graft rejection *in vivo*. Based on its immunosuppressive properties we tested Nti's effect on proliferation of MM cells. MM is an invasive plasma cell neoplasm responsible for 10% of all hematological malignancies. Nti inhibited the proliferation of human MM cell lines with an EC_{50} of 20 μ M, whereas other human cells lines were substantially less sensitive. To mimic the bone marrow environment localization of MM cells, co-culture of MM cells with bone marrow stromal cells did not affect the antiproliferative activity of Nti. [3 H]-Nti exhibits saturable, low affinity binding to intact MM cells and the pharmacological properties of the Nti binding site differ significantly from those of the DOR, suggesting that Nti inhibits proliferation of MM cells through a non-opioid receptor mechanism. RT-PCR assays confirmed the lack of delta, kappa and mu receptor mRNA in MM cells. The identity of the naltrindole binding site is currently under investigation. Nti does not induce apoptosis in MM cells, based on FACS analysis and caspase cleavage assays, but decreases the rate of cell division. While investigating the mechanism of action of Nti, we found that it increases intracellular calcium levels in MM cells, and the calcium appears to be released from the endoplasmic reticulum, based on inhibition of the response following thapsigargin treatment. This effect is specific to Nti as other opioids such as naltrexone and morphine do not affect the levels of calcium in MM cells, nor do they block the activity of Nti. Based on the anti-proliferative activity of Nti toward MM cell lines, an *in vivo* study was conducted. Nti injected IP daily at 30mg/kg significantly decreased tumor volumes in a murine SCID/human RPMI 8226 xenograft model over a 39-day period compared with saline injected controls. Further studies on Nti as a potential therapeutic agent for the treatment of human MM are warranted.

Delta agonist glycopeptides: CNS active drugs from endogenous neuropeptides

Y. Li¹, D. Giuvelis², J. Lowery², C. M. Kirkmire³, L. Z. Szabo¹, B. Anglin¹, M. Lefever¹, L. Yeomans-Maldonado¹, C. M. Keyari¹, D. Muthu¹, E. J. Bilsky³, J. M. Bidlack², R. Polt¹, ¹Univ. Arizona, Tucson AZ, ²Univ. of Rochester Medical Center, ³Univ. of New England, USA

Glycosylation methods developed in the Polt lab have led to a number of stable and systemically available glycopeptide drug candidates have been synthesized and purified on large scale. Key to greater stability, increased bioavailability and enhanced penetration of the blood-brain barrier (BBB) is the *biosian* activity of the glycopeptides. Essential to this concept is the notion that the glycopeptides can adopt two different conformational ensembles: a water-soluble random coil ensemble with a diverse range of backbone conformations, and a more restrictive membrane-bound ensemble of conformations that allows the glycopeptide to participate in membrane transport processes that ultimately lead to BBB penetration. Short enkephalin-derived glycopeptide drugs have been studied as analgesics. Three distinct classes of the enkephalins have been developed: mu-selective opiate agonists, delta-selective opiate agonists, and mixed mu/delta agonists. All of these morphine substitutes have a high potential for translation to the clinic, and a company has been formed to commercialize their application. Endorphin/Dynorphin-derived helical glycopeptides have been explored. While these glycopeptides have a much higher M.W. than the shorter enkephalins (~2500 vs ~1000), their apparent penetration of the BBB is much better. Amphipathic helices are used to achieve *biosian* behavior. Circular dichroism (CD), NMR and computational methods have been used to provide important biophysical information to aid in the design of these drugs. While we are still working on a more complete understanding of this new class of drugs, it seems clear that we can obtain analgesics that are potent at 600 µg/kilo, and recent studies show that the *biosian* approach is not limited to opioid peptides. Support: Office of Naval Research (N00014-05-1-0807 & N00014-02-1-0471), the National Science Foundation (CHE-607917) and the National Institute of Neurological Disorders and Stroke (R01-NS52727).

Delta opioid receptor agonists in Parkinson's disease: a reappraisal

O.S. Mabrouk, M. Volta, R. Viaro, M. Morari, Dept. of Exp. and Clin. Med., Sect Pharmacol., Ferrara Univ., Ferrara Italy.

The delta opioid peptide (DOP) receptor has been considered a target in Parkinson's disease (PD) based on evidence of plasticity of DOP transmission in the parkinsonian brain, and symptomatic efficacy of

DOP receptor ligands in models of parkinsonism and levodopa-induced dyskinesia. In contrast to the commonly belief that DOP receptor agonists act by reinforcing enkephalinergic transmission in globus pallidus, we proved that the site of their antiparkinsonian action is the substantia nigra reticulata (SNr) where they overinhibit the nigro-thalamic pathway at doses effective in attenuating parkinsonian-like symptoms (Mabrouk et al., 2008, 2009). Since nociceptin/orphanin FQ peptide (NOP) receptor antagonists also act in SNr (Marti et al., 2007), we investigated whether both drug classes synergize in attenuating parkinsonism. Combined administration of subthreshold doses of the DOP agonist SNC-80 and the NOP antagonist J-113397 synergistically attenuated motor deficits in 6-OHDA hemilesioned rats. Microdialysis coupled to behavioral testing revealed that the synergism took place in SNr and was associated with synergistic overinhibition of the nigro-thalamic projection. SNC-80 and J-113397 also synergistically reversed MPTP-induced motor impairment in mice. This effect was maintained over a subacute course of administration, and was not accompanied by sparing of dopaminergic terminals in striatum (i.e. neuroprotection). To finally prove the cross-talk between DOP and NOP receptor signaling in vivo, SNC-80 promoted motor behavior more potently in NOP receptor knockout than wild-type mice. These data add to previous evidence of antiparkinsonian efficacy of DOP receptor agonists, suggesting that the combination of low doses of a DOP agonist and a NOP antagonist may provide sustained therapeutic benefit to PD patients. Mabrouk OS, et al. (2008) J Neurochem 107, 1647-1659; Mabrouk OS, et al. (2009) Neuroscience 164, 360-369; Marti M, et al. (2007) J Neurosci 27, 1297-1307. Supported by a FIRB Internazionalizzazione grant n. RBIN047W33.

Identity of dorsal root ganglion and spinal neurons mediating delta opioid receptor analgesia

G. Scherrer (1), B.L. Kieffer (2), A.I. Basbaum (3), A.B. MacDermott (1), (1) Columbia University, USA, (2) IGBMC, France, (3) UCSF, USA

Opioid analgesics targeting the mu opioid receptor (MOR) have limited utility for the management of nerve injury-induced mechanical hypersensitivity (mechanical allodynia/touch-evoked neuropathic pain). While the mechanisms underlying neuropathic pain start to be elucidated our limited understanding of the neuronal circuits on which opioids act prevents us from developing more efficient opioid therapeutics against touch-evoked pain. We recently showed that the delta opioid receptor (DOR), in contrast to MOR, is predominantly expressed by sensory neurons with myelinated axons, and that DOR agonists reduce mechanical hypersensitivity in a mouse model of neuropathic pain. Here we used in situ hybridization,

autoradiography and a DOR-GFP reporter mouse to identify neurons expressing opioid receptors in the somatosensory pathways underlying pain and touch sensation. We found that DOR is present in mechanosensitive Adelta and non-peptidergic C nociceptors critical to acute mechanical pain. Importantly, we show that DOR is also expressed by low-threshold Abeta fibers that innervate hair follicles and Merkel cells in the skin and project to medullary dorsal column nuclei for transmitting touch information. As these fibers have been implicated in touch-evoked pain, our results provide a presynaptic mechanism of action for the anti-allodynic effect of DOR agonists. Additionally, we found that DOR is expressed by spinal neurons that are part of the polysynaptic pathway engaged by Abeta fibers to generate touch-evoked pain. Thus, a subset of lamina I projection neurons that express the substance P receptor NK1R, which are essential for neuropathic pain, coexpress DOR. Furthermore, in the most ventral part of inner lamina II, a region critical for mechanical hypersensitivity, DOR is expressed by calbindin- and PKCgamma-positive excitatory interneurons, which relay Abeta fiber input to nociceptive circuits. Altogether our results provide a cellular basis for the analgesic action of DOR agonists against neuropathic pain. Funding: NIH (ABM, AIB), IASP (GS)

NOVEL THERAPEUTIC APPLICATIONS OF KAPPA OPIOID RECEPTOR LIGANDS

Natural product derived KOP ligands as novel treatments for drug abuse

T. E. Prisinzano Dept. of Med. Chem., Sch. of Pharmacy, Univ. of Kansas, Lawrence, KS, USA
Natural products have played an important role in the development of medications for a number of diseases. However, the search for natural products with utility in the treatment of drug abuse is an area much less developed than the search for anticancer or anti-infective agents. Investigation of psychoactive natural products, such as salvinorin A, provides an opportunity to identify novel scaffolds and selective agents to better characterize known receptor types and study their role in drug abuse. It is relatively rare for natural products to have sufficiently attractive ADME/Tox (Absorption, Disposition, Metabolism, Excretion, and Toxicity) properties to be marketable, despite their excellent potency and selectivity. Thus, the ability to improve these properties by semi- or total synthetic chemistry is important in drug seeking campaigns. A growing amount of evidence suggests that kappa opioid (KOP) receptors are involved in the abuse related effects of CNS 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). As part of our continuing efforts toward developing effective natural product based drug abuse therapies, we report the synthesis and biological characterization of unique semisynthetic analogues of salvinorin A. These agents provide a better understanding of the structure-activity relationships of this unique KOP agonist. This information can then be used to aid in the development of KOP based drug abuse therapeutics with enhanced pharmacological properties. Supported by DA018151 and DA018151S1.

High throughput *in vivo* screening for the identification of novel analgesics

R. A. Houghten, C. T. Dooley, M. Giulianotti and J. P. McLaughlin, Torrey Pines Institute for Molecular Studies, USA

Typical compound screening used to identify potential drug candidates typically yields compounds that do not have desired drug like properties. Thus identified compounds found in this traditional manner have a high inherent rate of attrition in the later stages of drug development as evidenced by poor *in vivo* activity. One approach to circumvent this high attrition rate would be to directly use phenotypic *in vivo* models in the discovery phase to identify enhanced hits with desired biological profiles. Our working hypothesis is that the direct use of mixture-based combinatorial libraries for *in vivo* testing offers a unique opportunity to carry out successful preliminary studies in which 10s to 100s of thousands of compounds can be used in translational *in vivo* assays. Two studies will be presented involving the mouse tail flick test (8 animals per time point; times tested were 30 minutes, 1.0 hours, 2.0 hours, 3.5 hours, 5.0 hours, 8.0 hours and 24.0 hours; differences in mixture results were carried out by summing the area under the curve) of a tetra-peptide library which contains Dmt-DALDA as an internal control (the library in total is made up of 17,850,625 peptides with each mixture composed of 274,625 peptides—these were successfully tested at 25 and 5 mgs/kg). Additionally, a classic small molecule library was tested in the same tail flick assay (this library is made up of a total of 738,192 compounds; the single position defined mixtures were made up of 17-28,000 compounds each and were tested by IP administration at 5mgs/kg). The initial results of these studies were published in the AAPS Journal, 8 (2) E371-382, 2006 and AAPS Journal, 12 (3), p. 318-329. These results lead us to conclude that the direct *in vivo* screening of mixture-based libraries can yield highly active individual compounds having enhanced desired activity. These approaches can be utilized to identify mu, delta and kappa specific analgesics. The breadth and implication of these

approaches will be discussed. Funded in part by NIDA R21DA 019620 (to RAH).

Kappa opioid receptor ligands and development of antipruritic agents

A. Cowan and S. Inan, Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

Itch, for so long an orphan symptom of several systemic diseases, is in the news. It is a unique sensory modality that is closely related to, yet distinct from, pain. Recently, two high profile papers from Dr. Chen's lab at Washington University in St. Louis have raised the possibility of spinal gastrin-releasing peptide serving as a common itch neurotransmitter by relaying information to the somatosensory cortex in response to an array of pruritic stimuli, at least in mice. Chemicals selected to precipitate the particular behavior measured – compulsive scratching of the neck with hindlegs–included chloroquine and compound 48/80. We have found that 5'-guanidinonaltrindole (GNTI), a standard kappa opioid receptor antagonist, also provokes the same frenzied, repetitive scratching when injected s.c. behind the neck in male Swiss Webster mice. Might this be a useful animal model in developing structure-activity data on potential antipruritic agents? What are the alternatives? GNTI-induced scratching is dose-related (0.03-1 mg/kg), stable across at least 30 minutes, and mimicked by the less potent and less efficacious norbinaltorphimine. Critically, either s.c. pre-treatment (0.001-0.03 mg/kg) or post-treatment (0.01-0.03 mg/kg) with nalfurafine, a kappa agonist, attenuates the scratching caused by a standard dose of GNTI (0.3 mg/kg, s.c.). This is an important link to clinical pharmacology since nalfurafine is the first kappa opioid agonist to survive in the commercial arena (against pruritus in hemodialysis patients, in Japan). Our current research is focusing on the relationship between peripherally restricted kappa agonists and the suppression of scratch in mice. We call attention to the anti-scratch properties of asimadoline, an arylacetamide kappa agonist with limited CNS penetration, which is being developed by Tioga/Ono against diarrhea-predominant irritable bowel syndrome. This agent possesses dose-related anti-scratch activity against compound 48/80 and GNTI models of itch in mice. These promising results may hasten the formulation of asimadoline, or like compounds, as skin-directed antipruritics. (DA013429)

Disruption of kappa-opioid receptor function attenuates behavioral effects of stress in rodents

W. A. Carlezon Jr., Psychiatry, Harvard Medical School, McLean Hospital, Belmont MA, USA

Stress can induce profound changes in the brain that have immediate and long-lasting effects on behavior. We have shown that various stressors activate the transcription factor CREB in the nucleus accumbens (NAS). Using viral vectors, we have shown that elevated CREB activity in the NAS causes signs characteristic of depression (anhedonia) and anxiety (resistance to extinction of fear), producing a phenotype similar to that seen in people with post-traumatic stress disorder (PTSD). In contrast, disruption of CREB activity in the NAS has antidepressant-like effects. The mechanism of these effects is unknown, but may involve multiple factors. As one example, CREB may produce these effects by regulating the firing rate of NAS neurons that provide feedback inhibition of mesolimbic dopamine neurons, which in turn send projections to areas more classically implicated in stress responsiveness (amygdala, prefrontal cortex). CREB regulation of dynorphin, an endogenous ligand at KOR receptors, may play a key role in this process. CREB-induced elevation of dynorphin tone leads to increases in the stimulation of KORs located on mesolimbic dopamine neurons, thereby decreasing activity of this system. In support of this model, we now have considerable data indicating that blockade of KORs can prevent, attenuate, and reverse stress effects on behavior. KOR antagonists produce antidepressant-like effects in the forced swim test, regardless of whether they are given before or after exposure to stress. Likewise, KOR antagonists have acute anxiolytic-like effects in the elevated plus maze, and administration of these drugs before fear conditioning can prevent the development of PTSD-like changes in behavior. We have new data indicating that KOR antagonists reduce the disruptive effects of stress on attention in rats, as reflected by performance in the 5-choice serial reaction time task. Collectively, these data suggest that KOR antagonists might be particularly useful for producing protective effects in cases where it is possible to predict when stress will occur. Support: MH063266

Discovery and development of selective kappa opioid receptor antagonists

F. Ivy Carroll, Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC 27709, USA

Stress can induce despair and increase the risk of clinical depression and drug abuse. Dynorphin, the endogenous ligand for the kappa-opioid receptor, is a stress-related neuropeptide in the brain that may mediate these responses. Activation of the kappa-opioid receptor causes place aversion in rodents and

dysphoria in humans. The dynorphin/kappa-opioid receptor system is thought to be critical for stress-induced depression-like behaviors and reinstatement to drug-seeking behavior. Since kappa-opioid receptor activation contributes to stress-induced behavior, there is considerable interest in selective kappa-opioid receptor antagonists that possess drug-like properties. Studies from our laboratory led to the identification of JD_{Tic} as a potent, selective, orally active kappa-opioid receptor antagonist as a potential pharmacotherapy for treatment of depression, anxiety, and addiction (cocaine, alcohol, nicotine, and heroin). Several JD_{Tic} analogs have been identified that have in vitro efficacy similar to that of JD_{Tic}. The in vitro efficacy, pharmacokinetic properties, and potencies as an antagonist of U50,488-induced diuresis in rats will be presented. This research was supported by NIDA grant DA09045.

MOR REGULATORY PROTEINS

RGS9-2 actions in the Nucleus Accumbens modulate opiate addiction and analgesia

D. Terzi, A. Varidaki, M. Papachatzaki, K. Psifogeorgou and V. Zachariou, University of Crete, Greece

The signaling modulator RGS9-2 plays a potent role in dopaminergic and opioidergic transmission in the striatum via actions as a GTPase accelerating protein or as effector antagonist for the G protein alpha subunit. Evidence so far points to RGS9-2 as a potent modulator of antiparkinsonian, antipsychotic, psychostimulant and opiate drug actions. In this study we use genetically modified mice to further understand the role of RGS9-2 in addiction, analgesia and depression like behaviors associated with chronic pain or with long term exposure to opiates. Our results suggest that increased activity of RGS9-2 in the nucleus accumbens (NAc) following stereotaxic injection with an adeno associated virus-RGS9-2 construct blocks the rewarding and locomotor sensitizing actions of morphine and leads to a milder opiate withdrawal syndrome. Interestingly, manipulation of RGS9-2 levels in the NAc also affects analgesic tolerance to morphine. We examined changes in RGS9-2 complexes in the NAc following acute and chronic exposure to morphine and we identified changes in the composition of these complexes associated with morphine tolerance. We also examined the way RGS9-2 affects the actions of agents used to alleviate chronic pain symptoms. Using a neuropathic pain model (spared nerve injury) we show that mice lacking the Rgs9 gene develop tolerance to the antiallodynic actions of morphine much later than their wild type controls, and that they are more sensitive to the antiallodynic actions of tricyclic antidepressants. Tricyclic antidepressants may also improve depression like behaviors

associated with chronic pain in the mutant mice at lower doses than those required for their wild type controls. This phenotype is related to RGS9-2 actions in the NAc as it can be rescued by local overexpression of the protein. Our findings provide new insights into the cellular mechanisms of opiate and antidepressant drug actions and suggest that interventions in the formation of RGS9-2 complexes may be used to improve treatment efficiency. Funding was provided by the Greek Secretariat for Research and Technology (PENED03/860)

In vivo evidence for the role of PKC and other intracellular molecules in opioid tolerance

W. L. Dewey¹, H. Akbarali¹, and G. Henderson²,
¹Dept. of Pharm. and Tox. Virginia Commonwealth University, Richmond, Virginia, USA, ²Dept. Pharm. Univ. Bristol, U. K.

We hypothesize that the differences in the rate and level of tolerance development might well be due to differences in the effects of chronic mu receptor stimulation on intracellular signaling mechanisms. Recent history has shown that receptor phosphorylation which causes a desensitization and encapsulation of the receptor are both seen after chronic administration of mu opioid receptor agonists and have considerable acceptance as important properties of chronic opioid exposure that leads to tolerance. We have found in whole animal experiments that inhibitors of PKC and inhibitors of PKA both reverse but do not inhibit the development of tolerance to moderately efficacious opioids such as morphine. Neither of these specific kinase inhibitors reversed the tolerance produced by the highly efficacious opioid DAMGO. A combination of the doses of each inhibitor that reversed morphine tolerance when given together did not reverse the tolerance to DAMGO. Further studies with specific inhibitors showed that the gamma, alpha and to a lesser extent the epsilon isomer of PKC are involved in this effect. The studies with the PKC inhibitors were confirmed in electrophysiological experiments in isolate LC neurons. On the other hand GRK inhibitors were found not to alter tolerance to these moderately efficacious opioids but completely reversed tolerance to the highly efficacious opioid DAMGO. We conclude from these and related studies that opioid agonists induce tolerance by different mechanisms, that receptor desensitization plays a major role in both cellular and in vivo tolerance and high efficacy agonists induce tolerance independent of PKC but involve G protein-coupled receptor kinases. In addition we will present recently obtained evidence to suggest that the differentiation in the rate of the development and level of tolerance achieved depends on the role of other intracellular molecules. This work was supported by grants from NIDA.

CaMKII in opioid tolerance and opioid-induced hyperalgesia

Z. Jim Wang, Department of Biopharmaceutical Sciences, Cancer Center, & Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois, Chicago, IL, USA

Ca²⁺/calmodulin dependent protein kinase II (CaMKII) is a multifunctional, Ca²⁺/calmodulin-activated protein kinase. CaMKII is co-localized with the mu opioid receptor (MOR) in the spinal cord and dorsal root ganglion neurons. Not only does MOR contain predicted sequence that can be phosphorylated by CaMKII, desensitization of MOR was modulated by CaMKII in cellular models. In several rodent models of opioids tolerance, inhibition of CaMKII by chemical inhibitors, siRNA, or gene-mutation effectively prevented the development of, or reversed the established, tolerance to morphine. These effects correlated well with the biochemical evidence that tracked CaMKII activity. Taken together, these data strongly implicate a critical role of CaMKII in opioids tolerance. Similarly, we found that CaMKII appeared to be essential for the development of opioid-induced hyperalgesia, a phenomenon that is highly relevant for opioids tolerance. The talk will further present evidence for potential mechanisms that may synchronize the action of CaMKII and other kinases in opioid tolerance. Supported by NIH grants DA000505, HL098141, & AT003647

Mu opioid regulation by beta-arrestins and implications for drug development

L. M. Bohn, K. M. Raehal, C. E. Groer, J. M. Streicher, and C. L. Schmid, Departments of Molecular Therapeutics and Neuroscience. The Scripps Research Institute, Jupiter, FL, USA.

The mu opioid receptor (MOR), like most G protein-coupled receptors, interacts with beta-arrestins (Barrestins) upon agonist stimulation. Barrestins (Barrestins 1 and 2) are intracellular scaffolding proteins that can serve to disrupt receptor-protein signaling scaffolds or facilitate such interactions. The degree of interaction between these two proteins can be influenced by the chemical composition of the ligand. Using Barrestin-2 KO mice, our laboratory has studied this protein's contributions to MOR-mediated biological responses. We have found that in the absence of Barrestin2, morphine analgesia is enhanced and tolerance is attenuated suggesting that Barrestin2 plays a role in dampening signaling transduction events leading to antinociception. Other morphine-mediated behavioral responses, including dependence (as assessed by antagonist-induced withdrawal), respiratory suppression and constipation are attenuated in this animal model suggesting that Barrestin2 may play a facilitatory role in the signaling underlying these responses. The work

presented here further examines the role of individual Barrestins in the regulation of the mOR, including the contribution to ubiquitination and resensitization, of the MOR. Early developments in our drug discovery efforts to generate MOR agonists that are biased against Barrestin recruitment will also be introduced. According to extensive studies in the Barrestin2 mouse models, such a strategy may allow for the treatment of pain with fewer side-effects than seen with traditional opioid therapies. Funding for this work has been sponsored in part by R01DA14600, R01DA18860, R03DA025158 to LMB and F31DA021952 to KMR.

Desensitization and trafficking of mu-opioid receptors in locus coeruleus neurons: Modulation by kinases

S. Arttamangkul, H.W. Lu and J.T. Williams, Vollum Institute, Oregon Health & Science University, Portland, Oregon, USA

Mu opioid receptor (MOR) desensitization and internalization induced by many opioid agonists is thought to result from receptor phosphorylation by G-protein receptor kinases (GRKs) and an increase in affinity of arrestins to the phosphorylated receptor. Morphine is different from other agonists in that it is inefficient at recruiting GRKs and arrestins and thus results in little receptor desensitization and internalization. Nevertheless, morphine-induced desensitization can be facilitated by activation of protein kinase C. It is unclear if the activation of PKC also facilitates morphine-induced internalization. This study uses the combination of intracellular recordings and live cell imaging of Flag-tagged-MORs from rat and mouse locus coeruleus neurons to examine the role of PKC in acute desensitization and receptor trafficking. In addition several kinase inhibitors were studied to understand the effects of phosphorylation on MOR desensitization. Blocking GRK2 via a specific inhibitor (NaPP1) in the LC neurons from GRK2-mutant mice showed no effects on MOR desensitization. SB203580 and SP600125, drugs known to inhibit p38 MAPKs and JNKs did not prevent MOR desensitization, internalization or alter the recovery from desensitization. Interestingly, MOR desensitization still occurred but the trafficking of the receptors was altered from normal following pretreatment with staurosporine, at high concentrations. The modified trafficking of receptors was also observed in Flag-tagged-MOR mice lacking arrestin-3. The results suggest that agonist-selective desensitization may take place at an early step following agonist binding that is modulated by, but not dependent on kinase activity. The work was supported by NIH Grants DA08163, DA026617.

**YOUNG INVESTIGATOR SYMPOSIUM: OPIOID
MODULATION OF NEURAL CIRCUITS**

Stress regulation of kappa opioid receptor signaling in the extended amygdala

T.L. Kash, K.E. Pleil, C.J. Li, Department of Pharmacology, Bowles Center for Alcohol Studies, University of North Carolina Chapel Hill, School of Medicine, Chapel Hill, NC, USA

Strong evidence exists for endogenous stress and anti-stress systems in mammalian organisms. Chronic exposure to stress is hypothesized to modulate the relative balance of activities of these systems within key circuitry in the brain, leading to dysregulated emotional behavior. The kappa opioid receptor (KOR) and its endogenous agonist, the neuropeptide dynorphin, are one such 'stress' system. Dynorphin is expressed in the cell bodies and terminals of the bed nucleus of the stria terminalis (BNST), a brain region associated with anxiety and stress, suggesting that KOR activation in this region may play a role in the regulation of emotional behavior. However, the cellular actions of KOR in this region have not been characterized. Using whole-cell voltage clamp recordings in an *ex vivo* mouse brain slice preparation, we investigated the effect of KOR activation on inhibitory transmission in the BNST. We found that activation of KOR reduced GABAergic transmission via a presynaptic mechanism. We next examined the interactions between corticotrophin releasing factor (CRF) and KOR systems. Surprisingly, we found that CRF produced a KOR dependent inhibition of GABAergic signaling, suggesting that CRF can induce dynorphin release in the BNST. We next evaluated the impact of stress exposure on KOR systems. We found that the inhibitory effect of KOR activation on synaptic inhibition was significantly greater in DBA/2J mice compared to C57BL/6J mice. Further, we found that chronic, but not acute restraint, altered KOR modulation in C57BL/6J mice; while both acute and chronic restraint altered KOR modulation in DBA/2J mice. The results from this study add to a growing body of evidence suggesting that the KOR system is involved in the regulation of stress disorders. Supported by an ABMRF Young Investigator Award, a NARSAD Young Investigator Award, an INIA-Stress Pilot Project, R01AA01954 and R00AA17668 from the NIH, and PT090344 from the DoD.

Opioid enhancement of GABA_A receptor function in VTA dopamine neurons: A novel non-G protein mediated signaling mechanism induced by stress

Elyssa B. Margolis, Ernest Gallo Clinic & Research Center, University of California, San Francisco, Emeryville, CA, USA; Dept. of Neurology University of California, San Francisco, CA, USA

Opioid receptors are G-protein coupled receptors that typically signal through activation of inhibitory Gi/o proteins. However, recent themes in GPCR research, including ligand-directed signaling and G protein independent signaling pathways, suggest that a variety of conditions determine the *in vivo* signaling pathway activated when a ligand binds to an opioid receptor. We have discovered that novel postsynaptic delta opioid receptor signaling emerges in VTA neurons following acute footshock stress. This novel signaling pathway causes rapid insertion of postsynaptic GABA_A receptors into the synapse, increasing the ability of synaptically released GABA to inhibit VTA neurons. This effect is PI3K/AKT dependent, but G protein independent. This effect is in the opposite direction to the small DOR-mediated inhibition of GABA_A signaling in naïve rats. Therefore, not only does the magnitude of opioid effects depend upon the state of the animal, but the signaling pathway utilized by opioid receptors is also state-dependent. This novel change in DOR signaling provides a potential mechanism for endogenous opioid release to selectively amplify the inhibition produced by GABA release in a subset of VTA neurons while reducing such inhibition in other VTA neurons. Supported by P50 AA017072, DA-016782-06, DA-030529-01, sponsored by the Army under award numbers W81XWH-08-1-0017 and W81XWH-07-1-043, and funds from the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco.

Dopamine mediated synaptic transmission in the VTA

C. P. Ford, Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA

Opioids alter the activity and excitability of midbrain dopamine neurons, the net effect being an alteration dopamine release and signaling at downstream targets. In order to understand the consequences of downstream dopamine signaling we have been investigating the mechanisms that regulate how dopamine mediates synaptic transmission. In the VTA and SNc, dopamine neurons make dendro-dendritic synapses with adjacent dopamine neurons. The dendritic release of dopamine activates post-synaptic D2-type autoreceptors on adjacent dopamine neurons and induces a GPCR-mediated inhibitory

synaptic current. This talk will outline recent work characterizing the time course and concentration of dopamine that results from the phasic release of dopamine in the VTA that underlies the generation of this inhibitory synaptic current. This aims to understand the dynamics of temporal profile of dopamine during synaptic transmission. Dopamine is often believed to signal at low concentrations over extended periods at D2-receptors due to the high affinity of the D2-receptor. However, using the combination of whole-cell synaptic recordings, electrochemistry and the rapid application of dopamine to excised patches, we have found that post-synaptic D2-receptors are exposed to a relatively high concentration of dopamine (~10 μ M) for a brief period of time (maximum duration ~100 ms) during the peak of phasic transmission. By altering the duration of dopamine that was applied to excised patches we conclude that post-synaptic signaling mechanisms (D2-receptor/G-protein) not the duration of dopamine defines the timecourse of dopamine mediated synaptic transmission. This work suggests that despite being a GPCR agonist, dopamine may signal in a relatively localized manner. Support: NIH/NIDA DA026417 and NARSAD

Context-dependent sensitization to morphine alters hippocampal neuroplasticity

J. A. Moron Concepcion. Dept. of Anesthesiology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA

Evidence suggests that long-term adaptations to the neural substrates of learning and memory after repeated drug treatment may play an important role in drug addiction. For instance, alterations of hippocampus-dependent contextual learning by drugs of abuse may lead to context-evoked cravings or drug seeking behavior. Glutamatergic systems, including α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA), are thought to be involved in opiate-induced neuronal and behavioral plasticity, although the mechanisms underlying these effects are only beginning to be understood. The present study examines the effects of repeated morphine administration, using a paradigm that results in context-dependent behavioral sensitization, on the expression of AMPARs and the functional ramifications in the hippocampus. The learned association between morphine and the drug administration environment following context-dependent locomotor sensitization to morphine leads to persistent changes in the expression and synaptic redistribution of AMPARs. More specifically we find that following context-dependent sensitization we observe a persistent increased expression of AMPARs lacking the glutamate receptor 2 (GluR2) subunit in hippocampal synaptic fractions. In addition, we provide electrophysiological evidence

that this effect is associated with an increase in excitatory synaptic transmission. Interestingly, we also find that the expression of context-dependent sensitization is associated with an impairment in long-term potentiation (LTP). However, these alterations are reduced when morphine injections are received in a non-paired environment. We propose that the learned association between environment and morphine effects is mediated by changes in excitatory transmission and plasticity in the hippocampus. Overall, these data suggest that glutamatergic synaptic transmission in the hippocampus may play an important role in drug-induced behavioral sensitization and addictive processes in general. Supported by NIH grant R01 DA025036 to JMC.

Drug-induced GABA transporter currents enhance GABA release and produce opioid withdrawal behaviours

E.E. Bagley¹, J. Hacker¹, V.I. Chefer², C. Mallet¹, G.P. McNally³, B.C.H. Chieng¹, T.S. Shippenberg², M.J. Christie¹, ¹Brain & Mind Research Institute, University of Sydney, Australia. ²National Institute on Drug Abuse, Baltimore, USA. ³School of Psychology, University of NSW, Australia.

Neurotransmitter transporters can affect neuronal excitability indirectly via modulation of neurotransmitter concentrations or directly through transporter currents. A

physiological/pathophysiological role for transporter currents has not previously been described. Here we show both *in vivo* and *in vitro* that GABA transporter 1 (GAT-1) cation currents directly increase GABAergic neuronal excitability and increase synaptic GABA release in the periaqueductal gray (PAG) during opioid withdrawal. By contrast, GAT-1 did not indirectly alter GABA receptor responses via modulation of extracellular GABA concentrations. Importantly, we found evidence that this GAT-1-induced increase in GABAergic activity induced many of the PAG-mediated signs associated with opioid withdrawal. Together these data support the hypothesis that GAT-1 activity directly produces opioid withdrawal signs through direct hyperexcitation of GABAergic PAG neurons and nerve terminals, which presumably enhances GABAergic inhibition of PAG output neurons. These data provide the first evidence that neurotransmitter transporter currents can play a pathophysiological role. Supported by the National Health & Medical Research Council of Australia (project 512390, Fellowship to MJC 511914) and the Intramural Research Program of the National Institutes of Health, National Institute on Drug Abuse.

ORAL SESSION THURSDAY, JUNE 23

Regulation of opioid dependence by let-7 microRNAs

Y. He and Z. Wang, Department of Biopharmaceutical Sciences, Cancer Center, & Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois, Chicago, USA

MicroRNA (miRNA) has emerged as a critical regulator of neuronal functions. We previously reported that let-7 family miRNAs can post-transcriptionally regulate the μ opioid receptor (MOR) and opioid tolerance. The aim of this study was to test the hypothesis that let-7 family miRNAs can regulate the development of opioid dependence. We found that expression of let-7 was significantly increased in SH-SY5Y cells that were treated with morphine (1 μ M, for 48 h). LNA-modified antisense oligonucleotides (LNA-anti-let-7) not only inhibited endogenous expression of let-7 in SH-SY5Y cells, but also abolished morphine-induced cAMP overshoot. In agreement with the *in vitro* findings, let-7 levels were up-regulated by morphine in mice. Real-time PCR analysis further demonstrated a temporal correlation between let-7 up-regulation and the development of opioid dependence (one 75 mg morphine pellet/mouse, *s.c.*). Moreover, treatment with LNA-anti-let-7 decreased the supraspinal level of let-7 and attenuated naloxone-induced withdrawal in mice dependent on morphine. Morphine conditioned place preference (CPP) was also blocked by LNA-anti-let-7. Taken together, these data suggest let-7 plays an integral role in the development of opioid addiction. (Supported in part by NIH grants RO1HL098141 and KO7AT003647)

Mu opioid receptor biased ligands: delivering powerful analgesia and minimizing side effects

S. M. DeWire, D. Yamashita, C. J. LaBuda, M. W. Lark, and J. D. Violin, Trevena Inc., King of Prussia, PA

Many classical mu-opioid receptor (MOR) agonists are established analgesics, but all possess a number of limiting side effects, notably respiratory depression and constipation. Unlike “off target” side effects which can be eliminated by improving receptor selectivity, these “on target” side effects act directly through the MOR. Building on the findings that beta-arrestin2 knock-out mice exhibit increased analgesic responses to morphine, yet show reduced respiratory depression, tolerance, and constipation (Bohn LM *et al*, *Science* 1999, Raehal KM, *JPET*, 2005), we sought to discover and develop a biased MOR agonist that would recapitulate the genetic findings, i.e. a ligand which engages Gi signaling with similar potency and efficacy to morphine, but with significantly less beta-arrestin recruitment. After performing a high throughput screen and

iterative chemistry campaign, we have identified a collection of MOR G-biased ligands. These compounds are potent and fully analgesic in the mouse 56 degree hot plate model, and when compared to morphine, display an enhanced therapeutic window between analgesia and two models of constipation: glass bead colonic motility and fecal boli accumulation. Additionally, MOR G biased ligands exhibit less respiratory depression at equianalgesic doses, implying a greater safety margin. Taken together, these data indicate that MOR G biased ligands may offer improved safety and tolerability, potentially enabling a better risk/benefit profile for pain management.

Deciphering mu-opioid receptor phosphorylation and dephosphorylation

C. Doll(1), F. Pöll(1) and S. Schulz(1), (1)Institute of Pharmacology and Toxicology, University Hospital, Friedrich Schiller University Jena, Germany

The molecular basis of agonist-selective signaling at the mu-opioid receptor is poorly understood. We have recently shown that full agonists such as [D-Ala²-MePhe⁴-Gly-ol]enkephalin (DAMGO) stimulate the phosphorylation of a number of carboxyl-terminal phosphate acceptor sites including threonine 370 (T370) and serine 375 (S375) that is followed by a robust receptor endocytosis. In contrast, morphine promotes a selective phosphorylation of S375 without causing rapid receptor internalization. Here, we identify kinases and phosphatases that mediate agonist-dependent phosphorylation and dephosphorylation of the mu-opioid receptor using siRNA knock down screening. We found that DAMGO-driven phosphorylation of T370 and S375 was catalyzed by G protein-coupled receptor kinases (GRK) 2 and 3 whereas morphine-driven S375 phosphorylation was catalyzed by GRK5. As a functional consequence, siRNA knock down of GRK5 abrogated morphine-induced but not DAMGO-induced ERK activation. We also identified protein phosphatase 1gamma (PP1gamma) as mu-opioid receptor phosphatase that catalyzed T370 and S375 dephosphorylation at or near the plasma membrane within minutes after agonist removal. Together, the morphine-activated mu-opioid receptor is an efficient substrate for phosphorylation by GRK5 but a poor substrate for GRK2/3. GRK5 phosphorylates mu receptors selectively on S375, which is sufficient to stimulate ERK signaling but not sufficient to drive receptor sequestration. This study was supported by the Deutsche Forschungsgemeinschaft.

Differential binding of non-visual arrestins to the intracellular domains of the mu-opioid receptor

K. Saxena, Y.-J. Chen, I. Rodriguez-Martin, V. Gurevich^a, J. Benovic^b, G. Henderson, E. Kelly,

School of Physiology and Pharmacology, University of Bristol, Bristol, UK, ^aDepartment of Pharmacology, Vanderbilt University, School of Medicine, Nashville, Tennessee, USA, ^b Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA.

Upon agonist binding the μ -opioid receptor (MOPr) is phosphorylated and can recruit arrestin-2 and arrestin-3. Apart from promoting the desensitization and internalization, the binding of arrestins can also lead to the triggering of alternative signalling pathways. We have shown that kinases including GRK2, PKC and CaMKII can regulate MOPr. Here we used GST-fusion constructs of the intracellular regions of MOPr to investigate whether arrestin-2 and -3 can bind to these sequences *in vitro*, and secondly to determine whether phosphorylation of these sequences can alter their ability to bind arrestins. GST-fusion constructs of the intracellular loop2 (ICL2), loop3 (ICL3) and the COOH-terminus (CT) of MOPr coupled to GST beads were incubated with purified arrestin-2 or -3 before being subjected to SDS-PAGE and Western blotting. The degree of arrestin-2 or -3 binding was determined with specific antibodies. Our results indicate that arrestin-3 binds to the unphosphorylated MOPr-ICL2, ICL3 and the CT whereas arrestin-2 shows preferential binding to the GST-MOPr-CT over the intracellular loops. Phosphorylation of the GST-MOPr-CT by GRK2 increased the binding of arrestin-2 and -3. Phosphorylation of the GST-MOPr-CT by PKC and CAMKII increased arrestin-2 and -3 binding but to a lesser extent than GRK2. Non-visual arrestins can bind to intracellular regions of MOPr *in vitro*, with GRK2 phosphorylation increasing the ability of the CT to bind arrestins. These interactions may well be important for the association of arrestins with the intact, agonist-activated MOPr. Supported by Rusan Pharma Pvt. Ltd. (Mumbai, India)

Bivalent ligand MDAN-21 blocks receptor endocytosis by bridging mu-delta opioid heteromers

A.S. Yekkirala (1,2), A.E. Kalyuzhny (3), and P.S. Portoghese (1,2,3), (1) Dept. of Medicinal Chemistry, College of Pharmacy, (2) Dept. of Pharmacology, (3) Dept. of Neuroscience, Medical School., University of Minnesota, Minneapolis, Minnesota, USA

The regulation of opioid receptors by endocytosis has been suggested to play a significant role in the development of antinociceptive tolerance. Studies have indicated that the endocytosis of mu opioid receptors has an inverse relationship to tolerance, whereas the endocytosis of delta receptors correlates with increased tolerance. With focus on the role of opioid receptor heteromers in antinociception and the physiological relevance of heteromer trafficking, we

have employed MDAN-21, a bivalent ligand that contains mu agonist and delta antagonist pharmacophores linked through a 21-atom spacer, as a pharmacological tool to investigate this relationship. MDAN-21 has been reported to produce potent antinociception via mu-delta heteromers without tolerance, dependence, or place preference in mice. Here we show that MDAN-21 did not produce any internalization of mu-delta heteromers in HEK-293 cells. In contrast, both the corresponding mu monovalent ligand (MA-19) and a bivalent ligand MDAN-16 with a short spacer, produced robust internalization of mu opioid receptors. As it is known that a 16-atom spacer does not permit efficient bridging of heteromers, it is likely that MDAN-16, like MA-19, interacts univalently with mu-delta heteromers. These results suggest that MDAN-21 prevents endocytosis by bridging neighboring mu and delta protomers in these heteromers, whereas univalent interaction leads to internalization of mu. Given these results, it is suggested that internalization of mu-delta heteromers plays an important role in the production of opioid tolerance. Support: NIH DA01533

Changes in ligand-biased signaling are associated with opioid tolerance

E. N. Bobeck (1), T. A. Macey (2), K. L. Suchland (1), M. M. Morgan (1) and S. L. Ingram (1), (1) Department of Psychology, WSU Vancouver, Vancouver WA, (2) VA Hospital, Oregon Health & Science University, Portland OR, USA

Opioids, such as morphine, are the most effective treatment for pain, but their use is diminished with the development of tolerance following repeated administration. Recent data from our laboratory demonstrated that morphine activates extracellular signal-related kinase (ERK1/2) phosphorylation selectively in tolerant compared to opioid naïve rats. These results suggest that morphine activation of mu-opioid receptor (MOPr)-coupled effectors is altered following repeated morphine administration and the development of tolerance. The current studies tested MOPr coupling to multiple effectors in the ventrolateral periaqueductal gray (vlPAG) following acute administration of multiple opioid agonists in opioid-naïve or opioid-tolerant rats. We also tested the hypothesis that MOPr activation of ERK1/2 in the vlPAG is dependent on dynamin, a GTPase essential for vesicle endocytosis. A single microinjection of morphine did not activate ERK1/2 levels over background, even though it initiated antinociception that can be inhibited by alpha-dendrotoxin, an inhibitor of presynaptic Kv channels. However, in morphine tolerant rats, a single microinjection of morphine induced ERK1/2 activation that was blocked with microinjections of myristoylated dynamin dominant-negative peptide (Dyn-DN)

compared to rats given a myristoylated scrambled peptide (Dyn-scr) 20 min prior to the morphine microinjection. These results suggest that MOPr activation of ERK1/2 signaling occurs via a dynamin-dependent mechanism in tolerant rats and that repeated morphine administration increases MOPr recruitment of endocytic proteins in response to morphine. Funding by NIH grant DA 015498. Morphine sulfate was a gift from NIDA.

RGS9 knockout enhances MOR-mediated inhibition of adenylyl cyclase in a CNS region dependent manner

D.E. Selley (1), V. Zachariou (3), M.P. Cassidy (1), C.K. Chen (2), E.J. Nestler (4) and L.J. Sim-Selley(1), (1) Dept. of Pharmacology & Toxicology and (2) Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA, USA; (3) Dept. of Pharmacology, University of Crete, Faculty of Medicine, Heraklion, Crete, Greece; (4) Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA

Regulator of G-protein signaling (RGS) type 9-2 is a CNS-expressed splice variant of retinal RGS9. RGS9-2 is highly expressed in the striatum (caudate-putamen and nucleus accumbens), with lower levels expressed in hippocampus, periaqueductal gray (PAG) and spinal cord. Our previous work showed that antinociceptive and conditioned rewarding effects of morphine were enhanced in RGS9 knockout (KO) mice, without any difference in striatal mu opioid receptor (MOR) levels. In the present study, the effect of RGS9 knockout on MOR-mediated inhibition of forskolin-stimulated adenylyl cyclase activity was examined in various CNS regions. Results in nucleus accumbens showed that adenylyl cyclase inhibition by the MOR-selective full agonist DAMGO was unaffected by RGS9 genotype, however inhibition by the high efficacy partial agonist morphine was significantly enhanced in RGS9 KO mice. In contrast, DOR-mediated inhibition of adenylyl cyclase was unaffected by RGS9 KO. Interestingly, in caudate-putamen, neither DAMGO nor morphine-mediated inhibition of adenylyl cyclase differed between genotypes. DAMGO-mediated inhibition of adenylyl cyclase in hippocampus was also not different between genotypes, whereas this response was significantly enhanced in the PAG and spinal cord of RGS9 KO mice. These results indicate that RGS9-2 negatively regulates inhibitory signaling of the MOR to adenylyl cyclase in CNS regions that control motivational and antinociceptive effects of mu opioids, but not regions that mediate motor and memory effects of these drugs. Supported by grants DA014277 (LJS), DA10770 (DES) and DA08227 (EJN) from the National Institute on Drug Abuse

Mouse strain-specific analgesic responses in MOR-1 and DOR-1 KO mice

J. Pintar, M. Ansonoff, T. Wen, J. Nitsche, Dept. Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NY, USA

Initial analysis of MOR-1 and DOR-1 KO mice produced in our laboratory revealed unexpected analgesic responses when these mutant alleles were initially analyzed while being maintained on mixed 129S6/C57Bl6 backgrounds. Specifically we found retention on M6G analgesia in MOR-1 KO mice and a dramatic increase in BW363U86 analgesia in DOR-1 KO mice. To better understand these analgesic responses, both mutant alleles were backcrossed onto both C57Bl6 and 129S6 stains and analgesic responses assayed. Both the retained M6G analgesia and the dramatically increased Bw363U86 analgesia were present in MOR-1 and DOR-1 KOs on the 129S6 background, respectively, but were completely absent in the MOR-1 and DOR-1 strains maintained on C57Bl6. BW363U86 responses were also present in 129S6 triple KO mice, indicating the presence of MOR-1 did not account for this activity. To begin to understand the modifier genes regulating these strain-specific effects of mutation, MOR-1 C57 x MOR-1 129S6, DOR-1 C57 KO x DOR-1 129, and TKO 129S6 x TKO C57Bl6 mice were crossed to produce F1 and F2 generations. While F1 DOR-1 KO mice completely lack BW363U86 analgesia, this analgesia re-appeared at cut-off levels in ~1/4 of F2 offspring suggesting that at most a few genes regulate this response. Similarly, while F1 MOR-1 KO mice completely lacked M6G analgesia, this analgesia also re-appeared in F2 KO mice. QTL analysis has begun to identify loci with significant associations with these analgesic phenotypes. In addition, analysis of TKO mice is currently in progress to determine whether the MOR-1 and DOR-1 analgesic responses coordinately reappear in F2 mice. We conclude that strain-specific modifier genes can regulate phenotypes of both MOR-1 and DOR-1 KO mice and may reveal novel analgesic pathways. Supported by DA-18592, NJTBI, and the NJ Governor Council.

POSTERS (ALPHABETICAL ORDER)

L-theanine suppresses abstinence signs in morphine-dependent rhesus monkeys and has anxiolytic-like activity in the mouse elevated plus maze

M.D. Aceto, L.S. Harris, L.D. Hughes, I.D. Premaratne, L.E. Wise and A.H. Lichtman, Department of Pharmacology, School of Medicine, Virginia Commonwealth University, Richmond, Virginia, USA

L-theanine, 2-Amino-4-(ethylcarbamoyl) butyric acid, is an amino acid that found in green tea (*Camellia sinensis*) and is sold in the United States as a dietary supplement. L-theanine blocked caffeine-induced convulsions (Kimura and Murata 1971) and spontaneous motor activity (Kimura and Murata, 1980) in the mouse and inhibited caffeine's EEG stimulatory effect in the rat (Kakuda et al., 2000). These reports suggested that it might be a caffeine antagonist. In addition, Collier (1974) noted that xanthines mimicked opioid withdrawal signs in rats. We found that caffeine (4.0-32.0 mg/kg, s.c.) elicited many opioid withdrawal signs in normal rhesus monkeys (Aceto et al., 1978). Thus, we speculated that L-theanine might attenuate opioid withdrawal signs and have anxiolytic properties. In morphine-dependent rhesus monkeys in withdrawal, L-theanine dose-dependently (1, 4 and 8 mg/kg, s.c) attenuated the number of withdrawal signs designated slowing, fighting, rigid abdominal muscles, vocalizing on palpation of abdomen, pacing, tremors, coughing, retching, vomiting and wet-dog shakes (Kruskal-Wallis ANOVA and Mann-Whitney comparisons, $P < 0.05$). It had a quick onset and its duration of action was at least 2½ hours. In the mouse, L-theanine dose-dependently produced anxiolytic-like effects in the elevated plus maze (One-way ANOVA, $p = 0.004$). Mice treated with 16 mg/kg, i.p. of L-theanine 60 min before testing in a 5 min session spent more time in the open arms ($p < 0.01$) than mice treated with vehicle. Additionally, mice traveled the same distance in the closed arm ($p < 0.05$) regardless of treatment indicating that changes in motor behavior did not account for the anxiolytic-like actions. L-theanine may be of use in the pharmacotherapy of opioid drug abuse and anxiety. These studies were approved by the University IACUC Committee and supported by NIH (NIDA) 7-8859, DA017259 and DA009789.

Down-regulation of beta-arrestin2 contributes to morphine tolerance in the gastrointestinal tract

H. I. Akbarali, M. Kang, H. Maguma, T.H. Smith, G.R. Ross and W.L. Dewey. Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA

Inhibition of gastrointestinal peristalsis is a major side-effect of chronic morphine that significantly limits its clinical utility. Morphine and other mu-receptor opioids reduce neurotransmitter release by presynaptic inhibition of excitatory and inhibitory enteric neurons. We have previously reported that repeated administration of morphine results in tolerance development in the ileum but not the colon, reflecting the lack of morphine-induced tolerance to constipation (Ross et al., JPET, 2008: 327, 561-72). In this study we examined the role of β -arrestin2 in morphine-induced tolerance development in the ileum and colon. The longitudinal muscle-myenteric plexus (LMMP) preparations from guinea-pig ilea and colon were subject to electrical field stimulation (EFS). β -arrestin2 and ERK 1/2 expression was determined by Western blots in LMMP and isolated enteric ganglia. Tolerance to morphine-induced inhibition of EFS was observed following 2 hour incubation (10 μ M) in isolated ileum but not the colon in guinea-pig LMMP. The IC_{50} (-LogM) significantly shifted to the right from 5.7 ± 0.08 to 5.45 ± 0.09 ($n=9$) ($p < 0.0001$) in the ileum but not in the colon (5.43 ± 0.14 to 5.48 ± 0.17). A corresponding time-dependent down-regulation of β -arrestin2 occurred in the ileum but not the colon LMMP and isolated enteric ganglia. Pretreatment with naloxone prevented the down-regulation of β -arrestin2 in the ileum. In β -arrestin2 knock-out mice, repeated administration of morphine resulted in tolerance to muscle contraction in both ileum and colon. These findings suggest that down-regulation of β -arrestin2 by chronic administration of morphine is associated with development of tolerance in the ileum whereas genetic deletion of β -arrestin2 is required to induce morphine tolerance in the colon. Supported by NIH DA024009.

Unexpected opioid activity profiles of analogs of the novel peptide kappa opioid receptor ligand CJ-15,208

J.V. Aldrich (1), S.S. Kulkarni (1), S.N. Senadheera (1), N.C. Ross (2), K.J. Reilly (2), S.O. Eans (2), M.L. Ganno (2), T.F. Murray (3), J.P. McLaughlin (2), (1)Dept. of Med. Chem., Univ. of Kansas, Lawrence, KS, USA, (2)Torrey Pines Inst. for Molecular Studies, Port St. Lucie, FL, USA, (3)Dept. of Pharmacol., Creighton Univ. Sch. of Med., Omaha, NE, USA

The cyclic tetrapeptide natural product CJ-15,208 (cyclo[Phe-D-Pro-Phe-Trp]) is a novel kappa opioid receptor (KOR) antagonist (Saito et al., J. Antibiot.

2002, 55, 847). An alanine scan was performed on CJ-15,208 to determine which residues contribute to the potent *in vivo* agonist activity observed for the parent peptide. These cyclic tetrapeptides were synthesized by a combination of solid phase peptide synthesis of the linear precursors, followed by cyclization in solution. One alanine substituted analog exhibited higher KOR affinity than the parent peptide in binding assays, while the other analogs had much lower KOR affinities. Also like the parent peptide, each of the analogs exhibited agonist activity and KOR antagonist activity in the 55 degree C warm-water tail-withdrawal assay *in vivo* after intracerebroventricular (i.c.v.) administration. Unlike the parent peptide, the agonist activity of these analogs was predominantly mediated by mu opioid receptors, with agonist potency (ED₅₀ (95% confidence intervals)) varying from 0.1 (0.03-0.35) to 7.0 (1.0-47.4) nmol compared to 1.7 (0.6-4.8) nmol for the parent peptide. The two analogs in which one of the phenylalanine residues was replaced by alanine exhibited both potent agonist activity and KOR antagonist activity *in vivo*. These peptides represent novel lead compounds for the development of peptide-based opioid analgesics. Research supported by grants R01 DA018832 and R01 DA023924.

Using an operant orofacial assay to measure the analgesic effects of morphine and the hyperalgesic effects of withdrawal

E. M. Anderson (2) and R. M. Caudle (1,2). (1) Department of Oral Surgery, University of Florida, Gainesville, FL, USA (2) Department of Neuroscience University of Florida College of Medicine, Gainesville, FL, USA

A divergence exists between methods of measuring the effects of opioids in animals and in humans. In rodents, morphine analgesia and tolerance are generally measured with reflex-based procedures. Humans on the other hand are simply asked how much pain they feel. We present a procedure that allows an animal to report how opioids affect its pain. This is a reward/conflict assay which forces an animal to endure an aversive stimulus in order to receive a reward. A rat's adjustment of its behavior in this assay gives us a measure of their response to opioids.

Fasted hairless rats were trained to press their faces into two heated aluminum tubes in order to be able to receive a reward of diluted sweetened condensed milk. Sensors were attached to the tubes and feeding bottle so that each time a rat made contact with either a recording was made. After training at a non-aversive 37°C the temperature was changed to an aversive 46°C and rats were tested for a baseline reading. Rats were then injected twice daily with 10mg/kg of morphine and tested every 2-3 days at 46°C. After 10 days injections were ceased and rats

were tested through withdrawal. Data was analyzed by taking the number of licks and dividing it by the amount of facial contact time. Aversive temperatures of 46°C caused the ratio of licks per contact to decrease from the values taken at 37°C. Morphine reversed this effect and caused the ratio to increase significantly, demonstrating analgesia. This ratio decreased over the 10 days of injections due to tolerance. The ratio then dropped below baseline during withdrawal, demonstrating hyperalgesia. This assay may allow a more accurate measure of the effects of opioids in rodents than reflexive measures of pain as the animal is essentially reporting to us the amount of pain it can withstand. Funding for this project was provided by the NIDA. DA030044

Acute Tolerance to Etorphine and Morphine Dependence in MOPr Phosphorylation Deficient Mice

E. Barbier, J.B. Wang. Dept. of Pharmaceutical Sciences, Univ. of Maryland-Sch. of Pharmacy, Baltimore, USA.

Mu opioid receptor (MOPr) phosphorylation is a key event in the receptor internalization and desensitization *in vitro*, which underlie the development of tolerance and dependence induced by opioid treatment *in vivo*. Using the MOPr – T394A phosphorylation deficient (PD) knockin mice we previously reported that compared with their wild type (WT) littermate controls the MOPr-PD mice did not develop tolerance to the analgesic effect of acute morphine treatment, this was paralleled with reduced etorphine-induced internalization at the spinal cord level. In the present study we assessed the contribution of the T394 phosphorylation site in the development of acute tolerance to etorphine analgesia and in the development of naloxone-precipitated morphine withdrawal. We describe the development of acute tolerance to etorphine analgesia in WT mice submitted to the hot plate test in a paradigm of two administrations of etorphine separated by a three hours interval. Under the same conditions MOPr-PD mice did not display any tolerance. The preliminary study of cumulative dose-response effect of naloxone in chronically morphine treated mice revealed a higher frequency of physical signs of withdrawal in MOPr-PD mice compared with their WT littermates. These behavioral studies suggest that phosphorylation of the MOPr at the T394 site is a crucial mechanism in the development of acute tolerance to the analgesic effect of opioid and a key point in the development of morphine dependence. Supported by NIH grant DA011925 to JBW.

Activation of spinal Mu and Delta opioid receptors potently inhibits substance P release induced by peripheral noxious stimuli

H. Beaudry, D. Dubois and L. Gendron, Université de Sherbrooke, Canada

Over the past few years, delta (DOPR) and mu (MOPR) opioid receptors were shown to interact with each other. We have previously observed that expression of MOPR was essential for morphine and inflammation to potentiate the analgesic properties of selective DOPR agonists. *In vivo*, it is not clear if MOPR and DOPR are expressed in the same neurons. Indeed, it was recently proposed that these receptors are segregated in different populations of nociceptors, with MOPR and DOPR being respectively expressed by peptidergic and non-peptidergic fibers. In the present study, the role and the effects of DOPR and MOPR selective agonists in two different pain models were compared. Using PPTA^{-/-} mice, we first confirmed that substance P partly mediates intraplantar formalin- and capsaicin-induced pain behaviors. In these mice, we found a significant reduction in pain behaviors when compared to PPTA^{+/+} mice. We then measured the effects of intrathecal deltorphin II (DOPR agonist) and DAMGO (MOPR agonist) on pain-like behaviors, neuronal activation and substance P release following formalin and capsaicin injection. We found that both agonists were able to decrease formalin- and capsaicin-induced pain, an effect that was correlated with a reduction in the number of *c-fos* positive neurons in the superficial laminae of the lumbar spinal cord. Finally, visualization of NK1 internalization revealed that DOPR and MOPR activation strongly reduced formalin- and capsaicin-induced substance P release *via* a direct action on primary afferent fibers. Taken together, our results indicate that functional MOPR and DOPR are both expressed by peptidergic nociceptors. Supported by CIHR, NSERC and FRSQ

The superoxide-generating enzyme NADPH oxidase is required for the normal expression of opioid addictive behaviors

M. A. Beckerman (1), M. J. Glass (1, 2), (1) Department of Neurology and Neuroscience, and (2) Graduate Program in Neuroscience, Weill Cornell Medical College, New York, NY 10065, USA

Identifying novel signaling pathways that contribute to the development and persistence of drug-related neural plasticity is critical for elucidating the mechanisms of opioid addiction. Although reactive oxygen species (ROS) like superoxide have traditionally been viewed as deleterious byproducts of cellular metabolism, they have more recently been established to be important signaling molecules generated by specific and highly regulated enzymes in a variety of cell-types. There is also emerging

evidence that ROS play important roles in neural processes critical to addiction, including the modulation of dopamine, glutamate, and G-protein-coupled receptor signaling, in addition to synaptic plasticity, learning, and memory. However, there is little direct evidence that specific ROS-generating enzymes are involved in opioid addiction. We provide evidence that the superoxide-producing enzyme NADPH oxidase (NOX) plays a role in the normal expression of opioid addictive behaviors. Mice with a constitutive knockout of the catalytic Nox2 NOX subunit show alterations in opioid dependence and reward behaviors, as well as patterns of neural activity associated with opioid use. These findings demonstrate that a specific ROS-producing enzyme plays a critical role in opioid addictive behaviors; this information may enhance our understanding of opioid addiction by identifying novel free radical-mediated intracellular signaling pathways involved in opioid plasticity and provide new targets for the development of future addiction treatments. Supported by: DA-016735, DA-024030 (MJG)

Kinetics of fluorescent opioid ligand binding to the mu opioid receptor

W. Birdsong (1), S. Arttamangkul (1), K. Rice (2), J. Williams (1), (1) Vollum Institute, Oregon Health & Science University, Portland, OR, USA, (2) National Institute on Drug Abuse, Bethesda, MD, USA

In intact cells, opioid receptor function is often inferred from the activity of downstream effectors. Fluorescent ligands provide a tool for examining opioid receptor function at the receptor level directly. Here we use confocal microscopy and rapid solution exchange to characterize the binding kinetics of a previously described opioid peptide as well as novel fluorescent derivatives of an alkaloid agonist and antagonist. We have found that fluorescent conjugates of the opioid peptide dermorphin bind to FLAG tagged mu opioid receptors in a specific and reversible manner with affinity ranging from 20 to 100 nM in intact cells under physiological conditions. The affinity of dermorphin conjugated to alexa488 measured using kinetic binding in intact cells is at least 10 fold lower than that previously measured using competition radioligand binding assays of solubilized membranes. Fluorescent conjugates of the alkaloid agonist oxymorphone display variable affinity and potency depending on the identity of the attached fluorophore. Fluorescent naltrexamine maintains antagonist activity and binds with an affinity of approximately 50nM. Interestingly, naltrexamine derivatives unbind with a time constant of 1-3 minutes while dermorphin and oxymorphone unbind much more rapidly with time constants ranging from 5-30 seconds. Interestingly, the rate of unbinding of all ligands was dependent on

the length of agonist application suggesting that binding is not a simple first order process. In summary, we present data examining the kinetics of binding of functional fluorescent derivatives of an opioid peptide— dermorphin, a morphine like agonist— oxymorphone, and an antagonist— naltrexamine. Supported by DA08163, DA026617 (JTW) and DA007262-18 (WTB)

***Csnk1e* is a genetic regulator of sensitivity to psychostimulants and opioids**

C. D. Bryant¹, . Zhou^{3,4}, C. Olker^{3,4}, M. H. Vitaterna (3, 4), F. W. Turek^{3,4}, and A. A. Palmer^{1,2},¹Department of Human Genetics, University of Chicago, Chicago, IL USA, ²Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL USA, ³Center for Sleep and Circadian Biology, Northwestern University, Evanston, IL USA, ⁴Department of Neurobiology and Physiology, Northwestern University, Evanston, IL USA

Recent evidence suggests that *Csnk1e*, the gene encoding casein kinase 1-epsilon, modulates sensitivity to amphetamines in mice and humans. Additionally, a *CSNK1E* genetic variant is associated with heroin addiction, suggesting that it may also regulate opioid sensitivity. In this study, we used both a forward and reverse genetics strategy to test the hypothesis that *Csnk1e* is a genetic regulator of sensitivity to psychostimulants and opioids. Reciprocal congenic lines of C57BL/6J (B6) and DBA/2J (D2) origin capturing *Csnk1e* were phenotyped for methamphetamine (MA) and opioid sensitivity. We also tested the phenotypic consequence of a *Csnk1e* null or *tau* mutation. B6.D2^{*Csnk1e*} mice carrying a 4.63 cM introgressed region of D2 origin (78-86.8 Mb) on a B6 background showed an increase in MA sensitivity whereas D2.B6^{*Csnk1e*} mice carrying a 0.55 cM introgressed region of B6 origin (78.7-81.6 Mb; *Csnk1e* = 79.2 Mb) on a D2 background showed a decrease. Interestingly, B6.D2^{*Csnk1e*} mice were also more sensitive to the locomotor stimulant of fentanyl. Mice harboring a null *Csnk1e* mutation showed an increase in MA sensitivity whereas mice harboring the *tau* mutation showed a decrease in MA sensitivity. Mirroring the knockout results, the new selective *Csnk1e* inhibitor PF-4800567 increased the locomotor response to both MA and fentanyl. These results provide selective genetic and pharmacological evidence that *Csnk1e* regulates sensitivity to two distinct classes of abused drugs. We are currently testing the effect of PF-4800567 in *Csnk1e* knockout mice in order to demonstrate pharmacological specificity and examining *Csnk1e* gene expression differences in the congenic lines. FUNDING:5R01DA021336,2T32DA007255, 1F32DA026697, 1K99DA029635-01

Interactions between cortical cannabinoid and opioid receptors during neuropathic pain

I. Bushlin, A. Gupta, L. K. Miller, S. D. Stockton Jr., and L. A. Devi, Dept. of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, New York, USA

The expression and function of opioid and cannabinoid receptors are altered during neuropathic pain. Most studies thus far have examined changes in these receptors in peripheral sensory neurons (primary afferents and DRGs) and in spinal cord, as peripheral sensory signals are initiated through these circuits. However, neuropathic pain is also associated with neuroplastic changes in supraspinal brain regions, leading to enhanced anxiety, altered impulse control, and activation of descending analgesia. Exogenous activation of supraspinal opioid and cannabinoid receptors is known to lead to reduced anxiety and antinociception, however alterations in the expression and function of these receptors during a neuropathic pain state have not been well explored. We examined changes in the expression, function and interaction of these receptors in the cerebral cortex of rats experiencing neuropathic pain. We find that the expression of cannabinoid type 1 receptor (CB₁R) and delta opioid receptor (DOR) are increased in the cortex of lesioned animals; however, while CB₁R activity is increased, DOR activity is decreased. We hypothesized that this decrease could be due to interactions between these two receptors; this is based on previous *in vitro* experiments that had shown inhibitory interactions between CB₁R and DOR. We tested this by examining allosteric modulation of DOR activity by CB₁R ligands and determining if alterations in DOR activity could be blocked by a CB₁R-DOR heteromer-specific antibody. We find that in cortical membranes from neuropathic animals, low, non-signaling doses of CB₁R ligands significantly enhance DOR activity and this is selectively blocked by the heteromer-specific antibody. Together, these studies support a role for CB₁R-DOR heteromers in altered cortical function of DOR during neuropathic pain. Supported by NIH grants DA08863 and DA19521 to L.A.D.

***In vivo* modulation the behavioral effects of the kappa-opioid hallucinogen salvinorin A by p-glycoprotein ligands**

E.R. Butelman¹, S. Rus¹, K. Lovell², T.E. Prisinzano² and M.J. Kreek¹. ¹The Rockefeller University; New York NY USA, ²Dept. of Medicinal Chemistry, University of Kansas, Lawrence KS USA.

Salvinorin A is a kappa-agonist hallucinogen from the plant *Salvia divinorum*. Salvinorin A – based products are widely available in shops and on the internet. While there is little population-based data,

there appear to be variable responses to self-administration of salvinorin A-based products in humans. A recent *in vitro* study reported that salvinorin A was a potential substrate for the multi-drug resistance blood-brain barrier efflux transporter, p-glycoprotein (Teksin et al., 2009; Eur J Pharm Biopharm 72:471-477). It may thus be hypothesized that the functional status of the p-glycoprotein transporter influences the effects of salvinorin A *in vivo*. Unconditioned behavioral effects (ptosis and facial relaxation) of i.v. salvinorin A (0.01 mg/kg) were studied in rhesus monkeys (n=4). Salvinorin A alone (0.01 mg/kg; n=4) produced moderate, time dependent, ptosis (eye closure) and facial relaxation, similar to other centrally penetrating kappa-opioid agonists. Pretreatment with the clinically available p-glycoprotein substrate loperamide, which is a peripherally-selective mu-opioid agonist (0.032-0.32 mg/kg, i.v. 5 min pretreatment), resulted in a dose- and time-dependent enhancement in the effects of salvinorin A on ptosis, but not on facial relaxation. In a second experiment, the p-glycoprotein blocker tariquidar (0.32-3.2 mg/kg; 30 min pretreatment) also enhanced the effects of salvinorin A on ptosis, but less so on facial relaxation. Overall, these studies are consistent with the hypothesis that centrally mediated effects of salvinorin A are modulated by functional status of the p-glycoprotein transporter. These studies suggest that variability in the incidence and severity of salvinorin A effects may thus differ in individuals due to pre-existing (e.g., genetic) variation in p-glycoprotein function, or due to concurrent medications. We gratefully acknowledge funding by NIH-NIDA grants DA017369 (ERB), DA018151, and DA05130 (MJK).

Morphine-induced hyperalgesia is associated with AMPAR trafficking in the dorsal horn of the spinal cord

D. Cabañero¹, Y. Xia¹, A. Baker², S. Zhou², S. M. Carlton², J. Morón-Concepción¹. ¹Anesthesiol. Dept., Columbia Univ. Med. Center, New York, USA, ²Dept. of Neurosci. and Cell Bio., UTMB, Galveston, USA

Repeated morphine administration promotes the insertion of calcium permeable, GluR2-lacking AMPAR in hippocampal synapses (Billa SK et al, 2010). We find that the same treatment elicits cold and mechanical sensitivity (lasting 12 hrs and one week, respectively), which could be mediated by an increase in GluR2-lacking AMPAR in the spinal cord. In this work, we examine the effects of morphine on AMPAR subunit expression in the dorsal horns of the spinal cord. C57BL6 mice received saline or four escalating doses of morphine (5, 8, 10 and 15 mg/kg i.p.) administered at 12 hrs intervals, and were sacrificed 12 hrs or one week after the last injection. Using western blotting,

expression levels of GluR1/2/3/4 and phosphorylation levels of GluR1/2/4 were determined in the homogenate (H) or in a fraction enriched in the postsynaptic density (PSD) from spinal cord dorsal horns. In order to examine changes in AMPAR subunit composition, GluR4 was co-immunoprecipitated (co-IP) with GluR2 and GluR3 in PSD samples. Twelve hours after morphine, GluR4, pGluR1 and pGluR2 levels significantly increased in the H; GluR4 and pGluR4 levels were also increased at the PSD. One week after treatment GluR4 and pGluR4 returned to normal levels, but pGluR1 and pGluR2 were still increased in the H of morphine-treated mice. The co-IP study showed a significant decrease in the proportion of GluR4-GluR2 heteromers 12 hrs after morphine. Our results suggest that morphine exposure induces a postsynaptic increase in GluR4 homomers, which could trigger an early enhancement in the excitability of projection neurons of the dorsal horn. Interestingly, cold and mechanical sensitivity correlate respectively with the increases in GluR4 and GluR1/2 phosphorylation. This is the first report describing morphine-induced changes in AMPAR trafficking at the spinal cord dorsal horn, a mechanism that could participate in morphine-induced hyperalgesia.

Supported by NIH DA027460 to JMC

The effect of mastitis and milk congestion on the levels of beta-casomorphin-8 in milk and plasma samples from puerperal women

A. Carlsson (1), L. Righard (2), F. Nyberg (1), (1)Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, (2)Department of Paediatrics, University hospital, Malmö, Lund University, Lund, Sweden

In addition to the classical opioids, which are mainly produced in the CNS, the so-called atypical opioid peptides have been identified and characterized. One of these is β -casomorphin, derived by partial hydrolysis of the milk protein β -casein. β -casomorphin was originally detected in bovine milk, and in subsequent studies β -casomorphin-8 (H-Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro-OH) – like immunoreactivity has been measured in human plasma, CSF and milk. Atypical opioid peptides share many effects typical for the classical opioids. In addition, the β -casomorphins have been suggested to be involved in the interplay between mother and child during lactation but also in the etiology of some psychiatric diseases. Studies have implicated increased levels of β -casomorphin-like peptides in postpartum psychosis, a rare but serious condition following birth. It has also been postulated that an underlying reason for postpartum psychosis is milk congestion. In this study we examined whether milk congestion may induce increased levels of β -

casomorphin-8 in milk and in plasma from puerperal women. Milk and blood samples from fourteen women with mastitis were collected during the acute phase and after 2-3 weeks, when the symptoms had disappeared. Samples from ten women without problems served as controls. The samples were purified and analyzed for β -casomorphin-8-immunoreactivity using radioimmunoassay. The results demonstrate a significant increase in β -casomorphin-8-like immunoreactivity in milk samples from women with mastitis in the acute phase compared to controls. After recovery from the mastitis, the β -casomorphin-8-like immunoreactivity was restored to levels of control subjects. No significant difference, although a tendency, was seen in the plasma samples. This result suggests that β -casomorphin may be of importance in the development of mastitis. This study was supported by the Swedish Medical Research Council (Grant 9459).

Antinociceptive effects of NOP receptor agonists, nociceptin, Ro 64-6198 and (+)-5a Compound, given by intra-periaqueductal gray injection

L.-C. Chiou (1, 2, 3, 4), H.-J. Lee (1, 3) and Y.-Y. Liao (2). (1) Dept. Pharmacology, Coll. Medicine, (2) Grad. Inst. Pharmacology and (3) Zoology, National Taiwan University, Taipei, Taiwan

We previously showed that the functional heterogeneity of nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptors in ventrolateral periaqueductal gray (vlPAG) slices can be revealed by two non-peptide agonists of NOP receptors, Ro 64-6198 (Ro) (Chiou et al., J. Pharmacol. Exp. Ther. 2004) and (+)-5a Compound (5a) (Liao et al., Int. J. Neuropsychopharmacol. 2010). Both compounds activated K⁺ channels via one, and the same, subset of NOP receptors in vlPAG neurons while N/OFQ was effective in those Ro/5a-insensitive NOP receptors. Interestingly, most of 5a-sensitive neurons are GABAergic. The vlPAG is enriched with intrinsic GABAergic tone and inhibition of this tone via K⁺ channel activation can lead to analgesia. We, therefore, examined if Ro and 5a given by intra-vlPAG (*i.pag.*) injection would be antinociceptive in the mouse hot-plate test. Indeed, *i.pag.* injection of 5a (30-100 nmol) and Ro (30 nmol) increased paw withdrawal latency in the hot-plate test. These antinociceptive effects of 5a and Ro were markedly antagonized by *i.pag.* UFP-101 (10 nmol), a NOP receptor antagonist, but was also partially reversed by *i.pag.* naloxone (5 nmol). Surprisingly, *i.pag.* N/OFQ (1 nmol) was also antinociceptive. Its antinociceptive effect was effectively blocked by *i.pag.* UFP-101, and also partially reversed by *i.pag.* naloxone. UFP-101 and naloxone had not effect *per se*. Biphasic effects of *i.c.v.* N/OFQ have been reported in regulating pain, motor activity and learning memory. However, *i.pag.* N/OFQ only produced

antinociceptive, but no pronociceptive, effect at the dose ranged from 0.01 to 3 nmol. These results suggest that activation of NOP receptors in the vlPAG, instead of hyperalgesia, results in analgesia via a mechanism partly mediated by endogenous opioids. (Supported by grants NHRI-EX99-9506NI, NSC-98-2320-B-002-011-MY3, NSC-98-2323-B002-012 and NTU-99R81855).

Prolonged stimulation of μ -opioid receptors in locus coeruleus neurons induces β -arrestin-2-dependent heterologous desensitization of α_2 -adrenoceptors

M.J. Christie(1), B.Chieng(1), V.C. Dang(2), (1)Brain & Mind Research Inst. U. Sydney Australia. (2) Dept Psychiat UCSF, CA 94158, USA

Profound desensitization of μ -receptors (MOR) develops during exposure of locus coeruleus (LC) neurons to high concentrations of met-enkephalin or DAMGO for up to 5 min. At this time the efficacy of coupling of other GPCRs such as the α_2 -adrenoceptor (α_2 -AR) to activation of GIRK is little affected, ie. MOR desensitization is largely homologous. More prolonged activation of MOR produced greater heterologous desensitization of α_2 -ARs in mouse LC. Heterologous desensitization also reversed more slowly than homologous desensitization. Although homologous desensitization of MOR persisted in LC neurons from β -arrestin-2 (β arr2) k.o. mice (Dang et al. 2009, J Neurosci. 29:3322-7), heterologous desensitization of α_2 -AR was ablated. In wild type mice, heterologous desensitization of the α_2 -AR was blocked by intracellular application of GRK2 but not dynamin inhibitors, suggesting that β arr2 binding to MOR is required but endocytosis is not necessary. Heterologous desensitization was also blocked in wild types by ERK (UO126) and cSRC (PP2) inhibition, suggesting a β arr2-ERK1/2-cSRC-mediated mechanism. This mechanism may be physiologically significant because the adrenergic inhibitory post-synaptic current in LC neurons was also heterologously depressed after met-enkephalin exposure in wild type but not β arr2 knockout mice. Together, these findings demonstrate a novel mechanism by which β arr-2 regulates neuronal responsiveness to endogenous neurotransmitter release after exposure to high concentrations of opioids that mobilize β arr-2. Supported by the National Health & Medical Research Council of Australia

ALKS 33, a novel opioid receptor modulator, attenuates cocaine-induced increases in extracellular DA concentrations and cocaine self-administration in rats

J.I. Cunningham¹, M.S. Todtenkopf¹, R.L. Dean¹, M.R. Azar², G.Koob³, D.R. Deayer¹, D.J. Eyerman¹,
¹Alkermes, Inc., Waltham, MA, ²Behavioral Pharma, Inc., La Jolla, CA, ³The Scripps Research Institute, La Jolla, CA, USA

Cocaine increases extracellular concentrations of dopamine (DA_{ext}) in the nucleus accumbens shell (NAc-sh). In addition, opioid receptor agonists and antagonists have been found to alter cellular and behavioral responses to cocaine. We had two objectives: 1) to compare the ability of ALKS 33, a mu receptor antagonist with partial agonist/antagonist activity at delta and kappa receptors, and naltrexone (NTX) to inhibit cocaine-induced elevations in NAc-sh DA_{ext} when given by the subcutaneous (SC) and oral (PO) routes; and 2) to determine if ALKS 33 would inhibit cocaine self-administration in a fixed ratio 1 (FR1) or progressive ratio (PR) schedule of reinforcement. In the first experiment, *in-vivo* microdialysis was performed in rats (n=5-6 per group) with probes inserted into the NAc-sh. Dialysate samples were collected during baseline and for 3 hrs post-drug administration. Cocaine (5 mg/kg, IP) caused an approx. 300-350% increase from baseline in NAc-sh DA_{ext}. SC administration (1 mg/kg) of NTX 30 minutes prior to cocaine significantly (P<0.05) attenuated increases in DA_{ext}, but no reduction was observed when NTX was given orally (10 mg/kg). Importantly, regardless of the route of administration, ALKS 33 significantly (P<0.05) attenuated cocaine-induced increases in NAc-sh DA_{ext} (1 mg/kg, SC or 10 mg/kg, PO). In the second experiment, treatment with ALKS 33 (1 mg/kg, SC) did not significantly alter self-administration in the FR1 paradigm in rats (n=9-10 per group) trained to self-administer cocaine (0.5 mg/kg/inf, IV). However in the PR paradigm, ALKS 33 had a marked effect on cocaine self-administration; rats in the control group reached a break point of 62, whereas in rats treated with ALKS 33 the break point was 18 (p<0.005). The combined neurochemistry and self-administration results suggest that ALKS 33 might be an effective treatment for cocaine dependency. All work funded by Alkermes, Inc.

Using the transitive inference task to study the relational memory deficits associated with withdrawal from chronic nicotine in the C57BL/6 mouse

K.A. Cordero, R. C. Cassells, T. J. Gould, Dept. of Psychology, Neuroscience Program, Temple University, Philadelphia, PA, USA

Nicotine and opioid abuse show high comorbidity. This is the first in a series of experiments to investigate this relationship. The present study explored the effects of withdrawal from chronic nicotine on relational learning and memory by utilizing the transitive inference task (TI). TI is a form of relational memory characterized by the ability to infer that B is more likely to be rewarded than D after directly learning the following hierarchy: (A>B), (B>C), (C>D), and (D>E). Preference is also calculated for a novel control pair (A vs. E) which does not entail inference because (A) is always rewarded and (E) is never rewarded. During chronic administration of nicotine or saline, administered via osmotic minipumps implanted subcutaneously (SC), mice were tested on days 14 and 15 for their initial preference for the novel TI pair (B vs. D) and the novel control pair (A vs. E). Preliminary data indicate C57L/6 mice exhibit an enhanced preference for B over D during chronic nicotine administration (12mg/kg/day). To determine the effect of withdrawal from chronic nicotine on this task osmotic minipumps were removed to terminate nicotine treatment and the same animals were tested again at 24 and 48 hours for TI pair preference (B vs. D) and the control pair (A vs. E). Preliminary data indicate withdrawal produces cognitive deficits as exemplified by a decreased preference for the TI pair (B vs. D), but not the control pair (A vs. E). This study is the first to demonstrate that withdrawal from chronic nicotine impairs relational memory, whereas chronic nicotine enhances inferential memory in the same animal. Follow up experiments involving transitivity in mice can further explore the relationship between nicotine and opioid dependence. It has previously been demonstrated that smoking rates are positively correlated with heroin abuse. These findings could help in developing better substance abuse treatment programs for individuals suffering from multiple addictions. Supported by NIDA Grants DA024687 and DA017949

Investigation on DNA methylation status of opioid peptides promoters in PBMCs of subjects with bipolar disorder

C. D'Addario^{1,4}, M. Di Benedetto¹, B. Dell'Osso², S. Bastias Candia¹, F. Cortini³, D. Galimberti³, E. Scarpini³, S. Candeletti¹, M. Maccarrone⁴, A.C. Altamura² and P. Romualdi¹, ¹Dept of Pharmacology, University of Bologna, Bologna, ²Dept of Psychiatry, University of Milan and ³Dept of Neurological Sciences, University of Milan, Milano, ⁴Dept of Biomedical Sciences, University of Teramo, Teramo, Italy

The pathophysiology of Bipolar Disorder (BD) has not been clearly established. Many evidences support the hypothesis, in addition to dopamine and serotonin, of a role for the endogenous opioid peptides, in particular dynorphin and nociceptin, whose levels have already been found to be affected in psychiatric illness. It has been already proposed that altered expression of multiple mRNAs in psychotic subjects may be due to epigenetic mechanisms, thus we investigated dysregulation of DNA methylation in peripheral blood samples of subjects with BD. DNA was isolated from PBMCs of patients diagnosed with BD either type I or II (according to DSM-IV criteria), and from healthy control. Peripheral blood samples are easily accessible and an useful peripheral marker and model of epigenetic gene regulation in the brain. Following bisulfite conversion of DNA samples, Real-Time Methylation Specific PCR was used for the quantification of the methylated promoters. The percentage of methylation was calculated by the 2^{-DDC_t} method, where $DDC_t = (C_{t,Target} - C_{t,Myod})_{sample} - (C_{t,Target} - C_{t,Myod})_{fully\ methylated\ DNA}$ and multiplying by 100 where Myod is the internal reference gene to control for input DNA. A selective increase in DNA methylation of dynorphin promoter region was observed in BD II patients (23 %; n = 26, p<0,05), but not in BD I (15 %; n = 35) compared to controls (16 %; n = 32). No significant differences were found in DNA methylation status of nociceptin promoter of both BD I and BD II subjects. Our preliminary findings, showing selective changes in dynorphin regulation by epigenetic mechanisms, provide new insight in the possible involvement of dynorphin in mediating susceptibility to neuropsychiatric diseases. Grants from PRIN (SC) and RFO (PR).

Agonist-selective patterns of mu-opioid receptor phosphorylation revealed by phosphosite-specific antibodies

C. Doll(1), J. Konietzko(2), F. Pöll(1), T. Koch(2), V. Höllt(2), S. Schulz(1) (1)Institute of Pharmacology and Toxicology, University Hospital, Friedrich Schiller University Jena, Germany (2)Institute of

Pharmacology and Toxicology, University Hospital, Otto-von-Guericke-University Magdeburg, Germany Morphine activates the mu-opioid receptor without causing its rapid endocytosis. In contrast, full agonists such as [D-Ala²-MePhe⁴-Gly-ol]enkephalin (DAMGO) or etonitazene stimulate a rapid and profound internalization. However, the detailed molecular events underlying the differential regulation of receptor trafficking by distinct opioid agonists remain incompletely understood. Here, we have generated phosphosite-specific antibodies for the carboxyl-terminal residues serine 363 (S363), threonine 370 (T370) and serine 375 (S375) which enabled us to selectively detect either the S363-, the T370- or the S375-phosphorylated form of the receptor. We show that agonist-induced phosphorylation occurs at T370 and S375, whereas S363 is constitutively phosphorylated in the absence of agonist. We further demonstrate that DAMGO and etonitazene stimulated the phosphorylation of both T370 and S375. In contrast, morphine promoted the phosphorylation of S375 but failed to stimulate T370 phosphorylation. In the presence of DAMGO, S375 phosphorylation occurred at a faster rate than phosphorylation of T370 indicating that S375 is the primary site of agonist-dependent phosphorylation. Activation of PKC by PMA increased receptor phosphorylation only on T370 but not on S375 indicating that T370 can also undergo heterologous PKC-mediated phosphorylation. We also show that mu receptor dephosphorylation can occur within minutes at or near the plasma membrane, and that agonist removal is a major prerequisite for T370 and S375 dephosphorylation. Together, we show for the first time that distinct agonists stimulate site-specific patterns of phosphorylation, which are intimately related to their ability to elicit mu-opioid receptor sequestration. This study was supported by the Deutsche Forschungsgemeinschaft.

Orphanin FQ/Nociceptin activates Oct-2 in SH-SY5Y human neuroblastoma cells

C. L. Donica (1) and K. M. Standifer (2). (1) OK Center for Neuroscience, (2) Dept of Pharmaceutical Sciences, OUHSC, OKC, OK, USA

A natural reward pathway exists in the brain that provides an incentive to obtain different factors needed for survival, including food, water and reproduction. Activation of this pathway leads to increased dopamine transmission and positive reinforcement of these behaviors. Several abused substances, such as cocaine, ethanol, heroin and morphine hijack this pathway to produce positive reinforcement. These abused substances function, in part, by increasing tyrosine hydroxylase (TH) expression. TH is the rate-limiting enzyme in dopamine synthesis. Endogenous neuropeptide orphanin FQ/nociceptin (OFQ/N) modulates the

effects of abused substances, including a reduction in ethanol consumption as well as inhibition of alcohol-seeking behavior and inhibition of the rewarding properties of cocaine, yet it does not affect heroin-seeking behavior. We previously reported that OFQ/N inhibits chronic morphine-induced TH expression, with a simultaneous up-regulation of the transcription factor, Oct-2. Studies have shown that an Oct-2 binding site is located upstream of the TH promoter. To determine if OFQ/N modulates morphine-induced TH expression through Oct-2, SH-SY5Y human neuroblastoma cells were treated with OFQ/N in the presence and absence of morphine and assessed for changes in Oct-2 protein expression, nuclear accumulation and DNA binding. SiRNA is currently being used to confirm the effect of Oct-2 in this process. For the first time, we show that OFQ/N increases the protein expression, nuclear accumulation and the DNA binding of Oct-2 in a time-dependent manner, as determined by immunoblotting and electromobility shift assay. These studies are consistent with the hypothesis that OFQ/N inhibits morphine-induced TH expression in an Oct-2-dependent manner and will help elucidate the cellular mechanism(s) by which OFQ/N modulates the reward pathway. These studies were supported by DA017380 and OCAST HR08-152.

Correlating MOR ligand induced receptor internalization with acute antinociceptive tolerance

C. Dooley, J. Mislér, L. Li, K. Reilley, S. Eans and J. McLaughlin. Torrey Pines Institute for Molecular Studies, Port St. Lucie, Florida, USA

While many studies have been carried out *in vitro* on the relative propensity of MOR ligands to internalize, fewer have been carried out comparing internalization efficacy with induction of antinociceptive tolerance. To test the hypothesis that the inverse relationship between efficacy at internalization *in vitro* and ability to develop acute antinociceptive tolerance *in vivo* holds for diverse opioid structures (1), we examined three compounds with strong and three with poor MOR internalization efficacies in a mouse model of acute antinociceptive tolerance. We chose an acute model of opioid antinociceptive tolerance (2) as a starting point as it is less likely to be confounded by competing pathways and utilizes less material. Internalization efficacy was measured as fold increase in average particle number internalized (P.I.) using GFP-tagged mu-opioid receptors stably expressed in HEK293 cells. Antinociceptive tolerance was assessed by comparing dose response curves and ED50 values of tested compounds administered i.c.v with a second ED50 value generated from doses administered 8 hours later. Compounds with high efficacy for internalization did not produce the rightward shifts

observed for the compounds lacking ability to internalize receptors. In conclusion the inverse correlation of efficacy at internalization and induction of tolerance *in vivo* seems a valid predictor of agonist-induced acute tolerance in this model.

	Fold increase in internalization	ED ₅₀ (nmol)	95% C.I.	ED ₅₀ (nmol)	95% C.I.
DAMGO	3.4	1.4	(0.69-3.30)	4.29	(2.02-9.30)
YmFG-NH ₂	2.7	3.7	(1.43-10.4)	1.76	(0.87-3.56)
TPIMS6-32-4	2.9	0.2	(0.17-0.49)	0.68	(0.36-1.30)
Morphine	1.1	2.3	(1.13-5.03)	22.5	(8.48-61.9)
Ac-rfwrink-NH ₂	1.4	3.4	(1.85-6.56)	15.7	(8.25-30.0)
Herkinorin	1.0	5.4	(3.07-9.83)	23.6	(13.9-39.0)

1. Dooley CT, Houghten RA. Biopolymers. 1999;51(6):379-90. PMID:10797228
2. Mathews JL, Smrcka AV, Bidlack JM. J Neurosci. 2008 Nov 19;28(47):12183-9. PMID:19020012

Effects of A118G polymorphism and personality factors on HPA-axis response to metyrapone in normal volunteers

E. Ducat, B. Ray, M. Randesi, A. Ho and M.J.K. Kreek, Laboratory of the Biology of Addictive Disease, The Rockefeller University, New York, NY USA

We recently reported on our finding that the 118G variant of the mu-opioid receptor blunts the HPA-axis ACTH stress response to metyrapone challenge (Ducat et al, in press, Addiction Biology). This current study investigates the relationship between personality traits and stress hormone response differences. Healthy, normal subjects were recruited in our ongoing genetics studies at Rockefeller University. Subjects received medical and psychiatric evaluations during outpatient clinic visits. A subset of the subjects reported above completed the NEO-PI-R, a 240-item measure of the five factors of personality, the evening of admission to the Rockefeller University Hospital inpatient unit. On the test day, blood was sampled prior to administration of a standard dose of oral metyrapone,

2.25gm, up to 8 hrs afterward; plasma levels of ACTH and cortisol were assayed. Three time points were used in analyses of the ACTH response to metyrapone: time 0, 4hr and 8 hrs after administration. The data from 33 subjects who completed both the NEO-PI-R and metyrapone testing were analyzed; 22 with 118A genotype and 11 with 118G genotype. Subjects with the 118G genotype were found to score significantly higher ($p < 0.05$) on the Agreeableness factor of the NEO-PI-R than subjects with the 118A prototype. While we found no significant correlation between A118G genotype and the factor of neuroticism, subjects with the 118G variant showed an inverse relationship of Neuroticism score with ACTH plasma levels 8 hrs following metyrapone stress challenge ($r = 0.617$, $p < 0.05$). This was not seen in subjects with the 118A prototype. This result provides preliminary evidence that discrete personality factors such as neuroticism, may interact with specific genetic variants to affect HPA-axis stress responsivity. NIH-NIDA P60-DA005130 (M.J.K.), RR ULIRR024143 (B.C.) and The Adelson Medical Research Foundation.

Oral activity of cyclic tetrapeptide JVA-2802: short-acting KOR antagonism and prevention of stress-induced reinstatement of cocaine-CPP

S.O. Eans (1), M.L. Ganno (1), J.V. Aldrich (2), J.P. McLaughlin (1), (1)Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL; (2)Dept. of Med. Chem., Univ. of Kansas, Lawrence, KS

Cyclic peptides are resistant to proteolytic cleavage, potentially preserving activity after systemic administration. When administered centrally, by the intracerebroventricular (i.c.v.) route, the cyclic tetrapeptide JVA-2802 produced a dose-dependent, KOR-selective antagonism that lasted less than 18 h. We hypothesized that the cyclic structure of JVA-2802 would reduce proteolytic cleavage, facilitating retention of KOR antagonist activity after systemic administration by the subcutaneous (s.c.) or *per os* (p.o., or oral) routes. Pretreatment with JVA-2802 by either route (1-10 mg/kg, s.c. or p.o.) dose-dependently antagonized the antinociception induced by the selective KOR agonist U50,488 (10 mg/kg, i.p.) in C57Bl/6J mice tested in the 55 degC warm water tail withdrawal assay. KOR antagonism lasted less than 6 and 18 h after p.o. or s.c. administration, respectively, of a maximally-effective (10 mg/kg) dose. Furthermore, mice pretreated orally for 3 h with JVA-2802 demonstrated a dose-dependent (10-60 mg/kg p.o.) antagonism of U50,488 administered centrally (100 nmol, i.c.v.), strongly suggesting orally administered JVA-2802 crosses the blood-brain barrier to antagonize KOR in the central nervous system. From this, we further hypothesized that oral administration of JVA-2802 would prevent reinstatement of cocaine-seeking behavior. Mice

demonstrating cocaine-conditioned place preference (CPP) and subsequent extinction were pretreated daily with vehicle or JVA-2802 (60 mg/kg, p.o.), and exposed to repeated forced-swim stress or a single additional session of cocaine place conditioning. JVA-2802 prevented the stress-induced, but not cocaine-induced, reinstatement of cocaine-CPP, consistent with previous demonstrations with KOR antagonists. These data validate the use of modified, systemically active peptides such as JVA-2802 as potentially useful therapeutics. (Supported by the State of Florida and DA018832 & DA023924 from NIDA).

Social influences on morphine sensitivity in adolescent rodents

S. Eitan (1), S. R. Hofford (1), S. L. Cole (1), D. J. Evert (1), P.J. Wellman (1), (1) Behavioral and Cellular Neuroscience Program, Dept. of Psychology, Texas A&M University, College Station, Texas, USA Social/peer influences are among the strongest predictors of adolescents' drug use. Hence, we recently examined whether this phenomenon can also be modeled in rodents. Specifically, we examined how housing rodents with different social partners affected the subsequent activating (i.e. locomotion) and rewarding (i.e. conditioned place preference) properties of morphine. Both mice and rats were used in these studies. All animals were group-housed four per cage in one of two conditions. In the mixed treatment condition, morphine- and saline-treated animals were housed together (i.e. 2 rodents receiving morphine and 2 rodents receiving saline per cage). In the separated treatment conditions, all 4 animals in the cage received either morphine or saline, and cages were visually separated from each other. Animals were then individually examined for their responses to morphine. Our results demonstrate that housing with different social partners altered both the activating and rewarding properties of morphine (i.e. significant differences were found between animals treated identically but housed in the mixed versus separated conditions). Notably, in both mice and rats, the social effects on morphine sensitivity were prevalent among adolescents but were not observed in adults. Also, although the effects were observed in both species, the nature of the effect differed between mice and rats. This species-dependant difference seems to be due to the different effects of opioids on social contact and play in mice and rats. Thus, our results suggest that the quantity and quality of juvenile social contact and play (i.e. the nature of the 'social network' of adolescents) have an effect on both the activating and rewarding properties of morphine, and possibly of other drugs of abuse. Supported by NIH (DA022402 to SE and DA013188-07 to PJW).

Opioids block the effects of the HIV entry inhibitors Maraviroc and AMD-3100 in CNS glia

N. El-Hage, S. M. Dever, T. Ahmed, Y. Zhang, K. F. Hauser, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA

There are currently 27 HIV medications used in various combinations to treat HIV and AIDS, including inhibitors of viral entry. Maraviroc is a CCR5 inhibitor used with other HIV medications to treat CCR5 (R5)-tropic HIV and AMD-3100, a CXCR4 antagonist, is used to reduce CXCR4 (X4)-tropic HIV levels. Since these two inhibitors have the ability to inhibit HIV entry in target cells and opioid abusers are more susceptible to the neurodegenerative effects of HIV in the CNS, the goal of this study was to investigate to the impact of opioids such as morphine, widely abused drugs among people infected with HIV as well as DAMGO, on the inhibitory effects of Maraviroc and AMD-3100 on HIV entry in human microglia and astrocytes. We first confirmed that astrocytes and microglia express CCR5 and CXCR4 using flow cytometry. HIV binding and entry were directly visualized by confocal microscopy using GFP-labeled R5 (BaL) and X4 (NL4-3) virions and infection was further confirmed using a HIV Tat-activated luciferase reporter assay. As expected, we found that Maraviroc inhibited R5 HIV entry and reduced HIV infection levels by 95% in astrocytes and microglia. However, morphine and DAMGO treatment compromised the anti-HIV entry effects of Maraviroc leading to increased HIV levels in these cells. Similar results were found with opioids and AMD-3100 using X4 HIV. Our data suggest that opioids impair the effects of HIV entry inhibitors and may contribute to increased susceptibility of HIV entry in opioid abusers which could lead to accelerated CNS neuropathogenesis in these individuals.

Streptozotocin-induced type 1 diabetes impairs learning abilities in Barnes Maze and alters growth hormone receptor (GHR) but not prodynorphin (PDYN) mRNA expression in the prefrontal cortex of male mice

E. Enhamre, A. Carlsson, A. Grönbladh, H. Watanabe, B.-M. Johansson, M. Hallberg, F. Nyberg, Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, Uppsala University, Uppsala, Sweden

It is well known that chronic treatment with opiates and other drugs is associated with impaired cognitive abilities in humans as well as in rodents. By establishing a pathophysiological status of an impaired cognitive function, we here intend to simulate the negative effects of long-term opioid treatment in the brain. Previous studies report that

experimental diabetes in rodents, induced by streptozotocin (STZ), is associated with a reduced neurogenesis in combination with an increased neuronal apoptosis in the hippocampus. These animals also display cognitive impairments in several learning and memory tasks. Male C57BL/6J mice were injected with STZ 150 mg/kg i.v. and the control group received saline in the same volume. On day 21 the learning and memory function of the animals were tested in the Barnes Maze (BM) for 5 consecutive days including a probe trial. The day after the probe trial the animals were decapitated and brain tissue dissected and frozen for further analysis. RNA-preparation, cDNA synthesis and Taqman® real time PCR were conducted in order to measure the mRNA levels of prodynorphin (PDYN) and growth hormone receptor (GHR), both entities involved in cognition, in the prefrontal cortex of the animals. The results demonstrate a significant difference between diabetic animals and controls in their ability to locate the target hole in the BM. No significant differences were seen between the two groups in the probe trial. However, alterations in mRNA expression of GHR but not PDYN were seen in the prefrontal cortex of the diabetic mice. This study was supported by grants from the Swedish Medical Research Council (Grant 9459) and from Swedish Council for Working Life and Social Research.

Role of dynorphin/kappa opioid receptor in forced swim test behavior in rats

N.Z. Fang, Y. Zhou, S. Chen, B. Mayer-Blackwell, B. Reed, M.J. Kreek, Lab of the Biology of Addictive Diseases, Rockefeller University, NY, NY, USA

Antagonism of the kappa opioid receptor (KOR) has been reported to have anti-depressant-like properties. The dynorphin/KOR system is a crucial neurochemical substrate underlying the pathologies of addictive diseases and other disease states. However, the molecular underpinnings of the dysregulation of this system are not yet well understood. The aims of this study were: (1) to confirm if the selective KOR antagonist nor-binaltorphimine (nor-BNI) can have antidepressant-like effects in the forced swim test (FST); (2) to determine the extent of alteration of preprodynorphin (ppDyn) mRNA levels induced by FST; and (3) to elucidate other molecular mechanisms. Young adult male Sprague-Dawley rats were placed in a cylinder of water for 15 minute intervals. Immediately after the initial exposure, they were treated with vehicle or nor-BNI (5 or 10 mg/kg). One day after treatment, the rats were placed in the FST for five minutes and scored for immobility, swimming, and climbing. Nor-BNI increased climbing time (5 and 10 mg/kg both) and significantly reduced immobility (10 mg/kg only) in the FST, measures indicative of anti-depressant

activity. Rats were sacrificed under stress minimized conditions thirty minutes after the FST. Their brains were subsequently dissected. Several brain regions were analyzed, including: caudate-putamen, nucleus accumbens, hypothalamus, and amygdala. The regions were homogenized and the mRNA was isolated using Trizol. ppDyn mRNA levels were measured with real-time optical PCR and normalized to GAPDH mRNA. In comparison to control animals not exposed to FST, we observed a significant elevation in ppDyn mRNA levels following FST in the caudate-putamen but not in the nucleus accumbens, hypothalamus, and amygdala. In animals exposed to FST, nor-BNI treatment did not alter ppDyn mRNA levels in comparison to animals that received vehicle. Future studies will look at additional brain regions, additional gene expression levels, hormonal levels, and potential epigenetic mechanisms. Support: NIH-NIDA Grants P60-DA05130 (M.J.K.)

Interactions of gonadal steroids and acute stress on levels of phosphorylated mu opioid receptors in the rat hippocampus

K. L. Gonzales¹, J. D. Chapleau¹, D. Kelter¹, J. P. Pierce¹, T. J. Williams¹, A. Torres-Reveron^{1,3}, B. S. McEwen², E. M. Waters², T. A. Milner¹, ¹Dept. of Neurology/Neurosci. Weill Cornell Medical Col., NY, NY, USA, ²Lab of Neuroendocrin., Rockefeller Univ., NY, NY, USA, ³Col. of Pharm., Nova Southeastern University, Ponce Puerto Rico

Opioids play a critical role in hippocampally dependent behaviors and plasticity. In the hippocampal formation (HF), mu opioid receptors (MOR) are prominent in parvalbumin (PARV) containing interneurons. Previously we found that the trafficking of MORs in PARV interneurons is modulated by gonadal hormones (GH). Although sex differences in response to stress are well documented, the point at which opioids, sex and stress interact to influence HF function remains elusive. Thus, we used quantitative light and electron microscopic immunocytochemistry for the phosphorylated MOR (pMOR) in rats to assess these interactions. In both sexes, pMOR-immunoreactivity (ir) was prominent in axons and terminals in dentate gyrus (DG) hilus and CA3 stratum lucidum and in a few neurons, some of which contained PARV, in DG hilus. In unstressed rats, the levels of pMOR-ir in the DG or CA3 were not different between the sexes nor between females at any point in the estrous cycle. However, the levels of pMOR-ir following acute immobilization stress (AIS; 30 minute) were affected by sex and estrous cycle stage. In particular, males had higher levels of pMOR-ir following AIS whereas females at proestrus had lower levels of pMOR-ir within the DG. In contrast, the number and types of neuronal profiles with pMOR-ir were not altered by AIS in either sex.

These data suggest that although GH do not alter levels of pMOR-ir in non-stressed animals, GH may alter the expression of pMOR, and ultimately the effects of opioids following AIS. These interactions may be the foundation for reported sex differences in hippocampally dependent behaviors in acutely stressed animals. NIH grants DA08259 & HL096571 (TAM), T32 DA007274(JDC), DK07313 (EMW), NS007080(BSM), MSTP grant GM07739(TJW)

Analgesic tolerance to high efficacy agonists but not to morphine is reversed in phosphorylation-deficient S375A mu-opioid receptor knockin mice

G. Grecksch(1), A.-K. Imhof(2), C. Pierstorff(1), S. Just(2), C. Doll(2), A. Lupp(2), A. Becker(1), T. Koch(1), R. Stumm(2), V. Höllt(1) and S. Schulz(2) (1)Institute of Pharmacology and Toxicology, University Hospital, Otto-von-Guericke-University Magdeburg, Germany (2)Institute of Pharmacology and Toxicology, University Hospital, Friedrich Schiller University Jena, Germany

Morphine is one of the most potent analgesic drugs. However, the utility of morphine in the management of chronic pain is limited by its rapid development of tolerance. Morphine exerts all of its pharmacological effects via the mu-opioid receptor. We have recently shown that mu-opioid receptor phosphorylation occurs in an agonist-selective manner. High efficacy agonists such as [D-Ala²-MePhe⁴-Gly-ol]enkephalin (DAMGO) or etonitazene stimulate the phosphorylation of both carboxyl-terminal threonine 370 (T370) and serine 375 (S375) with S375 being the primary site of phosphorylation. In contrast, morphine promotes the phosphorylation of S375 but fails to stimulate T370 phosphorylation. Here, we have assessed the contribution of S375 phosphorylation to the development of antinociceptive tolerance to mu-opioid receptor agonists with different efficacy. We show that S375 phosphorylation of the mu-opioid receptor occurs *in vivo* in intact mouse brain shortly and transiently after administration of both morphine and etonitazene. In knockin mice expressing the phosphorylation-deficient S375A mutant of the mu receptor, antinociceptive tolerance after repeated subcutaneous application of etonitazene or repeated intracerebroventricular application of DAMGO was strongly reduced. In contrast, tolerance to the antinociceptive effect of morphine was retained. Thus, tolerance to high- and low-efficacy agonists develops through two distinct pathways. Whereas tolerance induced by DAMGO or etonitazene requires agonist-driven phosphorylation of S375, the development of antinociceptive tolerance to morphine occurs independent of S375 phosphorylation. This study was supported by the Deutsche Forschungsgemeinschaft.

Prodynorphin gene expression in rats treated with ethanol and growth hormone

A. Grönbladh, J. Johansson, E. Enhamre, B-M. Johansson, M. Hallberg, F. Nyberg. Division of Biological Research on Drug Dependence, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

Alcohol dependence is a neuropsychiatric disorder that may lead to severe consequences, cognitive impairment being one of them. Connections between alcohol dependence and the endogenous opioid system have been confirmed in several studies and ethanol has, in addition, been demonstrated to induce changes in the prodynorphin system. The prodynorphin system has been implicated in deficits in learning and memory and it has previously been demonstrated that dynorphins may impair spatial learning in rats. Growth hormone (GH), on the contrary, has been demonstrated to induce beneficial effects on memory and learning. Thus, GH might have an ability to counteract cognitive impairments that may occur in humans and other mammals in connection to alcohol dependence. In the present study, we investigated the effects on the prodynorphin gene expression and spatial learning and memory in rats after treatment with ethanol and GH. Male Wistar rats were treated once daily with ethanol (3.2 mg/kg) i.g and/or GH (1 IU/kg) s.c for six days. Controls were treated with water and saline. The Barnes maze, a circular platform with 18 holes, was used to test spatial learning and memory. Training sessions were performed with three trials per day for three days and a probe trial was conducted one day after the last training session. Three trials of reverse learning were performed one hour after the probe trial. The rats were then decapitated, brain tissues were dissected and immediately frozen. After RNA preparation and cDNA synthesis, quantitative real time PCR of prodynorphin was performed using a TaqMan® gene expression assay. Comparisons between ethanol and GH treated animals demonstrate a tendency of increased prodynorphin gene expression in the hippocampus after treatment with ethanol, a trend that seemed to be reduced in the presence of GH. Support: Swedish Medical Research Council (Grant 9459).

Antinociception of perineurally applied drugs via modulation of tight junction proteins in the perineurium

D. Hackel^{1,2}, S. Amasheh⁴, S. Krug⁴, S.A. Mousa³, E.J. Wrede¹, M. Fromm⁴, A. Brack², H.L. Rittner²,
¹Dept of Anaesthesiology, CBF, Charité–Berlin, Germany, ²Dept of Anaesthesiology, University hospital of Würzburg, Germany, ³Dept of Anesthesiology, CCV, Charité – Berlin, Germany, ⁴Institute of Clinical Physiology, CBF, Charite – Berlin, Germany

During postoperative pain treatment regional anesthesia aims at specifically blocking the transmission of signals in nociceptors to prevent pain. Most widely used substances in clinical use are local anesthetics blocking sodium channels in all neurons with the effect of numbness from touch receptors and paralysis from block of motor neurons. Nav 1.7 blockers or hydrophilic opioids which target only nociceptors could not be used *in vivo* because they do not penetrate into the peripheral nerve. The peripheral nerve is surrounded by the perineurium composed of perineurial cells, special membrane proteins for active transport and tight junction proteins to limit paracellular permeability. Previously we showed that hypertonic solutions applied in the subcutaneous tissue increase permeability and facilitate peripheral opioid analgesia. We examined the possibility to facilitate nociceptor specific analgesia using perisciatic injection of hypertonic saline. This allowed for an increase in the mechanical pain threshold after injection of ProToxin II (Nav 1.7 blocker) or DAMGO or DPDPE in rats. Furthermore we explored the downstream events to ultimately open tight junctions in the perineurium. We showed in rats a reversible and directed opening of the perineurial barrier via reduced expression of claudin-1 and under influence of metalloproteinase and its inhibitors. This presents a first step of the analysis of the molecular events following the perisciatic application of hypertonic saline to facilitate nociceptor specific analgesia. Our findings also put forward new targets for the specific and regulated opening of the perineurium for targeted drug delivery. This work was supported by the DFG (German Research Foundation) FOR 721.

Bivalent ligands for the characterization of opioid receptor heterodimers

J. Harvey (1, 2), R. van Rijn (1), P. England (2), J. L. Whistler (1, 2), (1) Ernest Gallo Clinic and Research Center, Emeryville, CA, USA, (2) University of California, San Francisco, San Francisco, CA, USA
Several recent studies have suggested that a heteromer of the mu (MOR) and delta (DOR) opioid receptors could be a relevant target for the treatment of several indications including pain, anxiety and alcoholism. However, elucidating the functional role

of the MOR/DOR heteromer *in vivo* has been hindered by the lack of pharmacological agents that selectively activate or inactivate the MOR/DOR heteromer without affecting activity at the MOR and DOR homomers. We have designed and synthesized a series of novel “tuned-affinity” bivalent ligands to selectively target opioid receptor heteromers. Here we report the characteristics of these ligands *in vitro* and how we have used them to probe the role of receptor heteromers *in vivo*. J.H. is supported by the AP Gianini Foundation and a PBBR Fellowship. This work was supported by NIH grant 1R21DA031574 and funds provided by the State of California for medical research through the University of California San Francisco both to JLW and PE.

Analysis of antinociceptive efficacy following microinjection of mu-opioid receptor agonists into the periaqueductal gray of the rat

R.A. Haseman, E.N. Bobeck, S.L. Ingram, M.M. Morgan, Washington State University Vancouver, Vancouver WA, USA

The antinociceptive efficacy of mu-opioid receptor (MOPr) agonists is important in predicting receptor signaling. High efficacy agonists, such as fentanyl, produce limited tolerance, whereas low efficacy agonists such as morphine produce rapid tolerance. However, tolerance develops to repeated microinjections of fentanyl or DAMGO into the ventrolateral periaqueductal gray (vlPAG) suggesting that fentanyl and DAMGO are not high efficacy MOPr agonists in the vlPAG or that the inverse relationship between efficacy and tolerance does not hold true in the vlPAG. To test these hypotheses, antinociceptive efficacy of morphine and fentanyl was determined using the irreversible MOPr antagonist, beta-funaltrexamine hydrochloride (Beta-FNA). Six hours after Beta-FNA (0, 0.1, 0.5, 5, or 10 microgram per 0.5 microliters) administration, cumulative doses of morphine or fentanyl were microinjected into the vlPAG. Nociception was assessed after each MOPr agonist injection. Increasing doses of Beta-FNA caused a graded decrease in antinociception for both agonists. However, the decrease in fentanyl was enhanced compared to morphine using % maximum possible effect (%MPE), demonstrating that morphine has higher efficacy than fentanyl when injected into the vlPAG. The comparison of morphine and fentanyl using % of analgesic animals, instead of %MPE, the agonists showed equal efficacy. These data are in contradiction with differences in efficacy following systemic administration. This study was supported by NIH grant DA 015498. Morphine sulfate was a gift from NIDA.

Pharmacological functional magnetic resonance imaging analysis for pain research with understanding the mechanisms within the brain that provoke pain

H. Horiuchi (1), K. Niikura (1,2), Y. Takemura (1,3), A. Yamashita (1), K. Torigoe (1), S. Imai (1), N. Kuzumaki (1), M. Yamazaki (3), T. Suzuki (1) and M. Narita (1,3), (1) Dept. Toxicol., Sch. Pharm. Pharmaceut. Sci., Hoshi Univ., Tokyo, Japan, (2) Lab. Biol. of Addict. Dis., Rockefeller Univ., New York, USA, (3) Dept. Anesthesiol., Grad. Sch. Med. Pharmaceut. Sci. Res., Toyama Univ., Toyama, Japan
Functional magnetic resonance imaging analysis (fMRI) has been introduced to detect spatial as well as temporal brain activation following experimental pain induced by various stimuli. This technique allows us to characterize a network in the brain that forms a pain matrix. Pharmacological MRI (phMRI) is the combination of fMRI with drug administration. phMRI is a promising tool that may greatly contribute to our understanding of the mechanisms within the brain that provoke pain. Furthermore, it appears that this technology may be useful for identifying molecules and developing drugs for the modulation of pain in clinical practice. We are currently attempting to image pain processing, based on molecular mechanisms in animal models using phMRI. The inflammatory pain stimuli induced by intraplantar injection of complete Freund's adjuvant caused robust positive signal activity in the cingulate cortex and somatosensory cortex. In contrast, these activations were diminished in prodynorphin knockout mice, indicating that the dynorphin A is responsible for the activation of these pain-related regions in the acute phase of inflammatory pain. The neuropathic pain-like stimuli induced by intrathecal injection of protein kinase C activator, phorbol 12,13-dibutyrate, caused a remarkable increase in the activity of the cingulate cortex and somatosensory cortex. Those activations were abolished in mice that lacked the PKC γ gene, suggesting that the activation of spinal PKC γ plays a role in direct acceleration of the ascending nociceptive pathway. These results suggest that phMRI is a powerful tool with a potential for high sensitivity and specificity for evaluating analgesics in early drug development and clinical studies.

Opioid & alcohol pharmacodynamics: Contributions of innate immune signaling to drug response

M.R. Hutchinson (1,2), Y. Wu (2), E.J. Jaehne (2), L. Liu (2), K.R. Diener (3), J.D. Hayball (3), K.C. Rice (4), L.R. Watkins (5), A.A. Somogyi (2), (1) Physiology, Univ of Adelaide, Adelaide, Australia; (2) Pharmacology, Univ of Adelaide, Adelaide, Australia; (3) School of Pharmacy & Medical Sciences, Univ of SA, Adelaide, Australia; (4) Chem

Bio Res Branch, NIDA & NIAAA, Rockville, MD, USA (5) Dept Psychology & the Center Neuroscience, Univ Colorado at Boulder, Boulder, Colorado, USA

Opioid & alcohol behavioral actions have classically been categorized as solely neuronal events. Recent discoveries have demonstrated that both opioid & alcohol activation of innate immune Toll Like Receptor 4 (TLR4) signaling also plays a critical role. However, the functional involvement of other TLRs & their signaling pathways remains to be determined. Given that both opioids & alcohol have TLR actions, this raises the possibility of a novel site of drug interaction that may contribute to their established synergistic interactions. The aim is to examine the impact of TLR signaling on opioid & alcohol action in mice. Acute (analgesia dose response) and chronic (analgesic tolerance & withdrawal) morphine actions in male wild-type (WT) Balb/c mice, and mice with knockouts (KO) of TLR4, TLR2, TLR2/4, MYD88, TRIF or TIR8 were quantified. Acute alcohol actions (loss of righting reflex [LRR] & rotorod performance), & morphine & alcohol drug interaction (LRR) were examined in the first 4 KO mice strains. Acute morphine analgesia was potentiated by KO of TLR2 &/or TLR4 signaling compared to WT. Tolerance did not develop in TLR4 or TLR2/4 mice. Withdrawal was not significantly influenced by TLR signaling. Acute alcohol action was reduced by KO of TLR2 &/or TLR4 signaling. Interestingly, alcohol & morphine synergism was dependent on TLR2 & MyD88 signaling, but not TLR4. Opioid radioligand binding data from the KO mice will also be presented. These data highlight an important role that TLR signaling has in a broad range of opioid & alcohol actions individually & on their drug interactions. Supported by NIH DA015642, DA023132, DA024044, DE017782; NHMRC ID465423; ARC DP110100297

Novel analogs of endomorphins provide antinociception without spatial and recognition memory deficits produced by morphine

J.N. Jernberg (1), X. Zhang (2), J.E. Zadina (1,2,3,4) (1) Graduate Neuroscience Program, Dept. of (2) Medicine & (3) Pharmacology, Tulane Univ. Sch. of Med., (4) SE LA Veterans HCS, New Orleans, LA, USA

Currently opioids that activate the mu opioid receptor are considered the gold standard treatment for moderate to severe pain. Due to a variety of side effects, such as reward/addictive potential, motor impairment, respiratory depression, and cognitive dysfunction, physicians are often reluctant to implement opioid therapy. Endomorphins and their analogs, however, have shown promise for producing potent antinociception with fewer adverse side-effects. The focus of this study is on cognitive

effects of endomorphin analogs relative to those of morphine in rats. Doses of the analogs were optimized to produce antinociception equal to or greater than that of morphine throughout the timeframe of the behavioral tests. The effects on spatial and recognition memory were examined using standard Morris water maze (MWM) and novel object recognition paradigms. Preliminary data demonstrates that an antinociceptive dose of morphine significantly impairs both spatial and recognition memory using measures that control for motor impairment. By contrast, novel endomorphin analogs at doses producing equal or greater antinociception produce no significant cognitive impairments. In the MWM, morphine, but not endomorphin analogs, impaired average swim speed as well as average distance from the platform, an index of spatial memory unaffected by swim speed. Morphine, but not the analogs, also impaired exploration of novel objects, an index of recognition memory. Studies of potential mechanisms of the differential antinociceptive/side effect profile are underway, and future studies will use similar methods to examine effects of acute and chronic opioids in an aging model. Since some side effects of opioid therapy are particularly serious in older adulthood, the results are anticipated to provide evidence that these novel endomorphin analogs have reduced side effects that may translate to safer pain medications for older adults. Supported by the VA, ONR, and DOD.

Opioid withdrawal induced hyperalgesia is mediated in the peripheral nervous system via Transient Receptor Potential Vanilloid 1 (TRPV1)

J.A. Jira (1), V. Spahn (2), O. Fischer (2), C. Zöllner (1), (1) University Hospital Hamburg Eppendorf, Center for Anaesthesiology and Intensive Care Medicine, Hamburg, Germany, (2) Charité Berlin, CBF, Department of Anesthesiology and Operative Intensive Care Medicine, Berlin, Germany

Vanilloid receptor type 1 (TRPV1) is a ligand-gated ion channel expressed in sensory neurons that responds to noxious heat, protons, and chemical stimuli such as capsaicin. TRPV1 plays a critical role in the development of pain after tissue injury, inflammation or nerve lesions and can be sensitized by phosphorylation. Opioid withdrawal following chronic activation of the mu-opioid receptor induces AC superactivation and subsequently an increase in cAMP and protein kinase A (PKA) activity. In the current project we investigated whether opioid withdrawal can increase TRPV1 activity in cells, animals and humans. Opioid withdrawal induces an increase of intracellular cAMP, resulting in phosphorylation and sensitization of TRPV1. In whole cell patch clamp and calcium imaging experiments opioid withdrawal significantly

increased capsaicin-induced TRPV1 activity in a naloxone and pertussis toxin sensitive manner. A decrease in paw withdrawal latency after peripheral opioid treatment was detected in male Wistar rats, indicating opioid induced hyperalgesia. Volunteers were enrolled in a randomized, double-blind, placebo-controlled study. Capsaicin stimulation induced acute pain and stable areas of mechanical hyperalgesia to pinprick stimuli and touch (allodynia). The magnitude of pain and area of hyperalgesia were assessed before, during, and after opioid infusion. Opioid treatment reduced pain and areas of mechanical hyperalgesia during infusion. In contrast, postinfusion pain and hyperalgesia were significantly higher than control. In summary, our results demonstrate that opioid withdrawal increases the activity of TRPV1. This mechanism is mediated via the PKA/cAMP pathway and delineates a new mechanism underlying hyperalgesia during opioid withdrawal. These projects are funded by the DFG (German Research Foundation).

The impact of long term GHB treatment on spatial learning in male rats

J. Johansson, A. Grönbladh, F. Nyberg, M. Hallberg. Dept Pharm Biosciences, Uppsala University, Sweden

The illicit "club drug" gamma-hydroxy butyric acid (GHB), is usually abused for its euphoric and sedative effects, but it is also commonly used by body builders for its ability to increase lean muscle weight. In humans, GHB is known to induce short-term amnesia and disruption of memory and learning has been reported in animal studies. In this present study, we investigated the effects of repeated treatment with GHB on spatial learning and memory using the Morris water maze (MWM), and related neurochemical changes in the brain, including the endogenous opioids. Thus, the expression of the opioid receptors in brain areas related to cognition, such as hippocampus and frontal cortex is being considered. Adolescent male Sprague Dawley rats were orally administered with 100 mg/kg and 300 mg/kg GHB or saline, daily during 16 consecutive days. Behavioral tests in the MWM were performed on day 10-15, one hour after administration. Data collected from biochemical analysis of opioid receptors using receptor autoradiography are compared with behavioral performance. Although, no significant difference in swim speed or percentage of time spent in the four different quadrants were detected, rats treated with the high dose of GHB required a significant longer time to find the hidden platform during acquisition. In the probe trial, the high-dose treatment group shows a tendency to spend shorter percentage time in the target quadrant and a longer latency in visiting target zone. This study was supported by SMRC, grant 9459.

Exploring bifunctional activity of 3-substituted piperidin-4-yl-1,3-dihydroindol-2-one class of NOP ligands at the mu-opioid receptor (MOP)

V. Journigan (1), W. Polgar (2), L. Toll (2), N. T. Zaveri (1), (1) Astraea Therapeutics, LLC, Mountain View, CA; (2) SRI International, Menlo Park, CA.

The nociceptin receptor (NOP/ORL-1) and its endogenous peptide N/OFQ play a significant role in the reward process and morphine abuse. N/OFQ administered i.c.v. decreases basal and morphine-stimulated dopamine release in the nucleus accumbens; moreover, this endogenous peptide blocks morphine-induced conditioned place preference (CPP). These results point to an "anti-opioid" role for N/OFQ agonism in reward. We hypothesize that bifunctional compounds with NOP full agonist activity and MOP partial agonist activity will result in analgesics with reduced dependence liability. *In vivo* studies of SR16835, a potent NOP agonist/MOP partial agonist developed in our laboratory, show attenuation of morphine-induced CPP when given prior to morphine, an effect that reverses upon treatment with selective NOP antagonist SB612111. In our laboratory, we have developed extensive structure-activity relationships (SAR) of our piperidin-4-yl-1,3-dihydroindol-2-one scaffold to produce potent NOP agonists. Interestingly, ethyl substitution at the 3-indolinone position of this scaffold resulted in increased MOP affinity while retaining potent NOP agonism. Our SAR also shows that substitution at the piperidine nitrogen with cyclic and aromatic-containing structures allows for modulation of MOP intrinsic activity while retaining the crucial NOP agonism/partial agonism component. The SAR and *in vitro* activity of these NOP/MOP bifunctional ligands will be presented. This work is supported by grant R01DA027811 (NZ).

Remifentanyl exposure produces prolonged hyperalgesia under certain pain conditions but not morphine tolerance in rats

E. M. Jutkiewicz, Y. Sun, J. S. Schimmel, and J. R. Traynor, Department of Pharmacology, University of Michigan Medical School, University of Michigan Substance Abuse Research Center, University of Michigan, USA

The mu-opioid receptor agonist remifentanyl is infused intravenously (i.v.) during surgery and other procedures to promote anesthesia and/or analgesia and has been reported to increase postoperative pain and morphine requirements. The present study investigated the effects of 1 h continuous i.v. remifentanyl administration to rats on noxious stimuli thresholds, antinociceptive doses of morphine, and opioid withdrawal signs. Rats were implanted with i.v. catheters and infused with remifentanyl (0-320

mcg/kg/h) for 1 h followed by exposure to noxious stimuli: an i.p. injection of diluted acetic acid, tail dip in 50°C water, or exposure to 50°C hotplate. Abdominal stretches or withdrawal latencies were measured as different time points following the infusion. Remifentanyl exposure produced a dose-dependent increase in the number and duration of acid-induced abdominal stretches, but it did not alter tail withdrawal or paw lick latencies in the warm water tail withdrawal or hotplate assays, respectively. The remifentanyl-induced increase in stretching persisted for at least 4 h after termination of the remifentanyl infusion. Naltrexone administration (10 mg/kg) did not precipitate any behavioral signs of opioid withdrawal following the remifentanyl infusion. In rats exposed to remifentanyl, larger doses of morphine were required to decrease stretches as compared with saline-infused rats. Injecting higher acid concentrations in saline-infused rats also required larger doses of morphine to decrease stretching, demonstrating that morphine requirements were related to noxious stimulus intensity. In conclusion, these data demonstrate that continuous exposure to remifentanyl produces long-lasting hyperalgesia under certain pain conditions, and higher morphine doses are also required to relieve pain, which is likely due to elevated pain thresholds rather than opioid tolerance. This study was supported by USPHS grant 04087.

Nicotine prevents neuropathic pain following peripheral nerve injury through the suppression of neuroinflammation.

S. Kishioka, N. Kiguchi, Y. Kobayashi, S. Tominaga, J. Nakamura, T. Maeda, Department of Pharmacology, Wakayama Medical University, Japan
Neuropathic pain is caused by peripheral nerve damage and is characterized by allodynia and hyperalgesia. Although growing evidence indicates that up-regulation of inflammatory mediators plays a crucial role in the pathogenesis of neuropathic pain, the detailed mechanisms are still unclear. It was reported that activation of nicotinic acetylcholine receptor (nAChR) on inflammatory cells improves inflammatory disease through the suppression of neuroinflammation. Therefore, we examined the role of nAChR in the nerve injury-induced neuropathic pain. Mice were given partial sciatic nerve ligation (PSL) under the pentobarbital anesthesia. PSL-induced tactile allodynia and thermal hyperalgesia were evaluated by von Frey test and Hargreaves test, respectively. Drugs were perineurally injected once a day for 4 days in a volume of 10 microliter under the pentobarbital anesthesia. PSL-operated mice showed long-lasting tactile allodynia and thermal hyperalgesia on the ipsilateral but not contralateral paws. By RT-PCR, up-regulation of pro-inflammatory cytokines (e.g., IL-1beta and

TNFalpha) and chemokines (e.g., MIP-1alpha and MIP-1beta) were observed in the injured sciatic nerve (SCN) following PSL. By western blotting and immunohistochemistry, nAChR subunit alpha4 were increased in the injured SCN. PSL-induced tactile allodynia and thermal hyperalgesia were prevented by the early phase injection of nicotine (1-20 nmol, day0-3) in a dose-dependent manner. The preventive effects of nicotine were inhibited by the co-injection of dihydro-beta-erythroidine, a selective antagonist for alpha4. PSL-induced up-regulation of inflammatory mediators (IL-1beta, TNF-alpha, MIP-1alpha, and MIP-1beta) in the injured SCN on day7 was suppressed by nicotine. In conclusion, activation of nAChR in the peripheral nerves prevents the pathogenesis of neuropathic pain through the suppression of neuroinflammation. This work was supported by a grant from the Smoking Research Foundation.

Stimulation of the brain reward system attenuates the analgesic effects of the NMDA antagonist LY235959

C. M. Knapp (1), L. Tozier (1,2), S. Tapan (1), C. Kornetsky (1,2), (1) Division of Psychiatry and (2) Dept of Pharmacology, Boston University School of Medicine, Boston MA, USA

Morphine elevates the threshold for escape from nociceptive stimulation delivered to the mesencephalic reticular formation (MRF). This anti-nociceptive action is attenuated by the simultaneous delivery of low intensity stimulation to the medial forebrain (MFB). Stimulation of the MFB with moderate current intensities results in rewarding effects. MFB stimulation has also been found to potentiate the nociceptive effects of MRF stimulation. These effects of combined MRF –MFB stimulation resemble those of an opioid antagonist. In order to determine if these observed phenomena reflect the presence of an endogenous opioid antagonist our previous experiment was repeated substituting the putative analgesic agent LY235959 for morphine. LY235959 is a competitive N-methyl-D-aspartate (NMDA) receptor antagonist. In the present experiment two electrodes were implanted into each rat, one in the MFB and one in the MRF. After establishing that MFB stimulation resulted in rewarding effects, MRF thresholds were determined using a modification of the rate independent psychophysics method of limits. The administration of LY235959 (0.5, 1, and 2 mg/kg s.c.) produced a significant dose dependent elevation of the MRF-escape thresholds in the absence of MFB stimulation. In contrast, delivery of 5 or 10 uA's of MFB stimulation concurrently with MRF stimulation produced a lowering of the escape-threshold significantly below control levels in animals treated with LY235959. These results indicate that LY

235959 may have anti-nociceptive effects at the supra-spinal level. They suggest that the anti-analgesic effects of combined MRF-MFB stimulation may not be restricted to opioid analgesics and point to the possible presence of an endogenous anti-analgesic system. Supported in part by NIDA Grant 5R21DA-025586 to CK.

Selective interaction of G-protein coupled receptors with isoforms of ADP-ribosylation factor (ARF)

T. Koch, M. Rankovic, J. Konietzko, E. Kahl, and Volker Höllt, Dept. of Pharmacology and Toxicology, Otto-von-Guericke-University, Magdeburg, Germany

ARF1 and ARF6 are distinct members of the ADP-ribosylation factor (ARF) small-G-protein subfamily. ARF1 is mostly cytosolic, with minor locations at the Golgi and plasma membrane, whereas ARF6 is restricted to the plasma membrane. In previous studies we have demonstrated that the opioid-mediated and ADP-ribosylation factor (ARF)-dependent activation of phospholipase D2 (PLD2) is a prerequisite for MOPr endocytosis. By coexpressing the MOPr and dominant negative or constitutively active ARF mutants in human embryonic kidney (HEK) 293 cells and primary cultured cortical neurons as well as with the use of siRNA-technology, we identified the ARF6 protein to be involved in PLD2 activation and regulation of MOPr endocytosis. Remarkably, ARF6 is involved in the endocytosis and PLD2 activation of several but not all GPCRs. We demonstrate here, that the activation of PLD2 and the induction of the 5-HT_{2A} receptor endocytosis is mediated via interaction with ARF1. A specific conserved motif, NPxxY, which is found at the junction of the tm7 and ct domains of many GPCRs, has been implicated as a determinant of ARF-receptor interactions and specificity. Therefore, in the present study we investigated the effect of the N332D, N332A, and Y336A mutations in the conserved NPxxY motif within the C-tail of the MOPr on the ARF-selectivity, PLD2 activation and endocytosis of the MOPr. This work was supported by Deutsche Forschungsgemeinschaft (KR1740/10-1) and by the Land Saxony-Anhalt from the "Europäischen Fond für regionale Entwicklung (EFRE 2007-2013).

Morphine-induced motor stimulation after repeated administration: age-related differences in mice

W. Koek, Departments of Psychiatry and Pharmacology, University of Texas Health Science Center at San Antonio, TX, USA

Given evidence for age-related differences in the effects of drugs of abuse, surprisingly few preclinical studies have explored effects of opioids in

adolescents (versus adults). The present study compared the motor stimulating effects of morphine in adolescent and adult mice, 2 days and 5 weeks after its repeated administration. Morphine (3.2 – 56 mg/kg, i.p.) increased locomotion along an inverted U-shaped dose-response curve in adolescent, late adolescent, and adult male C57BL/6J mice treated with saline once per day for 4 days. The maximum effect of morphine, assessed 2 days after the last injection of saline, was higher in adolescents than in adults. Repeated treatment with morphine (10-100 mg/kg) shifted the dose-response curve of morphine upward, and 17.8 mg/kg was the lowest dose to do so in each of the age groups. The maximum extent to which the dose-response curve shifted upward was similar in each age group (i.e., 1.6 – 1.9 fold). The enhancing effects of repeatedly administered morphine were still evident 5 weeks later, when the adolescents had become adult, but consisted of a smaller upward shift (i.e., 1.4-fold) that occurred only at a higher dose (i.e., 56 mg/kg). In animals treated repeatedly with morphine as adults, its enhancing effects were no longer evident 5 weeks later. Repeated administration of morphine produced similar short-term enhancement of its motor stimulating effects in all age groups, but evidence for long-term enhancement was obtained only in adolescents. These findings suggest that compared with adults, adolescents are more sensitive not only to the acute locomotor stimulating effects of morphine, but also to its long-lasting locomotor sensitizing effects. Supported by DA23261

μ -opioid control of P2X3 receptors in DRG sensory neurons of rat is crucially dependent on the experimental *in vitro* conditions

O. Krishtal, (1), I. Chizhnikov (1), V. Kulyk, (1), D. Simone (2) and G. Bakalkin (3), (1) Dept. of Cell. Membranol., Bogomoletz Institute of Physiol., Kiev, Ukraine, (2) School of Dentistry, Univ. Minnesota, Minneapolis, USA, (3) Dept. of Pharm. Biosci., Uppsala University, Uppsala, Sweden

P2X receptors in nodose sensory neurons of rat are under opioid control: μ -opioid agonists powerfully inhibit the currents generated by nodose neurons isolated from rat and kept in primary culture (Chizhnikov et al., 2005). This evidence has been obtained on the "slow" responses to ATP generated by P2X2 and P2X2/3 receptors. According to existing paradigm, homomeric P2X3 receptors (expressed almost exclusively in the sensory neurons) play especially important role in the nociception. In this study we have examined the effect of μ -opioids on the currents generated by P2X3 receptors in the neurons from DRG of rat. In acutely isolated cells, inward currents generated by P2X3 receptors were effectively blocked by μ -opioid agonists (endomorphin1 or leu-enkephalin, in concentrations

10-100 nM). However, the experiments on the neurons kept in primary culture demonstrated complete loss of sensitivity of P2X3 receptor-mediated currents to opioids after only 24 hours of culturing. Histochemical evidence indicates that chronic treatment with opioid receptor antagonists increases the density of μ -opioid receptors in cell culture and in the intact animal (Patel et al., 2002). Such increase in receptor density is possibly due to a reduction in internalization of opioid receptors. We have tested whether opioid effect on P2X3 receptors can be preserved when opioid antagonist is added to the culture medium. Addition of naloxone (1 μ M) for 24 hours completely restored the effect of opioids on P2X3 receptor. This simple procedure allows to achieve experimental conditions when histochemical and electrophysiological evidence successfully merge. Our data indicate at the necessary caution in the compiling *in vivo* and *in vitro* data when studying volatile mechanisms of opioid signaling. FIRCA grant 1R03TW008228-01A1 (1,2) and Visby grant 00697/200 (1,3)

Suppression of malignancy of gefitinib-resistant human non-small-cell lung cancer (NSCLC) cells by activation of δ -opioidergic system

N. Kuzumaki (1), A. Suzuki (1), M. T-Narita (1), K. Yamamizu (2), A. Nagasawa (1), Y. Okada (3), H.J. Okano (3), H. Okano (3), J.K. Yamashita (2), T. Suzuki (1) and M. Narita (1), (1) Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan, (2) Lab. Stem cell Differentiation, Stem Cell Research Center, Institute for Frontier Med. Sci., Kyoto Univ., Kyoto, Japan, (3) Dept. Physiol., Keio Univ. Sch. Med. Tokyo, Japan

The δ -opioidergic system has been recognized as a neurotransmitter system that could be directly involved in emotionality, immunity and the development of cells including neurogenesis. In this study, we found that H1975 cells, which are human non-small-cell lung cancer (NSCLC) cells and that are resistant to an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib, expressed δ -opioid receptors (DORs). Addition of the DOR agonist SNC80 produced a concentration-dependent decrease in H1975 cell growth. Under these conditions, the addition of SNC80 to H1975 cells produced a significant decrease in phosphorylated-signal transducers and activator of transcription 3 (p-STAT3). It has been reported that expression of the key pluripotent genes of embryonic stem (ES) and induced pluripotent stem (iPS) cells has been found in cancer stem cells. In the present study, the pluripotency genes were absent in normal human lung fibroblasts cells, whereas these were found in H1975 cells. The addition of SNC80 to H1975 cells produced a significant and concentration-dependent decrease in pluripotency gene expression. These

results suggest that the stimulation of DOR by agonist may help to reset the pluripotent state by inhibiting STAT3 activity. Furthermore, the present findings constitute promising support for the notion that DORs may be prime candidates for EGFR-TKI-resistant NSCLC therapy.

Role of $Gal\alpha_o$ protein in opioid agonist-dependent signaling and behavior

J. Lamberts (1), E. Jutkiewicz (1, 2) and J. Traynor (1, 2), (1) Department of Pharmacology and (2) Substance Abuse Research Center, University of Michigan, Ann Arbor, MI

Mu-opioid receptor (MOR) agonists elicit analgesia *via* activation of the $Gal\alpha_{i/o}$ subunit of heterotrimeric G proteins—most notably $Gal\alpha_o$. Signal termination is accelerated by the family of regulator of G protein signaling (RGS) proteins. To evaluate $Gal\alpha_o$ signaling and determine whether RGS proteins modulate $Gal\alpha_o$ activity *in vivo*, we have measured both biochemical and behavioral endpoints of MOR agonist action in heterozygous mice from two different 129S6 strains, in comparison with their respective wild type littermates: (1) a $Gal\alpha_o$ knockout strain ($Gal\alpha_o$ (+/-)), constituting a “loss-of-function” system and (2) a “knock-in” mutant strain expressing an RGS-insensitive (RGSi) $Gal\alpha_o$ protein ($Gal\alpha_o$ (+/GS)), constituting a “gain-of-function” system. In $Gal\alpha_o$ (+/-) mice, there was a 4-fold reduction in the potency of morphine to elicit antinociception, as measured in the hot plate test. In contrast, the potency of morphine was enhanced 3-fold in $Gal\alpha_o$ (+/GS) mice. In whole brain homogenates from $Gal\alpha_o$ (+/-) mice, the loss of $Gal\alpha_o$ protein was confirmed, and resulted in a reduction in both high-affinity MOR binding ($28.2 \pm 8.6\%$) and maximal MOR agonist-stimulated [35 S]GTP γ S incorporation (DAMGO, $14.4 \pm 6.4\%$; morphine, $39.2 \pm 9.4\%$), supporting the findings *in vivo*. Despite the observed enhancement in morphine antinociception in $Gal\alpha_o$ (+/GS) mice, whole brain homogenates from these mice showed a paradoxical reduction of $Gal\alpha_o$ protein ($39.3 \pm 2.8\%$), high-affinity MOR binding ($24.8 \pm 7.9\%$) and MOR-dependent G protein activation (DAMGO, $40.5 \pm 10.5\%$; morphine, $53.3 \pm 12.8\%$), presumably due to developmental compensation caused by the $Gal\alpha_o$ “gain-of-function” mutation. Differences in the development of tolerance and dependence between the mouse strains will also be presented. Together, these results highlight the importance of $Gal\alpha_o$ for MOR-mediated signaling and behavior. Supported by GM077667, DA007267 (JL) and DA04087 (JT).

Morphine tolerance, desensitization and recovery in locus coeruleus neurons from morphine-treated rats

E. Levitt and J. Williams, Vollum Institute, Oregon Health & Science University, Portland, OR, USA

Cellular tolerance can be observed following long-term morphine treatment either in vivo or in vitro. Tolerance to morphine-mediated G protein-coupled inwardly rectifying potassium conductance is observed in locus coeruleus neurons from rats treated with morphine (50 mg/kg/day) for 6 or 7 days. This tolerance is long-lasting since brain slices were washed in morphine-free buffer for several hours prior to testing. However, part of the tolerance induced in vivo reversed within a relatively short (< 2 h) wash, which was revealed by comparison to slices continuously incubated in morphine and is consistent with previous findings (Bailey et al., 2009). The rapidly recovering portion of tolerance was dependent on the time spent in morphine-free buffer and the concentration of morphine used to maintain desensitization. In the presence of the Ser/Thr phosphatase inhibitor okadaic acid, desensitization was still able to recover to the same extent as control after a long-term (> 2 h) wash. Cellular tolerance can also be induced by incubating brain slices from naïve rats in morphine for 4-6 hours. Okadaic acid enhanced the onset of morphine tolerance in slices, with significant effects observed following just 1-3 hours of morphine incubation. These results indicate that phosphorylation/dephosphorylation cycles are ongoing during the development of tolerance, in line with reports that PKC activity is required to maintain morphine tolerance. Together, these results identify two forms of tolerance induced in vivo distinguished by the time-course of recovery, and implicate phosphatase activity during the development of morphine tolerance. Supported by T32NS007381 and DA08163.

Pharmacogenetics of methadone dose requirement in opioid addiction treatment

O. Levran (1), E. Peles (2), S. Hamon (1), M. Randesi (1), M. Adelson (2), M. J. Kreek (1)(1)Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY, USA, (2)Dr. Miriam and Sheldon G. Adelson Clinic for Drug Abuse, Treatment and Research, Elias Sourasky Medical Center, Tel Aviv, Israel

The inter-individual differences in the efficacy and toxicity of methadone may be affected in part by gene variants in genes encoding proteins involved in pharmacokinetic processes and pharmacodynamic effects. We have recently shown that homozygosity to SNP 1236T allele in the *ABCB1* transporter gene is associated with high methadone dose requirement (>150 mg). Other studies suggested a role of *OPRM1*, *DRD2*, *BDNF*, and *KCNJ6* variants in

MMT response. To explore the role of additional genes in methadone dose requirement, we have genotyped variants in genes encoding the major methadone metabolizing enzymes CYP3A4, CYP2D6 and CYP2B6, as well as genes encoding the neurotrophins, BDNF and NGFB, that mediate synaptic plasticity. Our sample includes well-characterized Israeli former heroin addicts in MMT (n=74), with a stabilizing daily methadone dose range of 12.5 mg -260 mg (mean 140±52 mg) and no major co-medication that may affect methadone metabolism. The sample was shown to be primarily of Middle Eastern/European ancestry based on ancestry informative markers. Out of the 45 informative SNPs analyzed, homozygosity for the variant alleles of three SNPs showed significant association with a relatively low methadone dose requirement: *NGFB* intronic SNP rs2239622 ($P = 0.0002$), and *CYP2B6* SNPs 785A>G and 516G>T ($P = 0.01, 0.04$, respectively). Repeated analysis controlling for the *ABCB1* 1236 TT genotype (that showed an opposite effect on methadone dose) substantiated the result ($P = 0.0036, 0.019$, respectively). *CYP2B6* SNPs are in high LD and constitute the *CYP2B6**6 allele that was previously shown to be associated with slow methadone metabolism. No significant differences in trough plasma (*R/S*) methadone levels were identified between subjects with different genotypes of these SNPs. Support: NIDA-P60-05130 (M.J.K.) and the Adelson Medical Research Foundation.

Pharmacokinetic interaction and safety of naltrexone hydrochloride co-administered with oral opioids

N. Levy-Cooperman¹, B. Setnik², N.L. Chen¹, B. Chakraborty¹, K. Schoedel¹, M.K. Romach¹, E.M. Sellers¹, Sommerville, K², V. Goli^{2,3}, ¹Kendle Early Stage-Toronto, Canada, ²King Pharmaceuticals Inc., Cary, NC, USA, ³Duke University Medical Center, Durham, NC, USA

The effect of naltrexone hydrochloride on the pharmacokinetics of 120 mg morphine, 60 mg oxycodone, and 60 mg hydrocodone in healthy recreational drug users was investigated in 4 randomized blinded crossover studies (N= 98). Co-administration of increasing doses of naltrexone with 120 mg morphine dose dependently increased C_{max} , $AUC_{0-8\text{ hr}}$ and AUC_{0-inf} . Compared to morphine alone, percentage increase in peak and extent of exposure ranged between 7.9 - 35% for naltrexone doses ranging from 2.4 mg to 38.4 mg. Naltrexone also delayed T_{max} (up to 16%) and decreased clearance (5-21%). Similarly, co-administration of 2.4 to 14.4 mg naltrexone with 60 mg oxycodone resulted in a 16-32% increase in C_{max} , a 17-26% increase in $AUC_{0-8\text{ hr}}$, and a 6-19% increase in AUC_{0-inf} . The pattern of increase in C_{max} was also noted to a lesser extent (8-

17%) following administration of 60 mg hydrocodone with 2.4 to 7.2 mg naltrexone. Naltrexone did not substantially affect other pharmacokinetic parameters in these studies. In general, the incidence and severity of adverse events for all 3 opioids decreased with increasing doses of naltrexone. Naltrexone has previously been shown to increase the bioavailability of morphine but this has not yet been reported with other opioids. Despite the increase in plasma concentration, administration of naltrexone was associated with a reduction of opioid-induced subjective reports of drug-liking and high. The current studies found modest increases in the bioavailability of morphine, oxycodone, and hydrocodone suggesting a weak interaction. This finding may be the result of naltrexone displacing protein-bound opioids and saturating binding sites or possibly a result of accelerated gastric emptying. Changes in opioid bioavailability should be considered when planning bioavailability or bioequivalence studies of opioids in healthy volunteers where naltrexone blockade is often used.

Endorphin peptide & glycopeptide analogues with helix address domains provide potent antinociception in mice

Y. Li¹, M. Lefever¹, D. Muthu¹, C. M. Kirkmire³, D. Giuvelis², J. M. Bidlack², E. J. Bilsky³, and R. Polt¹,
¹Univ. Arizona, Tucson AZ, USA ²Univ. of Rochester Medical Center, ³Univ. of New England, USA

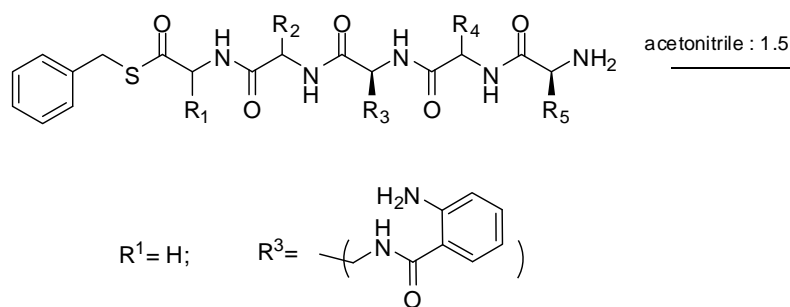
Opioid SAR of peptides related to endorphin or dynorphin has provided a rational & powerful approach toward the design of peptide therapeutics. Analogues had modified address domains with altered intrinsic helix stabilities. Unglycosylated peptides & glycopeptides bearing mono- & disaccharides were studied. The endorphin analogue ¹Tyr-²D-Thr-³Gly-⁴Phe-⁵Leu-⁶Pro(Linker)-⁷Asn-⁸Leu-^hAib-^cGlu-^cLys-^hAla-¹³Leu-^cLys-¹⁵Ser[β-O-Glc]-¹⁶Leu-NH₂, was modified at the indicated positions and binding affinities K_i measured using human receptors expressed in CHO cells. All the peptides and glycopeptides were pan-agonists, showing low nanomolar affinity for all 3 opioid receptors. Helix stability was altered by substituting ^hAib, ^hAla, and ^hGly, which alters membrane affinity which is correlated with helix stability. Charges on the address side chains were altered by substituting ^cAsn, ^cGlu, and ^cLys. The ¹⁵Ser residue bore either a lactoside, a glucoside or was unglycosylated. Peptides were studied by CD and by NMR in H₂O (pH = 5.5), in TFE/H₂O, or SDS micelles as a membrane model. In H₂O the glycosylated analogues showed nascent helix behavior and random coil conformations. Chemical Shift Indices and NOE confirmed helical structures in the presence of membrane mimics. Detailed backbone conformations were determined using distance

constraints provided by NOE volumes. Based on the CD experiments, most of the endorphin glycosides showed substantial helicity in the presence of micelles. Several glycopeptides demonstrated BBB penetration and produced potent antinociceptive effects in mice after *i.v.* injection. The amphipathic address played a major role in BBB penetration, as reflected by the *i.v.* activities. Acknowledgement: We thank the Office of Naval Research (N00014-05-1-0807 & N00014-02-1-0471), the National Science Foundation (CHE-607917) and the National Institute of Neurological Disorders and Stroke (R01-NS52727).

Fluorescent opioid peptides from a cyclic peptide combinatorial library

Y. Li, M. Cazares, J. Thompson, J. Misler, R. Houghten and C. Dooley, Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA

A positional scanning library of 30,420 cyclic peptides was prepared using a pentapeptide thioester scaffold on mercaptomethylphenyl-functionalized silica gel. Positions R¹ and R³ were fixed with glycine and Dap (diaminoproprionic acid; for subsequent addition of 2-amino benzoic acid). Positions R² and R⁴ contained 36 L and D- amino acids and position R⁵ contained 19 L- amino acids. Cyclization was performed in a mixture of acetonitrile and 1.5 M aqueous imidazole solution at room temperature for 5 days. The library was screened in binding assays for all three opioid receptors. Activity was greatest in mu and delta receptors. Combinations of amino acids found at each position yielded active cyclic peptides ($K_i = 16$ nM). Screening profiles of the cyclic peptide library, the individual sequences identified, selectivity and signaling behavior of these fluorescent peptides will be presented.



This work was funded in part by: NIH RO3 DA025850, NSF funding (CHE0455072) and the State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development

Mu-opioid receptor desensitization in the ventral tegmental area

J. D. Lowe and C. P. Bailey, University of Bath, Department of Pharmacy & Pharmacology, Bath, UK. A primary site for opioid-induced euphoria is activation of mu-opioid receptors (MOPrs) in the ventral tegmental area (VTA). The causes and mechanisms of tolerance to opioid-induced euphoria are poorly understood, but one hypothesis for tolerance in general is agonist-induced MOPr desensitization. In this study we have investigated MOPr desensitization in the VTA. In the VTA, MOPrs are located on GABAergic interneurons both somatodendritically (ie. on cell bodies and dendrites) and on nerve terminals that innervate dopaminergic neurons. Using whole-cell patch-clamp electrophysiological methods, we have investigated agonist-induced desensitization of both populations of MOPrs in mouse VTA slices. In common with many other neuronal cell-types, agonist-induced MOPr desensitization is readily seen at VTA cell bodies, where the DAMGO-induced response declined by approximately 50% during a 10-minute application. In contrast, we have consistently failed to observe agonist-induced MOPr desensitization at MOPrs located at nerve terminals. By recording miniature inhibitory post synaptic currents (mIPSC) from dopaminergic neurons, both morphine (30 microM) and DAMGO (10 microM) inhibited mIPSC frequency, an effect that was sustained during 10-minute applications, even in conditions where there was no receptor reserve. Evoked IPSCs (eIPSC) were also measured in the presence of potassium channel inhibitors tertiapinQ (250nM) and barium chloride (1mM) to eliminate contributions from cell body MOPrs. Again, the inhibition of eIPSC amplitude produced by either DAMGO or morphine did not desensitize over 10 minutes. In other cell types, morphine mediated desensitization can be enhanced by Protein Kinase C (PKC) activation, however, even after PKC activation with the phorbol ester, PMA (1 microM), MOPrs at the nerve terminal did not desensitize. These findings suggest that, in the mouse ventral tegmental area, 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).

Morphine-induced mu-opioid receptor mediated desensitization of GIRK conductance in locus coeruleus neurons of RMOR mice.

A. Madhavan, J. L. Whistler, Ernest Gallo Clinic & Research Center, Emeryville, California 94608, USA. Recently, we generated a novel knock-in mouse that expresses a mutant form of the mu opioid receptor (MOR) that undergoes endocytosis and recycling in response to activation by morphine (RMOR). Here, we examined receptor activation, desensitization and

resensitization following activation of the MOR and RMOR receptors with, met-enkephalin and morphine, in neurons of the locus coeruleus (LC). We find that the potency and efficacy of morphine and met-enkephalin are indistinguishable in wild type (WT) and RMOR LC neurons. However, while application of a saturating dose of morphine (30 μ M) induces little desensitization in WT LC neurons, the same dose induces significant desensitization of the GIRK conductance in RMOR neurons. We then assessed recovery from desensitization (resensitization) of the GIRK conductance in WT and RMOR mice. To examine resensitization, 30 μ M of either the endogenous ligand met-enkephalin or morphine, was applied for 10 minutes. Next slices were treated with 300 nM β -FNA, a membrane impermeant irreversible MOR antagonist to silence all remaining surface receptors. Resensitization of MOR was then tested 5 and 30 minutes later with application of 30 μ M of met-enkephalin. Both WT and RMOR showed resensitization following treatment with met-enkephalin and β -FNA, suggesting that a pool of receptors is endocytosed and recycled in response to met-enkephalin in both genotypes. However, only RMOR showed resensitization following treatment with morphine and β -FNA. Together, these data suggest that endocytosis in response to morphine produces a protected pool of receptors in the RMOR mouse that are recycled and resensitized in the LC. This work was supported by funds from the state of California for research on alcohol and substance abuse and NIDA grants DA019958 to J.L.W. A.M. is supported by an individual NRSA F32DA027286.

Truncated MOR-1 splice variants: targets for potent opioid analgesics lacking side-effects

S. Majumdar¹, S. G. Grinnell¹, V. Le Rouzic¹, M. Burgman¹, L. Polikar¹, M. Ansonoff², Y. Xiang Pan¹, J. E. Pinter² and G. W. Pasternak^{1,2}, ¹Laboratory of Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, NY, NY 10065, USA, ²Department of Cell Biology and Neuroscience, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, USA

We have been particularly interested in the vast array of splice variants of mu opioid receptors (MOR-1) gene (Oprm1). There are two primary classes of MOR-1 splice variants generated by different promoters. The primary promoter is associated with exon 1 and generates a large number of traditional 7 transmembrane domain receptors. The second promoter, associated with exon 11 generates a number of truncated, 6 transmembrane domains. We have previously shown that, heroin and M6G analgesia is present in exon 1 KO animals while it is markedly diminished in the exon 11 KO animals, indicating a role of truncated MOR-1 splice variants

in the analgesic response of these drugs. We now report another novel exon 11-associated binding site remaining in the exon 1 KO mice, but lost in the exon 11 KO mice using a novel radioligand ¹²⁵I-BNtxA synthesized in our laboratories. IBNtxA is an effective analgesic. This analgesia persists in the exon 1 KO mice, but is lost in the exon 11 KO mice. The selectivity of this site is quite unique, with morphinans and benzomorphans showing very high affinity while drugs like morphine and the antagonist naloxone bind very poorly. This selectivity is supported by antagonist studies in vivo. Preliminary studies indicate that IBNtxA lacks respiratory depression, significant constipation, physical dependence or reward. It shows no cross tolerance to morphine and can be given to morphine-dependent mice without a decrease in its analgesic actions or the precipitation of withdrawal. Thus, this ligand comes close to the ideal opioid analgesic through a novel receptor target comprising truncated 6 transmembrane domain splice variants of the mu opioid receptor MOR-1 and can be used as a lead compound to design a library of opioid analgesics. Supported by DA0641, DA02615 and DA00220.

Opioid-sensitive GABA inputs from RMTg neurons synapse on midbrain dopamine neurons

A. Matsui and J. T. Williams, Vollum Institute, Oregon Health and Science University, Portland, OR, USA

All drugs of abuse increase the activity of midbrain dopamine neurons. Opioids increase dopamine neuron activity through disinhibition mediated by a hyperpolarization of GABAergic neurons. However, the specific GABAergic neurons projecting to dopamine neurons are still not clear. Increasing evidence suggests that GABAergic neurons in Rostromedial Tegmental Nucleus (RMTg) also known as tail of VTA project to midbrain dopamine neurons and these neurons express mu-opioid receptor. RMTg neurons projecting to the ventral tegmental area (VTA) were identified using retrograde tracers injected into the VTA. The labeled RMTg neurons were characterized by whole-cell current clamp recordings. RMTg neurons were hyperpolarized by DAMGO and Met-Enkephalin. In addition, electrical and channelrhodopsin2 stimulation in RMTg evoked GABA IPSCs in dopamine neurons. The amplitude of GABA IPSCs was reduced upon DAMGO application. Thus, the GABAergic neurons in RMTg project to dopamine neurons in the VTA and are sensitive to opioids. Supported by DA08163

DOR-KOR heteromer signaling in peripheral sensory neurons

B. A. McGuire, W. P. Clarke, and K. A. Berg, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX, USA

Several studies have demonstrated that delta opioid (DOR) and kappa opioid receptors (KOR) can form heteromers. 6'-guanidinonaltrindole (6'GNTI) has been reported to be a selective DOR/KOR heteromer agonist which produces analgesia when administered into spinal cord, but not brain, supporting the notion that heteromer-selective ligands will provide improved specificity. In this study, we sought to determine if, in peripheral sensory neurons, DOR and KOR form heteromers. We also compared the signaling characteristics of the heteromer agonist, 6'GNTI, to those of selective agonists for the individual protomers, DPDPE (DOR) and U50488 (KOR). Co-immunoprecipitation and signaling experiments were done in primary cultures of adult rat sensory neurons. Following cell surface crosslinking and immunoprecipitation with KOR antibody, a single, 120kd immunoreactive band for DOR was visualized with western blot. The signaling characteristics were determined for inhibition of adenylyl cyclase (AC) activity and activation of Extracellular Signal-Regulated Kinase (ERK). All three agonists produced an inhibition of AC in a pertussis toxin (PTx, 24h, 400ng/ml) sensitive manner. The DPDPE AC response was blocked by the DOR antagonist, naltrindole (20 nM), but not the KOR antagonist, nor-BNI (3nM). The U50488 AC response was blocked by nor-BNI but not naltrindole. The 6'GNTI AC response was blocked in the presence of either naltrindole or nor-BNI. All three agonists elicited ERK activation in a sustained manner (2.5 – 30 min incubation). Whereas DPDPE and U50488-mediated ERK activity was PTx sensitive, 6'GNTI-mediated ERK activity was PTx insensitive. These data indicate that, in peripheral sensory neurons, DOR and KOR form heteromers with different signaling mechanisms in comparison to the individual receptor protomers. Targeting opioid heteromers on peripheral sensory neurons may provide new approaches for the pharmacological treatment of pain. Supported by DA026619 and DA024865

Differential KOR agonist-induced activation of ERK1/2 MAP kinase mediates paradoxical potentiation of cocaine-conditioned place preference (CPP)

J.P. McLaughlin(1,2), M.R. Hoot(2) and K. Rasakham(1,3), (1)Northeastern University, Dept. of Psychology, Boston, MA USA, (2)Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA 34987 (3)Temple University, Dept. of Pharmacology, Philadelphia, PA, USA

Acute administration of kappa opioid receptor (KOR) agonists suppresses drug reward, but prolonged exposure has been observed to paradoxically potentiate drug reward. We hypothesized that while two structurally distinct KOR agonists (U50,488 and salvinorin A) would acutely suppress cocaine-CPP, the differential ability of these agonists to activate ERK1/2 MAP kinase would correlate with their respective ability to potentiate cocaine-CPP. Initial place conditioning with equianalgesic doses of the KOR agonists alone produced conditioned place aversion, and a 30-min pretreatment with each agonist suppressed cocaine-CPP in a nor-BNI-sensitive manner. However, when the agonist pretreatment interval matched antinociceptive abatement (90 min), U50,488-pretreatment paradoxically produced a 2.5-fold potentiation of cocaine-CPP, whereas salvinorin A-pretreated mice demonstrated normal cocaine-CPP responses. Western Blot analysis of mouse brain revealed that U50,488 increased ERK1/2 MAP kinase activity only after a 90-min treatment, whereas salvinorin A did not at any time. We then examined the effects of agonists on pERK1/2 in KOR-GFP/HEK293 cells. Immunolabeling (Western blot and ELISA) experiments after timed incubations and graded doses (0.1-10 μ M) of each agonist demonstrated that both increased pERK1/2 labeling after a brief (5 min) incubation, whereas only U50,488 (not salvinorin A) increased late-phase (>15-90 min) pERK1/2 labeling in a mechanism mediated by KOR internalization. Notably, and supporting the hypothesis, pretreatment of mice with either the KOR antagonist nor-BNI or the ERK1/2 MAP kinase inhibitor SL-327 prevented the U50,488-induced potentiation of cocaine-CPP. Overall, these results suggest differential KOR agonist-induced activation of MAP kinase may mediate potentiation of cocaine-CPP. (Supported by NIDA (DA016415) and the State of Florida)

Mu and delta opioid receptor heteromerization: the importance of being trafficked.

L. Milan-Lobo, J. Enquist & J.L. Whistler, Ernest Gallo Clinic and Research Center, Department of Neurology, University of California San Francisco, UCSF, Emeryville, CA, USA

Heteromerization of mu (MOR) and delta (DOR) opioid receptors has been shown to alter opioid

receptor pharmacology and receptor trafficking. The observation that heteromerization may affect receptor trafficking is of particular relevance for heteromers of the MOR and DOR, since the MOR is primarily recycled after endocytosis and the DOR is degraded in the lysosome. We examined the endocytic and post-endocytic fate of MORs, DORs and DOR/MOR heteromers in HEK293 stably expressing each receptor alone or co-expressing both receptors. We found that the clinically relevant MOR agonist methadone promotes endocytosis of MOR but also of the DOR/MOR heteromer. Furthermore, we show that DOR/MOR heteromers that are endocytosed in response to methadone are targeted for degradation, while MORs in the same cell are significantly more stable. Importantly, we found that the DOR-selective antagonist naltriben (NTB) could block both methadone- and DAMGO-induced endocytosis of the DOR/MOR heteromers but did not block signaling from this heteromer. Together, our results suggest that the MOR adopts novel trafficking properties in the context of the DOR/MOR heteromer. In addition, they suggest that the heteromer shows "biased antagonism", whereby DOR antagonist can inhibit trafficking but not signaling of the DOR/MOR heteromer. Here we describe how we have used this biased antagonism for trafficking versus signaling to assess the role of the DOR/MOR heteromer in nociception. LML was supported by a Schrödinger fellowship from the Austrian Science Fund (FWF) (J2967-B09). This study was also supported by NIH grant DA015232-07 and funds provided by the State of California for medical research through the University of California San Francisco both to JLW.

Kappa opioid tetrapeptides from expanded deconvolution of a positional scanning library

J. Misler, M. Cazares, T. LaVoi, T. Gibbins, A. Morales, L. Maida, M. Giulianotti and C. Dooley, Torrey Pines Institute for Molecular Studies, Port St. Lucie, Florida, USA

We have previously identified novel tetrapeptides for the three opioid receptors from a single tetra-peptide positional scanning combinatorial library (1). The library contained over 13 million peptides from which we synthesized only 24 peptides to identify novel KOR ligands. The active sequences identified were all D amino acid peptides lacking an N-terminal tyrosine (D-Phe-D-Nal-D-Nle-D-Arg-NH₂). With the knowledge that the library contained additional active sequences not identified in the first screen we have used a similar tetrapeptide library (63 amino acids versus 60 in the first library) and employed a new mixture linking analysis to assist in the library deconvolution. Positional scanning deconvolution can be prohibitive when combination of all active amino acids requires the synthesis of a large number of peptides. Mixture linking analysis allowed the

identification of three clusters of active peptides from the library. The deconvolution of the tetrapeptide library, the sequences identified from the new analysis their relative activities, selectivity and agonist/antagonist behavior will be presented.

1. Dooley CT, Ny P, Bidlack JM, Houghten RA. *J Biol Chem.* 1998 Jul 24;273(30):18848-56. PMID:9668060

Enkephalinergic system is involved in cocaine induced behavioral sensitization and the associated increase in AMPA receptor surface expression in nucleus accumbens and caudate putamen

B. Mongi Bragato¹, M. A. Assis¹, M. Bartos¹, A. Zimmer³, L. M. Cancela¹, ¹IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. 5000 Córdoba. Argentina, ² Institute of Molecular Psychiatry, University of Bonn. 53105 Bonn, Germany

Opioid receptors and endogenous opioid peptides, mainly enkephalin, are largely distributed in the mesolimbic system. However, their contribution to cocaine - induced sensitization on behavioral and associated molecular parameters has been poorly studied. Male C57B/6J wild type (WT) and preproenkephalin knockout (KO pENK) mice were daily treated with cocaine (15mg/Kg i.p.) and vehicle for 9 days followed by a cocaine challenge (7,5mg/Kg) on days 15 and 21 of the treatment. The locomotor activity was measured on days 15 and 21. In another set of experiments, male C57B/6J WT mice received the same treatment but 30 min. before each cocaine injection, the animals were administered with a naloxone injection (1mg/Kg s.c.). The locomotor activity was measured on days 1, 15 and 21. On this day, mice were killed for biochemical analysis. The nucleus accumbens, striatum, hippocampus and prefrontal cortex were dissected and GluR1, dopamine transporter, ERK and CREB levels were measured by western blot. Penk KO mice did not show sensitization to the behavioral effects induced by cocaine and failed to show the cocaine-induced increases in ERK activation and AMPA cell surface expression evidenced in the WT mice. However, the locomotor activity in response to an acute injection of the drug and the levels of dopamine transporter were similar in both KO and WT mice. Wild type mice pretreated with naloxone did not show the cocaine - induced increased in ERK and CREB phosphorylation. These results indicate that preproenkephalin-derived opioid peptides, possibly through the activation of opioid receptors in mesolimbic areas, are strongly involved in the long-term plasticity underlying behavioral sensitization to cocaine. Financial Support: FONCYT, Ministerio de Ciencia y Tecnología, CONICET

Morphine resistance and its underlying mechanisms in an experimental mouse model of fibromyalgia

T. Mukae, M. Nishiyori, K. Araki, H. Ueda, Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, Japan

Fibromyalgia (FM) is a common condition with generalized or widespread allodynia that affects at least 2% of the US, European and Japanese populations. Although the etiology of this disease remains to be fully understood, physical and psychological stressors have been assumed to play a role in the development of FM. Previously, we have established a novel mouse model of FM, using intermittent cold stress (ICS) exposure (*Mol Pain*, 2008, 4, 52). This model was found to show long-lasting mechanical allodynia and thermal hyperalgesia in a female-predominant manner, as often observed in FM patients. In contrast, constant cold stress (CCS) produced a transient allodynia. Importantly, we found that anticonvulsant agent gabapentin, especially when injected intracerebroventricularly, produces potent anti-allodynic and anti-hyperalgesic effects in the ICS-exposed mice. Furthermore, we have recently found that ICS model mice show morphine resistance (*Neurosci Lett*, 2010, 472, 184-187), as often observed in FM patients. In this study, systemic or intracerebroventricular, but not intrathecal or intraplantar, injection of morphine causes no significant analgesia in the ICS-exposed mice. In addition, we found that intracerebroventricularly administered morphine increases the 5-hydroxytryptamine turnover ratio in the dorsal half of the spinal cord of control mice, but not in the ICS-exposed mice. Taken together, these results indicate that ICS model well reflects pathological and pharmacotherapeutic features of FM patients, and the loss of descending serotonergic activation seems to be a key mechanism underlying the absence of morphine-induced analgesia in the ICS model.

Involvement of long-chain fatty acid receptors, GPR40 and GPR120, in the induction of antinociception of docosahexaenoic acid

K. Nakamoto¹, T. Nishinaka¹, K. Matsumoto¹, M. Mankura², S. Tokuyama¹, ¹Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University, Japan ²Ikeda Tohka Industries Co., Ltd.

We have previously demonstrated that the n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) has an antinociceptive effect on various pain stimuli in a naloxone-reversible manner. Recently, it is reported that G-protein receptor (GPR) 40 and GPR120 can be activated by polyunsaturated fatty

acid (PUFA) including DHA. However, the relationship between pain and GPR40 or/and GPR120 is still unknown. In this study, we estimated whether GPR40 and GPR120 are involved in the mechanism underlying DHA-induced antinociceptive effect. To evaluate the antinociceptive effect of DHA and GW9508, a GPR40 and GPR120 selective agonist, we employed the tail flick test, acetic acid writhing test and formalin test in mice. DHA (15 and 75 nmol/mouse) or GW9508 (0.1 and 1.0 mg/mouse) were intracerebroventrically (i.c.v.) or intrathecally (i.t.) administered at 10 min before measurement, respectively. The i.c.v., but not i.t., injection of DHA induced significant antinociceptive effect in formalin test. Similarly, the i.c.v., but not i.t., injection of GW9508 dose-dependently reduced pain-related behavior observed in formalin test and acetic acid writhing test. In the present study, it is suggested that the antinociceptive effect of DHA may be partially mediated by GPR40 or/and GPR120 in the supraspinal area. Furthermore, these findings may provide valuable information towards a therapeutic approach for pain control. Acknowledgment: Part of this work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology for Young Scientists (B) (grant number 22791459).

Different μ -opioid receptor activation profiles of oxycodone and morphine at specific brain regions in mouse femur bone cancer pain model

A. Nakamura^{1,2}, M. Hasegawa¹, T. Katayama¹, T. Tomii¹, K. Minami¹, A. Nishiyori¹, M. Narita², T. Suzuki², G. Sakaguchi¹ and A. Kato¹, ¹Pain & Neurology, Discovery Research Laboratories, SHIONOGI Co., Ltd. 1405, Gotanda, Koka-cho, Koka-shi, Shiga 520-3423, Japan, ²Department of Toxicology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41 Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan

Oxycodone, an opioid analgesic, is prescribed to control moderate to severe pain related to cancer or neuropathy. Previous study showed that the efficacy profile of oxycodone in the mouse femur bone cancer (FBC) model does not overlap those of morphine and fentanyl. Here, mechanism of the analgesic effect of oxycodone was investigated in the FBC model. The anti-hyperalgesic effect of oxycodone was more effective compared with that of morphine in the FBC model. The anti-hyperalgesic effects of both opioids were antagonized by a μ -opioid receptor (MOR) antagonist, but not by a κ -opioid receptor antagonist, suggesting an involvement of the MOR in the anti-hyperalgesic effect. In the FBC model mice, maximal efficacy and potency of MOR activation by oxycodone and morphine were attenuated at the several brain regions compared with the sham control mice. Interestingly, the degree of attenuation differed

between the two opioids, and the MOR activation by morphine was more affected at the mediodorsal thalamus, periaqueductal gray matter (PAG) and region ventral to PAG compared with those by oxycodone. No significant difference was observed in the levels of the total MOR mRNA at those brain regions between the FBC model mice and the control mice. Our results showed that in the FBC model the activation of MOR by oxycodone is relatively unaffected compared with that by morphine at several brain regions related to the pain transmission, and suggested that this agonist dependent MOR function may be responsible for the unique analgesic effect of oxycodone.

Possible change in microRNAs associated with mesolimbic motivation/valuation circuitry under neuropathic pain

M. Narita, S. Imai, M. Saeki, M. Yanase, H. Horiuchi, M. Abe, M.T-Narita, N. Kuzumaki and T. Suzuki, Dept. Toxicol. Sch. Pharm. Pharmaceut. Sci., Hoshi Univ., Tokyo, Japan

Neuropathic pain is the most difficult type of pain to control, and patients lose their motivation with a decrease in their quality of life. Using a functional magnetic resonance imaging analysis, we demonstrated that blood oxygenation level-dependent signal intensity was increased in the ipsilateral nucleus accumbens (N.Acc.) in nerve-ligated mice, indicating that neuropathic pain induces neuronal plasticity in the mesolimbic dopaminergic system. microRNAs (miRNAs) are small, noncoding RNA molecules that direct the post-transcriptional suppression of gene expression, and play an important role in regulating synaptic plasticity. In this study, we found that sciatic nerve ligation induced a drastic decrease in the expression of miR200b and miR429 in N.Acc. neurons. The expression of DNA methyltransferase 3a (DNMT3a), which is the one of the predicted targets of miR200b/429, was significantly increased in the N.Acc. at 7 days after sciatic nerve ligation. Double-immunolabeling with antibodies specific to DNMT3a and NR1 showed that DNMT3a-immunoreactivity in the N.Acc. was located in NR1-labeled neurons, indicating that increased DNMT3a proteins were dominantly expressed in post-synaptic neurons in the N.Acc. area under a neuropathic pain-like state. The results of these analyses provide new insight into epigenetic modification that is accompanied by a dramatic decrease in miR200b and miR429 along with the dysfunction of "mesolimbic motivation/valuation circuitry" under a neuropathic pain-like state. These phenomena may result in an increase in DNMT3a in neurons of the N.Acc. under neuropathic pain, which leads to the long-term transcription-silencing of several genes.

Effects of morphine on acetic acid-induced suppression of appetitive and reward related behaviors in mice

H. Neelakantan (1), S.J. Ward (1), E.A. Walker (1), (1) Department of Pharmaceutical Sciences, Temple University, Philadelphia, USA

Pain, an affective state, is known to depress behaviors and the reversal of these behaviors is an important marker for the efficacy of pain medications. In preclinical rodent models, pain states can depress behaviors such as locomotion, feeding and operant responding for positive reinforcers. In the present study, we hypothesized that while acetic acid-induced acute nociception will suppress the rate of responding for food and the rewarding effects of drugs, morphine will reverse the acetic acid-induced effects. To study the effects of exposure to acetic acid nociception on food reward, mice were trained to respond for a 50% Ensure solution under a fixed ratio 10 schedule of reinforcement. Response rates were measured following IP injection of saline, acetic acid (0.2-0.4%), morphine (3.2-10 mg/kg) and a combination of 0.4% acetic acid and 3.2 mg/kg morphine. Results demonstrated that: a) acetic acid produced concentration-dependent suppression of appetitive responding in mice; b) low to intermediate doses of morphine alone maintained intermediate to high response rates; and, c) pretreatment with morphine reversed the acetic acid-induced attenuation of response rates for food reward. Morphine and cocaine reward were measured under the influence of chemical nociception using the condition place preference (CPP) procedure. Following pretreatment with either water, acetic acid, or a combination of morphine and acetic acid, the mice were conditioned for 6 days with saline and drug on alternative sides of the CPP chambers. Results demonstrated that while acetic acid significantly and selectively attenuated conditioned morphine but not cocaine reward, pretreatment with morphine showed a trend towards reversing the depressive effects of acetic acid on contextual reward. In conclusion, assessment of pain-depressed behaviors in mice may be a useful measure for determining the role of pain as a subjective state on various behaviors including the propensity for future drug reward. (Supported by Peter F. McManus Charitable Trust)

Effects on proopiomelanocortin (POMC) expression and conditioned place aversion during protracted spontaneous withdrawal from chronic intermittent escalating-dose heroin in POMC-EGFP promoter transgenic mice

K. Niikura, Y. Zhou, A. Ho, M. J. Kreek, Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY, USA

In opiate-dependent individuals, heroin “highs” are inexorably followed by a severe negative emotional state (e.g. dysphoria, anxiety, irritability, aversion) when access to the drug is prevented (withdrawal state). In this study, we examined both POMC expression in hypothalamic and extra-hypothalamic regions, using POMC-EGFP bacterial artificial chromosome (BAC) transgenic mice, and heroin withdrawal-induced aversion (using conditioned place aversion, CPA) during withdrawal from chronic escalating-dose heroin administration (3x2.5 mg/kg/day on day 1 up to 3x30 mg/kg/day on day 14). The CPA pre-test was conducted before the drug exposure. In conditioning sessions, mice were confined for 30 min to the conditioning chamber following 12 h withdrawal from the last heroin injection across days 11–14. The CPA post-tests were conducted in three separate groups of POMC-EGFP (+) and (-) mice at 12-hour, 7-day or 14-day of withdrawal from chronic heroin exposure. Immunohistochemistry for ACTH- or beta-endorphin-immunoreactivity demonstrated ~90% colocalization with the EGFP-expressing neurons in the arcuate nucleus of hypothalamus (ARC). The POMC-EGFP neurons were visualized in the basomedial amygdala (BMA), nucleus accumbens and caudate-putamen, in addition to those in the ARC and dentate gyrus of the hippocampus. In 12-h acute withdrawal, both POMC-EGFP (+) and (-) mice displayed significant CPA, an effect persistent into chronic 14-day withdrawal. Chronic 14-day withdrawal from 14-day escalating-dose heroin resulted in an increased number of POMC-EGFP cells in the BMA, but not ARC. Our results suggest that increased POMC expression in BMA in withdrawal after 14-day chronic heroin is associated with negative emotional behavior. 3, 6 diacetylmorphine HCl (heroin) was generously provided by NIH-NIDA Division of Drug Supply and Analytical Services. NIH-NIDA P60-DA05130 (MJK)

Differences in opioid peptide levels in Wistar rats from five different suppliers

S. Palm, E. Roman, I. Nylander, Dept Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

The opioid system is involved in ethanol-induced reward and in the development of addiction but there is a gap in our knowledge of effects on opioids after long-term voluntary ethanol intake. We have previously shown that there are profound differences in voluntary ethanol intake within the Wistar strain depending on supplier. Therefore, it was of great interest to examine endogenous opioid peptides in rats from these suppliers. Dynorphin B and met-enkephalin-Arg⁶Phe⁷ were measured in naïve animals and in ethanol-drinking animals. Voluntary ethanol consumption over six weeks in outbred Wistar rats from BK Universal UK, Charles River Germany, Harlan Laboratories US, Harlan Laboratories the Netherlands and Taconic Denmark was measured using an intermittent access paradigm. Age-matched water drinking controls from each supplier were also included. Different brain structures and the pituitary gland were dissected and the levels of opioid peptides were analyzed using radioimmunoassay. Similar ethanol induced effects were found in brain areas related to reward pathways in most groups. The group that had the lowest ethanol intake generally showed little or no ethanol-induced effects. Basal differences were found in for example the hypothalamus, the pituitary and the hippocampus, indicating differences in the hypothalamic-pituitary-adrenal axis. In the hypothalamus, the ethanol-induced effects differed between groups. The results show that although all rats are of Wistar origin, the levels of opioid peptides are different in certain areas, both basally and after voluntary ethanol consumption. The choice of Wistar can therefore have implications for the outcome and make comparisons between studies difficult. The present findings highlight an important parameter to consider when planning and performing preclinical animal studies in the field of addiction research. Funded by the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (SRA 07-21:3; E.R.) and the Swedish Medical Research Council (K2008-62X-12588-11-3; I.N.).

Detection of nor-BNI in mouse brain weeks after administration using LC-MS/MS

K. A. Patkar, M. L. Ganno, H. D. Singh, N. C. Ross and J. P. McLaughlin, Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA

Therapeutic development of the kappa opioid receptor (KOR) selective antagonist nor-BNI has been hampered by prolonged pharmacological activity (days to weeks), the mechanisms of which are currently being investigated. We evaluated the potential accumulation of nor-BNI in brain tissue following direct intracerebroventricular (i.c.v., 1-100

nmol) or systemic intraperitoneal (i.p., 1-10 mg/kg) administration to C57Bl/6J mice, determining if the chronic presence of this compound in brain matched the prolonged duration of KOR antagonist activity *in vivo*. Additional mice were administered saline or the short acting, non-selective opioid antagonist naloxone (100 nmol i.c.v.). In the mouse warm-water tail-withdrawal assay, a single administration of nor-BNI (30 nmol i.c.v., 10 mg/kg i.p. or greater) significantly antagonized U50,488-induced antinociception at least 7 d, whereas naloxone- (100 nmol i.c.v. or 10 mg/kg i.p.) mediated antagonism lasted less than 24 h. The mice were euthanized at various time points ranging from 30 min to 21 days post administration, and brain extracts prepared from isolated tissues using a simple organic extraction for LCMS/MS analysis. Nor-BNI was detected in brain up to 21 days after a single i.c.v. injection of 30 or 100 nmol, and up to 24 h following administration of a 10 nmol dose. Likewise, nor-BNI was detected in brain 6 h after an i.p. administration of 10 (but not 1) mg/kg. In contrast, naloxone was not detected in brain after 6 h. Additional data will discuss comparison of mouse brain and plasma from same animal for the presence of nor-BNI and naloxone post administration. However, to the best of our knowledge, this is the first direct physical determination of nor-BNI in brain after pretreatment. The continued presence of nor-BNI (but not naloxone) in mouse brain days after a single injection correlates with the prolonged antagonism of U50,488, and may offer insight into the long duration of KOR antagonism mediated by nor-BNI. (Supported by the State of Florida.)

Clinically insignificant QTc changes among former opiate addicts during first years of Methadone Maintenance Treatment (MMT)

E. Peles¹, S. Linzy² M.J. Kreek³, M. Adelson^{1,2,3}, ¹Dr. Miriam and Sheldon G. Adelson Clinics for Drug Abuse Treatment and Research, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, and ²La Vegas, NV, USA, ³The Rockefeller University, New York, NY, USA

Because of the suspected but unproven risk for TdP due to QTc prolongation in MMT patients, we prospectively studied QTc changes during MMT from admission. Between Oct/2007- Dec/2010, 435 new patients were admitted to the two Adelson clinics - Tel Aviv (TA) and Las Vegas (LV) (TA-101, LV-334). Of them 345 were included, excluding 64 admitted from other MMT, and 26 with no ECG, or ECG >28 days in MMT. The study included 3 groups: (A) 215 addicts who underwent an ECG for QTc before 1st dose; (B) 46 patients whose ECG was done following 1-28 days in MMT; and (C) 84 addicts who had used illicit methadone prior to their admission. QTc values were restudied before the first

take-home dose (steady dose and negative urines for opiates, cocaine, BDZ, cannabis, amphetamines for at least 3 months), or at steady methadone dose (at least 3 months and negative urines for opiate and cocaine), and then annually. At admission mean \pm S.D QTc was 410.0 \pm 19.5ms in group A; 425.4 \pm 23.5ms in group B; and 417.7 \pm 21.1ms in group C. No patient had QTc >500ms. Following 10.1 \pm 7.2 months, 91 patients were restudied. Mean QTc increased significantly in all the 3 groups with no differences amongst them (repeated measures, time p <0.0005, groups p =0.5); 428.8 \pm 25.6ms in 41 patients from group A, 435.7 \pm 19.5ms in 20 patients from group B, and 431.6 \pm 18.8ms in 30 patients from group C. No patient had QTc >500ms. Their mean stabilized methadone dose was 117.7 mg/d, median 120, (range: 28-210mg/d). No one died during study period. However, one patient who had never become stabilized during treatment left at his request was given no further methadone doses and committed suicide 2 weeks later. We conclude that there is significant QTc prolongation during early MMT, but this prolongation has no obvious clinical significance. Support: Adelson Family Foundation

Differential desensitization of pre- and postsynaptic mu opioid receptors regulating POMC neurons

R. L. Pennock and S. T. Hentges, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Mu opioid receptors located on terminals presynaptic to proopiomelanocortin (POMC) neurons are not desensitized by acute application of opioid agonists. In the present study, whole-cell voltage clamp recordings were made in POMC neurons of the arcuate nucleus to closely examine pre- and postsynaptic desensitization in response to the application of opioid agonists. Application of a high dose of [Met5]-enkephalin (ME) induced an outward current in the postsynaptic cell as well as a robust inhibition of the amplitude of evoked inhibitory postsynaptic currents (eIPSCs). After ten minutes of continuous superfusion of ME the outward current had desensitized to near control levels, however the presynaptic inhibition of eIPSC amplitude showed no such desensitization. Similar effects were observed with the partial mu agonist morphine. As has been shown in previous studies, the lack of presynaptic desensitization also persisted after 5 days of chronic treatment with morphine and after reduction of receptor number through beta-chlornaltrexamine treatment in POMC neurons. Together these data suggest that neither rapid receptor recycling or large receptor reserve are likely to explain the lack of acute desensitization of presynaptic receptors. Similar to the mu receptor, kappa opioid receptors presynaptic to POMC neurons also show no acute desensitization.

Altogether these findings suggest that pre- and postsynaptic opioid receptors may play differing roles in the development of tolerance and withdrawal from chronic opioid treatment. Interestingly, differences in pre- and postsynaptic desensitization extend to other Gi coupled GPCRs regulating POMC neurons, including GABA_B and ORL1 receptors. Exploring the similarities between these receptors may provide a means for determining whether differential coupling or covalent modifications may underlie differences in desensitization of Gi coupled GPCRs found in distinct cellular compartments. NIH Grant R01DK0798749

Hypothalamic KOP-r and MOP-r expression in Fischer and Lewis rats after dose escalation preference paradigm of heroin self-administration

R. Picetti, A. Ho, and M. J. Kreek, The Rockefeller University, New York, NY, USA

During the withdrawal from drugs of abuse, dysregulation of HPA axis occurs in humans and rodent models. Rats that self-administer drugs of abuse over extended periods of time increase the number of infusions over time. Recently, we published a new self-administration paradigm whereby rats choose between different doses of a drug and escalate dose as well as number of infusions. Using this model, we measured the mRNA levels of MOPr and KOPr in the hypothalamus of Fischer and Lewis self-administering heroin. Rats were trained to self-administer heroin (50 ug/kg/injection) in two-hour daily sessions (10-14 d). After acquisition, rats were exposed to extended (18h) self-administration sessions for 14 days. Rats had access to two active levers associated with two doses of heroin. If a rat preferred the lever associated with the higher dose for two consecutive days, then a higher dose was available. During escalation, four heroin doses were used ranging between 20 to 250 ug/kg/infusion. Total RNA was isolated from each hypothalamus, and gene expression was assessed by RT qPCR. After 14 days, Lewis rats escalated the total amount of heroin infused per day, whereas Fischer rats minimally escalated the unit dose and, in general, self-administered very low quantities of heroin. Lewis and Fisher rats self-administered an average of 5.2 \pm 0.9 mg/kg/session and 1.3 \pm 0.2 mg/kg/session of heroin, respectively. MOPr and KOPr mRNA levels in controls and in rats preferring to self-administer 50 ug/kg/infusion were lower in Lewis than in Fischer rats. Two-way ANOVA (strain x drug) shows a significant main effect of strain for both genes, with MOPr and KOPr mRNA levels being lower in Lewis than Fischer rats in both heroin self-administering rats and controls. In conclusion, Lewis rats have lower levels of MOPr and KOPr mRNA than Fischer rats and self-administered more heroin. This work was supported by a NIH-NIDA

P60-DA05130 grant and the Carson Family Charitable Trust. Heroin was generously provided by NIH-NIDA Division of Drug Supply and Analytical Services.

Regulation of Tat-mediated neurotoxicity and glial inflammatory signaling by CCR5 and the mu-opioid receptor

E.M. Podhaizer (1), Y. Zhang (2), T.E. Prisinzano (3), P.E. Knapp (1,4), K.F. Hauser (1), Depts. of (1) Pharmacol. & Toxicol., (2) Med. Chem., (4) Anat. & Neurobiol., Virginia Commonwealth University, Richmond, VA 23298, (3) Dept. of Med. Chem., The University of Kansas, Lawrence, KS, USA

The CCR5 receptor is critical to HIV-1 infection through interaction with HIV-1 gp120, but may also be an important mediator of HIV-1 Tat signaling, which elevates the CCR5 ligand, RANTES in glia. Additionally, mu-opioid receptor and CCR5 interactions could influence the exacerbation of neurotoxicity seen with combined morphine and Tat treatments. We hypothesized that inhibition of CCR5 would suppress Tat-mediated neurotoxicity by interfering with glial inflammatory signaling and may modify the response of opioid convergent effects. To address this hypothesis, co-cultures of neurons and glia were subjected to morphine (500 nM) and Tat (100 nM) alone or in combination, and treated with and without the CCR5 antagonist Maraviroc (50 nM). Time-lapse microscopy over 72 h revealed Maraviroc suppression of neurotoxicity mediated by Tat and Tat + morphine treatments, illuminating the importance of CCR5 to Tat's effects. To elucidate the underlying mechanism(s), NF-kappaB p65 nuclear translocation was assessed due to its importance in astrocyte-mediated inflammatory signaling. Maraviroc suppressed Tat-induced p65 translocation at 6 and 12 h time points. Additionally, while morphine did not affect p65 activation by Tat, full internalizing and non-internalizing mu-opioid receptor agonists, DAMGO and herkinorin respectively (1 microM each), suppressed Tat's response at 12 h but not 6 h. This result infers that mu-opioid receptor signaling converges with Tat to modulate the maintenance of Tat-mediated inflammation, but not response induction. Herkinorin had divergent effects on chemokine release by Tat, suppressing MCP-1 production, while elevating RANTES release, suggesting alternative transcriptional regulation of inflammatory signaling. Ongoing studies are aimed at examining interactions between mu-opioid signaling and Tat's actions through CCR5. Support: NIH P01 DA019398 & T32 DA007027

Repeated morphine administration alters contextual learning, synaptic plasticity, and requires phosphorylation of gluR1-containing AMPA receptors in the hippocampus

G.S. Portugal, Y. Xia, J. Liu, and J.A. Morón Concepcion. Dept. of Anesthesiology, College of Physicians and Surgeons, Columbia University Medical Center, New York

The effects of drugs of abuse on the neural substrates of learning and memory plays an important role in drug addiction, and numerous studies have demonstrated that alterations of hippocampus-dependent learning by drugs of abuse can lead to context-evoked cravings, drug seeking behavior and relapse. The trafficking of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) towards and away from the synapse plays an important role in NMDAR-dependent long-term potentiation (LTP) in the hippocampus. Thus, changes in synaptic AMPAR expression by drugs of abuse may lead to maladaptive changes of both learning and memory and synaptic plasticity. In the present study, we investigated the effects of context-dependent sensitization to morphine on synaptic plasticity and synaptic AMPAR expression. We find that the association between morphine administration and the drug administration environment leads to persistent changes in behavior, neuroplasticity, and AMPAR expression. Specifically, the results demonstrate that context-dependent psychomotor sensitization to morphine increases synaptic GluR1 subunit expression, increases basal synaptic transmission, and severely disrupts hippocampal LTP. Importantly, these changes in behavior, AMPA receptor expression, and synaptic plasticity were not observed or were less robust when morphine administration was not paired with the drug administration environment. Furthermore, the infusion of a viral vector that blocks GluR1 phosphorylation in the hippocampus impairs the acquisition and expression of context-dependent behavioral sensitization. These data suggest that glutamatergic synaptic transmission in the hippocampus may play an important role in drug-induced changes to associative learning. This work was supported by NIH grant R01 DA025036 to JMC.

Variants of mu opioid receptor influence HIV viral load change in individuals before initiating HAART

D. Proudnikov (1), M. Randesi (1), M. Dorn (2), V. Yuferov (1), H. Crystal (3), A. Ho (1), J. Ott (4, 2), MJ Kreek (1) (1) Lab. of Biol. of Addict. Diseases, The Rockefeller Univ., New York, USA, (2) Institute of Psychology CAS, Beijing, China, (3) Dept. of Pathol., SUNY Downst. Med. Center, Brooklyn, USA, (4) Lab. of Statist. Genet., The Rockefeller Univ., New York, USA

The mu opioid receptor (*OPRM1*) is involved in a number of important physiological functions including modulation of HPA axis by tonic inhibition. Ligands of *OPRM1* may alter the expression of chemokines and chemokine receptors that are involved in the mechanisms of penetration of HIV-1 into the cell. We suggest that the variants of *OPRM1* may affect the pathophysiology of HIV infection. Using DNA samples of HIV positive subjects of African American, Hispanic and Caucasian ethnicities recruited for the Women Interagency HIV Study (WIHS) from 1994 to 2002 and who survived as of April 2006 (704 subjects total), we performed regression analysis of 18 genetic variants of *OPRM1* with change of viral load (VL) between two study points: admission to WIHS and start of HAART. During this interval subjects were on no-therapy, mono-therapy or combination therapy. The duration of this time interval was 30.3 ± 1.6 months in African Americans, 26.8 ± 2.2 months in Hispanics and 34.9 ± 3.3 months in Caucasians. Overall, we found significant decrease of the VL in individuals of all ethnicities between admission to WIHS and start of HAART. A significant effect of genotype on decrease of VL was found in Caucasians for variants rs1799971 (118A>G), rs510769, rs524731 and rs9479757 ($0.01 < p < 0.04$). The decline of VL was greater in individuals with at least one allele A of functional variant 118A>G. These findings may provide new insights in functionality of *OPRM1*. Supported by NIH-NIDA P60-DA05130 (MJK), NIH-NIMH MH79880 (MJK), NIH-NIMH MH076537 (HC) and NSFC from the Chinese Government 30730057 (JO). We also acknowledge NIH-NIAID supported Women Interagency HIV Study for specimens and limited clinical data.

Differential role for beta-arrestin2 in the development of antinociceptive tolerance and physical dependence in response to distinct opioid analgesics

K. M. Raehal, L. M. Bohn, Departments of Molecular Therapeutics and Neuroscience, The Scripps Research Institute, Jupiter, FL, USA
Morphine and other clinically relevant opiates effectively treat pain but also produce several undesirable side effects including analgesic tolerance and physical dependence via activation of the mu opioid receptor, a G protein-coupled receptor (GPCR). We have previously shown that mice lacking beta-arrestin2, an important GPCR receptor regulatory protein, display enhanced analgesia in response to morphine whereas other opiate agonists like methadone and fentanyl do not produce different antinociceptive responses in these animals. Therefore, we investigated whether the loss of beta-arrestin2 would also affect the development of antinociceptive tolerance and physical dependence in

an agonist-dependent manner. Beta-arrestin2 knockout mice and their wild-type littermates were chronically infused with morphine, methadone, fentanyl or oxycodone as equi-efficacious doses using subcutaneously implanted osmotic pumps, and tolerance was evaluated by measuring antinociceptive responses using a hot plate test. Physical dependence was assessed by measuring naloxone-precipitated withdrawal responses following chronic infusion with all four drugs. While beta-arrestin2 knockout mice develop very little antinociceptive tolerance in response to chronic morphine administration, tolerance develops at the same rate and to the same degree in both genotypes with methadone, fentanyl, or oxycodone treatment. Similarly, the beta-arrestin2 mice develop physical dependence to a lesser degree than wild-type mice following chronic infusion with morphine, but not methadone, fentanyl, or oxycodone at several doses tested. Collectively, these studies lend further support to the idea that the nature of the agonist can differentially promote beta-arrestin2 regulation of the mu opioid receptor. Work Supported by NIDA grants RO1 DA18860 (LMB) and F31 DA021952 (KMR)

Variants of kappa opioid receptor influence viral load of HIV positive females on HAART

M. Randesi (1), D. Proudnikov (1), M. Dorn (2), V. Yuferov (1), H. Crystal (3), A. Ho (1), J. Ott (4, 2), and MJ Kreek (1), (1) Lab. of Biology of Addictive Diseases, The Rockefeller Univ., New York, USA, (2) Institute of Psychology, CAS, Beijing, China, (3) Dept. of Pathol., SUNY Downstate Medical Center, Brooklyn, USA, (4) Lab. of Statistical Genetics, The Rockefeller Univ., New York, USA

Many studies have found cross-desensitization interactions between opioid and chemokine receptors both *in vivo* and *in vitro*. Opioids selectively promote pro- or anti-inflammatory effects depending on the involvement of mu and kappa opioid agonists, which previously were found to be acting in opposition to each other. In this study, we tested whether genetic variants of the kappa opioid receptor (*OPRK1*) may influence the outcome of HAART. The study was conducted using DNA isolated from HIV positive subjects of African American, Hispanic and Caucasian ethnicities, who were recruited by the Women's Interagency HIV Study (WIHS) from 1994 through 2002 and who survived through April 2006 (N = 704). We performed a regression analysis of 16 genetic variants of *OPRK1* with change of viral load between two clinical measurement points: start of HAART and the most recently available visit from which we received biological specimens. The duration of this interval was 95.4 ± 1.7 months in African Americans, 106.5 ± 3.5 months in Caucasians, and 108.7 ± 2.7 months in Hispanics. In Caucasians, the decline in viral load was significantly affected by

genotype for variants rs997917 and rs1365098, which are in complete linkage disequilibrium, and for rs6985606 ($0.001 < p < 0.01$). The decline of viral load was greater in subjects with at least one copy of the A allele of rs6985606, thus providing evidence of possible involvement of *OPRK1* in pathophysiology of HIV treatment. Supported by: NIH-NIDA P60-DA05130 (MJK), NIH-NIMH MH79880 (MJK), NIH-NIMH MH076537 (HC), NSFC from the Chinese Government 30730057 (JO). We also acknowledge the NIH-NIAID supported Women's Interagency HIV Study for access to these specimens and limited clinical data.

Microdialysis-mass spectrometry quantification of vasopressin in the hypothalamus and amygdala of freely moving rats

B. Reed^{1,2}, B. T. Chait², M. J. Kreek¹, ¹The Laboratory of the Biology of Addictive Diseases, ²The Laboratory of Mass Spectrometry and Gaseous Ion Chemistry. The Rockefeller University, New York, NY, USA

Stress responsivity plays a crucial role in the development of addiction to all drugs of abuse, with our understanding of this role especially prominent for heroin, cocaine, and alcohol. Rodent models of drug abuse and addiction, in both our and others' laboratories, have recently demonstrated important changes in the vasopressinergic systems in crucial stress-responsive brain regions such as the amygdala and hypothalamus. Our understanding of the roles of vasopressin, the opioid peptides, and other neuropeptides in the development of drug addiction stands to be considerably advanced with the development of robust methodology for the quantification of these molecular entities *in vivo*. We have developed innovative targeted mass spectrometry methodology combined with microdialysis for the measurement of vasopressin in select brain regions of awake, freely moving animals, with the goal of investigating the timecourse of the extracellular peptide level response to exposure to drugs of abuse. In these initial studies, we have characterized the levels of vasopressin in microdialysis experiments with probes targeting the basolateral amygdala and the paraventricular region of the hypothalamus of Fischer rats, demonstrating that the peptides are, as expected, responsive to 1 M NaCl (>10-fold increase). In preparation for future drug exposure studies, we performed mock injections of the rats in the microdialysis chambers, a procedure which is inherently stressful, and observed an increase in vasopressin levels in the hypothalamus, ranging from 150-2200%, with more modest and variable responses observed in the amygdala. These observations of vasopressin increases in response to a mock injection (with no actual injection) will serve as an important guide in interpreting any observed

increases in response to drugs of abuse in future studies. **Support:** NIH-NIDA Grant P60-DA05130 (M.J.K.) and NCRG Grant RR00862 (B.T.C.).

Oral availability of CJ-15,208, an opioid mixed agonist/antagonist analgesic with fewer liabilities *in vivo*

N.C. Ross (1), S. Kulkarni (2), J. V. Aldrich (2) and J. P. McLaughlin (1), (1) *TPIMS, Port St. Lucie, FL, USA*, (2) *Dept. of Med. Chem., Univ., of Kansas, Lawrence, KS, USA*

Opioid analgesics with a mixed agonist/antagonist activity profile such as pentazocine and nalbuphine are thought to produce antinociception with fewer liabilities such as reduced tolerance, psychostimulation and drug abuse. Recently, the cyclic tetrapeptide CJ-15,208 was characterized *in vivo* as a mixed mu-opioid receptor (MOR) and kappa-opioid receptor (KOR) agonist and short-acting (>24 h) KOR-selective antagonist using C57Bl/6J mice in the 55°C warm-water tail withdrawal assay. We hypothesized that CJ-15,208 would be orally active and that the dual opioid receptor activity of CJ-15,208 would produce antinociception with fewer liabilities of use, specifically a lack of antinociceptive tolerance, locomotor activity and reinforcing properties. Oral administration of CJ-15,208 produced dose-dependent antinociception. Liability potential was then assessed, starting with acute antinociceptive tolerance to repeated *i.c.v.* administration of morphine or CJ-15,208. Morphine pretreatment (3 nmol, *i.c.v.*) produced a 9.6-fold rightward shift in the D50 value of morphine administered a second time 8 h later (22.5 (8.48-61.9) nmol vs 2.35 (1.13-5.03) nmol initially), a demonstration of acute antinociceptive tolerance. In contrast, pretreatment with CJ-15,208 (3 nmol, *i.c.v.*) did not significantly change the dose response of subsequently administered CJ-15,208 8 h later (5.23 (2.76-9.93) nmol vs an initial D50 value of 1.82 (0.58-5.77) nmol). CJ-15,208 produced a smaller dose-dependent bidirectional effect on spontaneous locomotor activity than morphine or U50,488. Moreover, whereas morphine (10 nmol *i.c.v.*) produced conditioned place preference (CPP), and U50,488 (100 nmol, *i.c.v.*) produced conditioned place aversion, CJ-15,208 (3, 10 or 30 nmol, *i.c.v.*) did not produce significant place preference or aversion. This data suggests the potential utility of CJ-15,208 as a clinically relevant, orally available low liability analgesic (Supported by NIDA grants R01 DA018832 and DA023924).

Opioid and gp120 interactive neuropathogenesis in HIV-1

K. L. Samano (1), P. E. Knapp (2), K. F. Hauser (1), (1) Depts. of Pharmacology and Toxicology, (2) Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, 23298, USA

Opioid use and HIV-1 are globally linked epidemics and some clinical studies have demonstrated that opioid abusers show an accelerated development towards neuroAIDS. The HIV-1 coat glycoprotein, gp120, is neurotoxic alone and it is suggested that the presence of glia, especially microglia, are instrumental in this damage. While our lab has shown co-morbidity of opioids with HIV Tat through effects on glia, few studies have addressed interactive effects of morphine and HIV-1 gp120. We hypothesize that morphine will potentiate the reactive gliosis caused by the viral protein gp120. To test this in vivo, C57BL/J6 mice were treated with systemic pellets of morphine (5 mg/day) \pm naltrexone (12 mg/day) and were stereotaxically injected with gp120 (100 ng) into the striatum since it has high levels of the μ -opioid receptor (MOR) and is a preferential target of HIV. At 48-h, immunohistochemical studies co-localizing Iba-1 (a microglial marker) and 3-nitrotyrosine (a marker of nitrosative stress) revealed increases in activated microglia with morphine + gp120 treatment, but not in animals receiving morphine + vehicle injection. Additionally, the proportion of GFAP immunopositive astrocytes in which MOR was co-detected increased after morphine + gp120 treatment, but not in morphine + vehicle injected animals. This suggests that HIV-1 gp120 may alter the CNS response to opioids. Future in vitro studies will explore how neuron-glia signaling pathways involved in glial activation and inflammation modulate HIV infection in the presence of opioids. Collectively, the above findings suggest opioids and gp120 interact through novel mechanisms to influence the neuropathogenesis of HIV. Support: NIH DA019398 & DA007027

Modulation of behavioral responses to stress by opioid receptor systems

K. Sasaki (1, †), M. Shimada (1, †), Y. Kasahara (1), S. Ide (2), H. Komatsu (3), F.S. Hall (4), G.R. Uhl (4), H. Nagase (5) and I. Sora (1). (1) Dept. of Biol Psychiat, Grad. Sch. Med., Tohoku Univ., Sendai, Japan, (2) Dept. of Pharmacol, Grad. Sch. Pharmaceut. Sci., Hokkaido Univ., Sapporo, Japan, (3) Dept. of Psychiat, Grad. Sch. Med., Tohoku Univ., Sendai, Japan, (4) NIDA-IRP, Baltimore, MD., USA, (5) Dept. of Medicinal Chem. Sch. Pharmacy, Kitasato Univ., Tokyo, Japan. †These authors contributed equally to this work.

Recently, an increasing number of findings have suggested that stress responses are modulated by

opioid receptor systems. In response to physiological stressors transgenic μ -opioid receptor knockout (MOR-KO) mice displayed significantly decreased responses compared with wild-type (WT) mice. Similarly, chronic social defeat stress induced aversion to social contact in WT mice, but this consequence of psychosocial stress was decreased in MOR-KO mice. These results suggest that the μ -opioid receptor (MOR) at least partially mediates these behavioral sequelae of exposure to stressful stimuli. In addition, the selective non-peptide δ -opioid agonist SNC80 has recently been demonstrated to reduce stress responses in the forced swim test. We report here an investigation of the interactions between μ -opioid receptor and δ -opioid receptor systems in response to forced swim stress. In forced swim stress procedure, MOR-KO mice exhibited decreased immobility time compared with WT mice. Administrations of the δ -opioid receptor agonist to WT mice decreased immobility time compared to saline-treated control mice. Administration of KNT-127 to MOR-KO mice before the forced swim stress procedure enhanced the reduction of immobility time compared with saline-treated MOR-KO mice and produced a greater reduction that in KNT-127 treated control mice. These findings may suggest that activation of the δ opioid receptor has synergistic effects on the reduction of stress responses produced by the absence of μ -opioid receptors in MOR-KO mice. Supported by Grants-in-Aid from MECSS and Health Sciences Research Grants from MHLW, Japan and intramural funding from the NIDA-IRP, NIH/DHHS (GRU/FSH).

The kinetics of priming-induced functional competence of delta opioid receptors

L. Scarlota, M. Rowan, K. Berg, W. Clarke, Dept. of Pharm., Univ. of Texas Health Sci. Center, San Antonio, TX, USA

Unreliable opioid receptor analgesia in the periphery represents a major challenge in pain management; however, analgesic effects of opioids are enhanced in inflamed tissues, providing insight into how efficacy may be improved via ancillary pathways. Consistent with these findings, our lab has shown that prior administration of the inflammatory mediator, bradykinin (BK), promotes delta opioid receptor (DOR)-mediated responses in a primary culture of trigeminal ganglion (TG) neurons and a behavioral model of pain. To further characterize the kinetics of this interaction, we measured the ability of a DOR agonist, DPDPE, to reduce prostaglandin E₂ (PGE₂)-mediated responses following multiple BK pretreatment times (0-90min). In rats that received hindpaw injections of BK prior to co-administration of DPDPE and PGE₂, DPDPE attenuated PGE₂-induced thermal allodynia, but only following 15-

30min of BK pretreatment. Parallel studies in TG cultures mimicked the in vivo effects, with DPDPE producing a significant reduction in PGE2-stimulated cAMP accumulation when primed for 10-30min, but not 60min. Considering the relatively short time frame of the priming effect, we next examined if functional competence to DPDPE could be re-induced with an additional application of BK. In rats, a second injection of BK (60min after initial BK) reestablished DPDPE-mediated thermal analgesia. Similarly, reapplication of BK in TG cultures (60min after initial BK) promoted DPDPE-mediated inhibition of PGE2-stimulated cAMP accumulation. The time-dependent reduction in DOR competence following longer BK pretreatments could reflect desensitization of the BK system or BK metabolism; however, the ability of a second application of BK to re-induce DOR activity suggests the system is not refractory to the induction of functional competence and may be amenable to more prolonged maintenance. Future studies will address the mechanisms underlying priming-induced competence to identify potential adjuvants to promote persistent peripheral opioid receptor analgesia. *Supported by DE14318 (COSTAR) and DA24865*

Novel peptide and non-peptide opioid agonists lacking a positively charged nitrogen

P.W. Schiller, G. Weltrowska, I. Berezowska, T.M.-D. Nguyen, B.C. Wilkes, C. Lemieux, N.N. Chung, Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Que., Canada

All classical peptide and non-peptide opioid agonists contain a positively charged nitrogen, the ionic interaction of which with the Asp residue in the third transmembrane helix of opioid receptors has been thought to be indispensable for receptor binding and activation. Here we describe novel opioid receptor ligands derived from endogenous opioid peptides or from classical non-peptide opiates that lack a protonatable nitrogen. While substitution of novel non-nitrogenous Tyr or Phe analogues for Tyr1 in opioid peptides produced neutral high-affinity antagonists with various opioid receptor selectivities, the first known examples of delta opioid agonists lacking a positively charged nitrogen were also obtained. In particular, the negatively charged DTLET analogue Bcpp-D-Thr-Gly-Phe-Leu-Thr-OH (Bcpp = 4-[N-((4'-phenyl)-phenethyl)-carboxamido]-3-phenylpropanoic acid) turned out to be a delta-selective opioid agonist with potency comparable to that of leu-enkephalin. Elimination of the positive charge of normorphine through formylation of its nitrogen resulted in a moderately potent mu and kappa opioid antagonist. On the other hand, analogues of fentanyl and carfentanyl in which the nitrogen was replaced with a carbon ("carba"-

analogues) showed full or partial mu agonist activity with potencies that were reduced as compared to their nitrogen-containing parents, but were still significant ($K_i[\mu] = 85 - 225 \text{ nM}$). These results indicate that elimination of the positively charged nitrogen in opioid agonists may have a divergent effect on the efficacy (agonism, partial agonism or antagonism), depending on the receptor binding interactions of other moieties present in the molecule. Finally, the inability of these compounds to engage in a salt bridge may result in the stabilization of distinct receptor conformations, leading to functional selectivity with regard to receptor signaling and internalization. Supported by grants from the NIH (DA-004443) and CIHR (MOP-89716)

Regional mRNA expression of the endogenous opioid and dopaminergic systems in brains of C57BL/6J and 129P3/J mice: Strain and heroin effects

S.D. Schlussman, J. Cassin, Y. Zhang, O. Levran, A. Ho and M.J. Kreek, The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York NY, USA

We have previously shown strain and dose differences in heroin-induced behavior, reward and regional expression of *Sstr* mRNAs in C57BL/6J and 129P3/J mice. Using Real Time PCR we examined the effects of five doses of heroin on the levels of the transcripts of endogenous opioid peptides and their receptors and dopaminergic receptors in the mesocorticolimbic and nigrostriatal pathways in these same mice. Compared to C57BL/6J animals, 129P3/J mice had higher mRNA levels of *Oprk1* in the nucleus accumbens and of *Oprd1* in the nucleus accumbens and a region containing both the substantia nigra and ventral tegmental area (SN/VTA). In the frontal cortex of 129P3/J mice, lower levels of both *Oprk1* and *Oprd1* mRNAs were observed. *Pdyn* mRNA was also lower in the caudate putamen of 129P3/J mice. Strain differences were not found in the levels of *Oprm1*, *Penk* or *Pomc* mRNAs in any region examined. Within strains, complex patterns of heroin dose-dependent changes in the levels of *Oprm1*, *Oprk1* and *Oprd1* mRNAs were observed in the SN/VTA. Additionally, *Oprd1* mRNA was dose-dependently elevated in the hypothalamus. Also in the hypothalamus, we found lower levels of *Drd1a* mRNA in 129P3/J mice than in C57BL/6J animals and lower levels of DAT (*Slc6a3*) mRNA in the caudate putamen of 129P3/J animals than in C57BL/6J counterparts. Heroin had dose-related effects on *Drd1a* mRNA in the hypothalamus and on *Drd2* mRNA in the caudate putamen. Significant strain- and region-specific correlations were found between *Oprk1* mRNA levels and heroin-induced locomotion and between both *Pdyn* and *Penk* mRNA levels and heroin-

induced conditioned place preference. Acknowledgements: 3, 6 diacetyl-morphine HCl was generously provided by NIH-NIDA Division of Drug Supply and Analytical Services. This work was supported by grants from NIH-NIDA (DA05130), the Arcadia Charitable Trust and The Carson Family Charitable Trust to MJK

Dynorphin gene expression in the amygdala after stress exposure

J. T. Silveira, S. Gouty, G. Bull, and B. M. Cox, Dept of Pharmacology, Uniformed Services University, Bethesda MD 29814, USA.

The role of dynorphin (DYN) in the aversive response to severe stress is unclear, although it has long been known that there is significant expression of the DYN gene in the amygdala, a brain structure intimately involved in the elicitation of fear-induced behaviors. Dynorphin is expressed in discrete groups of neurons within the amygdala of rats, with most concentrated expression in parts of the central amygdala (CeA). The CeA is a critical output nucleus sending information from the amygdala to the peri-aqueductal gray area, a brain region regulating the expression of fear, pain, and autonomic reactions to aversive stimuli. Exposure of rats to a sustained severe stress (restraint with random mild tail-shocks for 2 hrs per day for 3 days) resulted in a significant increase 24 hr later in expression of the processed product of dynorphin gene expression, DYN A(1-8), in fibers in the lateral division of the anterior CeA, but not in the medial CeA or in more caudal parts of the amygdala. Neurons expressing the mRNA for DYN were found throughout the anterior CeA with noted expression in its lateral division. DYN A(1-8) fibers were also noted to be present in close proximity to the mu-opioid receptor-expressing neurons of the intercalated nuclei of the amygdala that are arrayed around the basolateral amygdala (BLA), although there were few DYN A(1-8) fibers in the BLA itself. The relationship of the expression of DYN to other endogenous opioids in amygdala is being evaluated. Our results suggest that DYN may play a role in the response of the brain to aversive stress. Supported by a grant from USAMRMC, #W81XWH-08-2-0575).

Effects of dextromethorphan/morphine on treatment of neuropathic pain in mice

P.-L. Tao^{1,2}, P.-H. Lee², E. Y.-K. Huang², ¹Div. of Mental Health and Addic. Med., Inst. of Popul. Health Sci., Natl. Health Res. Inst.; ²Dept. of Pharmacol., Natl. Def. Med. Ctr., Taipei, Taiwan
Neuropathic pain is chronic pain results from primary lesions or dysfunction in the nervous system. Increasing evidences indicate that inflammatory and immune mechanisms play a role in these processes. In clinic, morphine is not very effective in treating

neuropathic pain, and chronic morphine alone results in tolerance and dependence. Dextromethorphan (DM) is a well known N-methyl-D-aspartate (NMDA) receptor antagonist and has long history of clinical safety as an antitussive drug for 5 decades. Our previous studies have shown that co-administration of DM and morphine could potentiate the antinociceptive effect of morphine and also attenuated the tolerance and dependence to morphine. In the present study we have used a partial sciatic nerve ligation (PSNL) model to produce neuropathic pain in male ICR mice and investigated the effects of DM by itself or combined with morphine. Treated drug(s) was (were) administered 2 hours after surgery and twice a day for 5 days or longer. We found that: (1) Chronic morphine treatment attenuated PSNL-induced allodynia (measured by von Fey test) at a dose of 10 mg/kg, but not at 5 mg/kg (s.c.); (2) repeated administration of DM (20 mg/kg; i.p., bid) alone significantly reduced allodynia on post-operative day 5; (3) Co-administration of morphine (5 mg/kg; s.c.) and DM (20 mg/kg; i.p.) did not show greater effects than DM alone. (4) Chronic co-administration of morphine (10-20 mg/kg) + DM (20 mg/kg) for long term (more than 10 days) significantly attenuated the PSNL-induced allodynia. We also found that repeated administration of morphine, DM or co-administration of morphine and DM attenuated the PSNL-induced expression of GFAP (astrocyte activation marker), Iba1 (microglial activation marker), inducible nitric oxide synthase (iNOS), at L4-L6 spinal cord (determined by immunofluorescence). Therefore, the beneficial effect of chronic morphine or DM on the development of allodynia may be related to its effect on inhibiting the activation of glia cells. (supported by NSC-97-2320-B-016-005-MY3, Taiwan)

Regulation of prodynorphin expression in human brain: Transcription factors targeting SNPs associated with alcohol dependence

M.M. Taqi, I. Bazov, H. Watanabe, T. Yakovleva, G. Bakalkin, Dept. Pharmaceut. Biosci., Uppsala Univ., Sweden

Several *PDYN* SNPs are associated with alcoholism. Here we analyzed whether three (rs1997794, rs6045819, rs2235749) of them showing high significance of association, may serve as targets for transcription factors (TFs) regulating *PDYN* in human brain. First, we demonstrated that the T allele of *PDYN* promoter SNP (rs1997794) resides within noncanonical AP1-binding element, and may be targeted by AP1 TF. The T and C alleles of this SNP differ in AP1 DNA-binding affinity. Analysis of human brain AP1 that interacts with *PDYN* demonstrated that the complex consists of JUND and FOSB, the dominant AP-1 constituents in this tissue. The C allele of this SNP forms a CpG site that is

methyated at low levels in the human brain. Evaluation of association of the promoter SNP variants with *PDYN* expression in brain of human alcoholics and controls using the principal component analysis suggested that *PDYN* expression in the dl-PFC may be related to alcoholism, while in the hippocampus may depend on the genotype. Analysis of the exon 4 SNP (rs6045819) demonstrated that its C allele forms a noncanonical E-box which may be targeted by USF2 TF in human brain. CpG site formed by this allele was methylated in human brain but a limited number of subjects precluded analysis of its influence on *PDYN* expression. The T allele of 3'-UTR SNP (rs2235749) forms T-box, an E-box variant and may be targeted by the 63 kDa, T allele specific binding factor which has differential binding affinity for T and C alleles. This SNP also forms a CpG site, which methylation is elevated in dl-PFC of human alcoholics, where it positively correlates with *PDYN* mRNA and peptides. Thus, *PDYN* SNPs associated with alcoholism may function as targets for TFs regulating *PDYN* transcription, and may be regulated through methylation of their C alleles. These SNPs, their methylation and the identified TFs could mediate effects of long-term alcohol consumption on brain area specific pattern of *PDYN* expression in human brain. Supported by the Swedish FAS and VR.

Buprenorphine/naltrexone by iontophoresis: a transdermal approach to drug abuse treatment

A. Taverner, S. Cordery, R.H. Guy, M.B. Delgado-Charro, C.P. Bailey, S.M. Husbans, Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

There has long been interest in the ability of buprenorphine to decrease cocaine use, but the clinical utility of this approach has been limited by the obvious problems of giving an opioid to a cocaine user. A combination of buprenorphine and naltrexone, where the naltrexone is present in sufficient quantity to block the mu partial agonist activity of buprenorphine, also appears to have utility in reducing cocaine use, as well as preventing relapse to opioid use. A problem with this treatment strategy arises from the need to administer buprenorphine and naltrexone by different routes. However, both buprenorphine and naltrexone can be delivered transdermally using iontophoresis, and we are currently using this technique to develop a buprenorphine/naltrexone combination therapy. Using conditioned place preference (CPP) in male Sprague-Dawley rats, the rewarding properties of buprenorphine and naltrexone combinations were assessed. The ability of buprenorphine/naltrexone to inhibit drug-primed reinstatement of morphine-induced CPP was then tested. Buprenorphine alone (0.3 mg/kg) was rewarding, whereas a buprenorphine

to naltrexone ratio of 1:10 (0.3 and 3 mg/kg) was aversive. However, a ratio of 1:3 (0.3 and 1 mg/kg) was neither rewarding nor aversive. A morphine priming dose of 2.5mg/kg reinstated morphine CPP (animals were trained to demonstrate CPP with 10mg/kg morphine followed by extinction training), an effect that appeared to be inhibited by prior administration of buprenorphine/naltrexone (0.3 and 1 mg/kg respectively). All drugs were administered i.p. We have demonstrated a combination of buprenorphine/naltrexone with no rewarding or aversive effects that appears to inhibit reinstatement in an animal model of drug-seeking behaviour. Our ongoing work will test further the ability of buprenorphine/naltrexone to prevent CPP reinstatement, particularly to cocaine, and to optimise conditions for their transdermal delivery. Acknowledgments: We gratefully acknowledge funding from the Medical Research Council (G0802728).

The novel opioid antagonist, ALKS 37, reduces morphine-induced slowing of gastrointestinal transit in rodents and hydrocodone-induced slowing in dogs

M.S. Todtenkopf¹, R.L. Dean¹, D. Arnelle¹, K.A. Heang¹, K.S. O'Neill¹, J.M. Bidlack², B.I. Knapp², D.R. Deaver¹, ¹Life Sciences and Toxicology Dept, Alkermes, Inc., Waltham, MA, USA, ²Dept of Pharmacology and Physiology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, NY, USA

ALKS 37 is a novel, orally active, peripherally-restricted, gastrointestinal (GI) tract targeted, metabolically stable, mu-opioid receptor antagonist being developed for the treatment opioid-induced bowel dysfunction (OBD), including constipation and associated GI abnormalities resulting from chronic opioid use. Here, we characterize the pharmacodynamic effects of ALKS 37 in rats and dogs. In rats, the ability of ALKS 37 to block morphine-induced delay in GI transit was assessed following oral (PO) administration. Rats were pretreated with ALKS 37 (0–10mg/kg,PO) 30min prior to morphine (14mg/kg,PO). A charcoal suspension was administered (PO) 60min later, and the small intestine was removed 20min following the charcoal meal. Morphine decreased the distance traveled by a charcoal meal by approximately 25%. Oral administration of ALKS 37 dose-dependently attenuated morphine's effects with the highest dose tested (10mg/kg) returning GI motility to 90.6% of controls. In addition, we utilized a novel method for assessing opioids effects on oro-caecal transit time (OCTT) in dogs and tested the effects of ALKS 37 on hydrocodone-induced increases in OCTT. OCTT was determined by appearance of sulphapyradine in plasma after PO administration of sulphasalazine.

Dogs received ALKS 37 (0 or 20mg,PO) 30 min before hydrocodone (0.33mg/kg,PO). Sulphasalazine (120mg/kg) was administered 30min later and blood samples were collected for 6 hrs. ALKS 37 pretreatment prevented hydrocodone-induced delay in OCTT. In conclusion, ALKS 37 demonstrated efficacy in these models following oral administration, and is currently being investigated in clinical trials for the treatment of OBD. All work funded by Alkermes, Inc.

(+)-5a Compound, but not nociceptin, excited projection neurons in rat periaqueductal gray slices

L.-W. Tung (1) and L.-C. Chiou (1, 2). (1) Grad. Inst. and (2) Dept. Pharmacology, Coll. Medicine, National Taiwan University, Taiwan
(+)-5a Compound (5a), ((3aS, 6aR)-1-(cis-4-isopropylcyclohexyl)-5k-methyl-2k-phenylhexahydrospiro[piperidine-4,1k-pyrrolo[3, 4-c]pyrrole]), is a non-peptide agonist of nociceptin/orphanin (N/OFQ) peptide (NOP) receptors with the structure backbone similar to Ro 64-6198 (Ro). We have previously shown that both 5a and Ro activated K^+ channels via one, and the same, subset of NOP receptors in vlPAG neurons while N/OFQ was effective in those Ro/5a-insensitive NOP receptors, and most of 5a-sensitive neurons are GABAergic. (Liao et al., Int. J. Neuropsychopharmacol. 2010). Here, we further compared effects of 5a and N/OFQ on synaptic transmission and subsequent neuronal activity of the PAG neurons which project to the rostral ventral medulla (RVM). PAG-RVM projection neurons in the PAG of Wistar rats (P7-P11) were retrogradely labeled by stereotaxically injecting retrobeads into the RVM (DV-7.5 mm, AP-1.6 mm, ML-0.0 mm) 3-4 days ahead. Visualized whole cell patch clamp recording was conducted in PAG-RVM projection neurons in 300 μ m-thick PAG slices in the current clamp mode. N/OFQ (300 nM) caused membrane hyperpolarization (7.67 ± 0.81 mV, $n=11$) in 11/12 (92%) recorded neurons, while 5a (10 μ M) only hyperpolarized 29% (4/14) of the recorded neurons (6.47 ± 1.86 mV, $n=4$). Local stimulation-evoked postsynaptic potential (PSP), which is a summation of excitatory and inhibitory PSPs, was depressed by N/OFQ (300 nM) from 9.82 ± 1.95 mV to 5.86 ± 1.9 mV ($n=5$, $p < 0.05$) after membrane potential correction. However, 5a (10 μ M) caused each tested projection neurons firing action potentials when evoked PSPs before drug treatment were at the same level (7.85 ± 1.62 mV, $n=6$) as that (9.82 ± 1.95 mV) for treating N/OFQ. In the group with smaller PSPs, 5a (10 μ M) had no significant effect on PSPs (5.04 ± 1.23 mV vs. 4.85 ± 1.33 mV, $n=7$, $p=0.56$). These results suggest 5a, but not N/OFQ, exerts an overall excitatory effect on PAG-RVM projection

neuronal activity. (Supported by grants NHRI-EX99-9506NI, NSC-98-2320-B-002-011-MY3, NSC-98-2323-B002-012 and NTU-99R81855).

HDAC inhibitors recover the epigenetically silenced mu-opioid receptor expression in neuropathic pain model

H. Ueda, H. Uchida, K. Araki, Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, Japan

Peripheral nerve injury causes chronic neuropathic pain, which is often refractory to treatments with conventional painkillers, including morphine. Previously we have demonstrated that injury down-regulates mu opioid receptor (MOP) expression in the dorsal root ganglion (DRG), thereby causing loss of peripheral morphine analgesia (J Pharmacol Exp Ther, 309, 380-387, 2004). Moreover, we have recently reported that neuron-restrictive silencer factor (NRSF, also known as REST), a negative gene regulator, causes the loss of peripheral morphine analgesia via epigenetic silencing of MOP gene in the DRG (J Neurosci, 30, 4806-4814, 2010). This study has revealed that NRSF is recruited to the NRSE (NRSE, also known as RE-1) site within MOP gene post-injury, and then causes histone hypoacetylation, which is closely related to transcriptional suppression. It has been known that NRSF, when it binds to NRSE, recruits histone deacetylase (HDAC) through its corepressors, mSin3 and CoREST, for generating a repressive chromatin environment. Therefore, we here tested whether HDAC inhibitors, including trichostatin A (TSA), could block NRSF-mediated epigenetic silencing of MOP gene, and then ameliorate the loss of peripheral morphine analgesia. We successfully found that HDAC inhibitors prevent the development of loss of peripheral morphine analgesia as well as ameliorate established morphine resistance in neuropathic pain. Taken together, we provided first evidence that epigenetic therapy with HDAC inhibitors is effective against morphine resistance in neuropathic pain.

A naturally occurring genetic model of human mu-opioid receptor genetic variation

E.J. Vallender, Z. Xie, D.M. Platt, G.M. Miller, Division of Neuroscience, New England Primate Research Center, Harvard Medical School, Southborough, MA, USA

Human variation has long been recognized in the mu-opioid receptor; two variants, A118G (N40D) and T17C (V6A), both occur in the extracellular N-terminal domain and seem to be functionally relevant. Their ethnic distributions, however, are extremely heterogeneous with the A118G polymorphism being most common outside of Africa and the T17C polymorphism almost exclusively seen

in African populations. Both of these variants have been associated with substance use disorders and pharmaceutical treatment, though the results have been mixed. The aforementioned ethnic genetic heterogeneity may contribute as well as the more common environmental variation that plagues human studies. We have identified a polymorphism in rhesus macaques, C77G (P26R) that occurs at moderate frequencies and appears to functionally parallel the human variation. We and others have also successfully demonstrated that this variant is associated with alcohol preference and consumption in rhesus macaques as well as their response to treatment with naltrexone. G77 homozygote animals demonstrate an ~40% increase alcohol consumption across concentrations (0.5%-4.0% w/v). These animals also respond to naltrexone at 30-fold lower concentrations than C77 homozygotes. In both cases heterozygous animals show intermediate phenotypes indicative of an additive genetic model. This development of an animal model of the genetic variation seen in human has allowed us to further explore the underlying causes for the phenotypic variation in humans. Notably our current work confirms and extends the functional similarities across mu-opioid receptor polymorphisms regardless of species. We have also leveraged the animal model to explore the differential sensitivity to naltrexone conferred by the polymorphisms. Together this allows us to better interpret the human findings and to explore the functional effects of mu-opioid receptor polymorphism in an experimental model system. Supported by NIH grants AA019688 (EJV), AA016828 (DMP), AA016184 (GMM), DA025697 (GMM) and RR000168

Lead optimization studies of n-(2-[1,1'-biphenyl]-4-ylethyl)-8-cac

M. A. VanAlstine (1), M. P. Wentland (1), D. J. Cohen (2), J. M. Bidlack (2), (1) Dept. of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY, USA; (2) Dept. of Pharmacology and Physiology, University of Rochester, Rochester, NY, USA

N-(2-[1,1'-biphenyl]-4-ylethyl)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocine-8-carboxamide has very high affinity for opioid receptors. This lead compound is an analogue of the well-known opioid cyclazocine where its prototypic (of opioids) phenolic-OH is replaced by an N-substituted carboxamido group. We now report analogues of this lead compound where a) the ethylene group linking the carboxamide N to the biphenyl group has been substituted with a mono-methyl or a fused-cyclopropyl group and b) a methylene or oxygen spacer has been inserted between the phenyls of the biphenyl group. Compared to the lead compound,

analogues in the first group displayed considerably reduced binding affinity against all receptors while several in the second group displayed comparable or enhanced binding affinity [K_i (μ) as low as 0.059 nM]. (Supported by NIDA grants R01 DA012180 and KO5-DA00360)

Pharmacological profile of delta and kappa opioid receptor subtypes in spinal cord.

R. M. van Rijn, D. I. Brissett, J. L. Whistler
Ernest Gallo Clinic and Research Center, Department of Neurology, University of California San Francisco, CA, USA

The opioid receptor family consists of four types: mu (MOR), delta (DOR), kappa (KOR) and nociceptin. While no functional splice variants have been cloned for the DOR and KOR pharmacological evidence *in vivo* suggests the existence of multiple opioid receptor subtypes. Opioid receptors play an important role in the perception of pain and are abundantly expressed in brain and spinal cord. Recently, it was shown that MOR and DOR are distinctly located in circuits mediating thermal and tactile pain, respectively. However, the expression of the KOR or opioid receptor subtypes in these circuits is unclear. Therefore, we set out to elucidate the molecular basis of the DOR and KOR subtypes. We injected C57BL/6 mice intrathecally with type and subtype selective drugs and measured thermal pain and tactile antinociception using tail-flick and Von Frey filaments, respectively, in wild-type as well as opioid receptor knockout mice. Our data suggests that in naïve mice KOR and MOR are present in circuits mediating thermal pain and that there are no pharmacological differences between DOR and KOR subtype selective agonist. We find evidence for the existence of opioid receptor homomers as well as DOR-MOR, KOR-DOR and KOR-MOR heteromers in circuits mediating tactile pain. Additionally, we find that mice that have been voluntarily drinking ethanol express functional DOR-MOR heteromers in circuits mediating thermal pain. Our data provides a better understanding of the distribution of opioid receptor types and subtypes in the spinal cord which could assist designing more selective and efficacious analgesic drugs, with reduced side effects. This work was funded by the Foundation for Alcohol Research-ABMRF (RvR), the Department of Defense (JW), NIDA (JW.) and funds provided by the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco (JW).

Dynorphin mutations cause human neurodegenerative disorder spinocerebellar ataxia type 23

D.S. Verbeek (1), H. Watanabe (2), J. Jeziarska (1), K.A. Artemenko (2), T. Yakovleva (2), Kurt F. Hauser (3), and Georgy Bakalkin (2), (1) Dept. Genetics, Univ. Groningen, The Netherlands, (2) Dept. Pharmac. Biosci., Uppsala Univ., Sweden, and (3) Dept. Pharmacol. Toxicol., Virginia Commonwealth University, Richmond, USA

Neuropeptides have not been previously identified as causative factors for neurodegenerative disorders. The spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar ataxia, dysarthria and loss of the Purkinje cells. The SCA23 locus has been previously located on chromosomal region 20p12.3-p13. We here report the identification of four missense mutations in prodynorphin (*PDYN*) located in this region. SCA23 families and 1100 ataxia patients, and 500 control individuals were screened for *PDYN* mutations. In cellular experiments, expression and processing of mutant PDYNs and effects of wt- and mutant peptides on striatal neurons were analyzed. Cellular pathways were studied by shotgun proteomic and key-node analyses in autopsy samples. Three mutations were located in Dyn A, a peptide with non-opioid neurodegenerative actions. Two mutations resulted in excessive generation of Dyn A. Two Dyn A mutants induced toxicity above that of wild type peptide. The fourth mutation was located upstream of dynorphins and affected expression of components of the opioid and glutamate system in the cerebellum. *PDYN* and Dyn A were located in Purkinje cells. Elevated non-opioid actions of Dyn A mutants or impairment of secretory pathways by mutant PDYNs may lead to glutamate neurotoxicity that underlies Purkinje cell degeneration and ataxia. This is the first demonstration of causative link between mutations in neuropeptides

and neurodegenerative/neuropsychiatric disorders. Identification of such mutations will also provide further insight into neuropeptide functions. Acknowledgments: R. Franklin Fellowship, University of Groningen, the Netherlands; the Swedish VR and FAS.

Both JNK and β -arrestin 2 play a role in ligand dependent signaling of the mu opioid receptor

N. Mittal^{1,2}, M. Tan¹, O. Egbuta¹, N. Desai¹, C. Crawford², T. Xie¹, C. Evans¹, W. Walwyn¹, ¹Dept. Psychiatry & Biobehavioral Sciences, Stefan Hatot Ctr Neuropharmacol., Semel Institute, UCLA, CA 90095, ²Dept of Psychology, California State University, San Bernardino, CA 92407

c-Jun N-terminal kinase (JNK), a member of the stress activated map kinase family, is best known for

its role in differentiation, apoptosis and neurodegeneration. More recently JNK has also been found to play a role in ligand directed signaling by the mu opioid receptor, particularly desensitization and tolerance induced by the clinically important opioid agonist, morphine. As one of the members of this family, JNK3, binds with β -arrestin 2, this scaffolding and signal transduction molecule may form an important component of JNK- and ligand-dependent opioid receptor signaling. Using mice lacking β -arrestin 2 (β -arr2^{-/-}) we first determined the role of β -arrestin 2 in the nuclear-cytosolic shuttling of the major downstream target of JNK, cJun, as a measure of activity within the JNK cascade. We found that morphine, but neither DAMGO nor Fentanyl, increased the nuclear import of the phosphorylated, therefore activated form, of cJun in β -arr2^{-/-} neurons. This enhanced activation of the JNK cascade explained the enhanced thermal analgesia induced by a single dose of morphine in β -arr2^{-/-} mice. By contrast the acute analgesic effect of fentanyl was neither β -arrestin 2 nor JNK dependent. This profile of JNK and β -arrestin 2 dependent signaling can be explained by a morphine-specific conformational change of the mu receptor affecting the confirmation of the recruited arrestin isoforms. This allows the arrestin associated upstream members of the JNK cascade to align, phosphorylate and activate JNK. (DA-05010)

Mu- and delta-opioid receptor agonists mediate up-regulation of RGS19 protein in SH-SY5Y cells

Q. Wang and J.R. Traynor, Department of Pharmacology and Substance Abuse Research Center, University of Michigan, Ann Arbor, USA
Regulator of G protein signaling protein 19 (RGS19), also known as G α -interacting protein (GAIP), was the first mammalian RGS family member identified. RGS19 acts as a GTPase accelerating protein (GAP) for *Galpha*₁₁₋₃ and *Galpha*_o subunits. In addition, interactions with GIPN (GAIP-interacting protein N-terminus), and GIPC (GAIP-interacting protein C-terminus), link RGS19 to a variety of intracellular proteins, ranging from receptors, including opioid receptors, to transporters and scaffolding molecules. Here we show that RGS19 is abundantly expressed in SH-SY5Y cells that endogenously express mu-opioid and delta-opioid receptors (MOR and DOR respectively). Overnight treatment with 10 microM of either MOR (DAMGO) or DOR (DPDPE) agonist increased RGS19 protein by 3-4 fold, but did not change the level of RGS19 mRNA, suggesting an effect on protein stabilization. Up-regulation of the level of RGS19 protein by DAMGO and DPDPE was both time- and dose-dependent. Lentiviral delivery of shRNA against RGS19 reduced RGS19 protein levels by at least 50% in SH-SY5Y cells, compared to cells infected

with lentivirus encoding shRNA against GFP. This led to an increase in the ability of acute MOR (morphine) and acute DOR (SNC80) agonists to inhibit forskolin-stimulated adenylyl cyclase. These findings indicate a role for RGS19 in modulating acute opioid receptor signaling and suggest that the increase in RGS19 protein level may be implicated in the chronic opioid state. Supported by DA04087

OPRM1 A118G SNP reduces MOPR expression in some, but not all, brain regions in a mouse model

Y-J. Wang¹, P. Huang¹, A. Ung¹, J. Blendy², L-Y. Liu-Chen¹,¹Dept. Pharmacol., Temple Univ, ²Dept. Pharmacol., Univ of Pennsylvania, Philadelphia, PA, USA

OPRM1 A118G, a common single nucleotide polymorphism (SNP) in the human mu opioid receptor gene, is associated with high morphine doses for postoperative pain and better treatment outcome for alcohol addiction. A mouse model possessing the equivalent substitution (A112G) in the *Oprm1* gene was generated. The mutant mice displayed reduced antinociceptive and locomotor responses to morphine and female mice showed attenuated morphine reward. The alteration in MOPR expression in A112G variant may play a role. In this study, [³H]DAMGO binding was examined in mice homozygous for the A allele (A/A) or G allele (G/G) using quantitative in vitro autoradiography. Brain sections (20 μm) were incubated with 5 nM [³H]DAMGO with or without 10 μM naloxone. Sections were exposed to [³H]sensitive screens and the images were quantitated. Data were analyzed with a 2-way ANOVA followed by Bonferroni post-hoc test. There was genotype effect in some brain regions, but no sex effect or genotype x sex interaction. A/A mice exhibited markedly higher [³H]DAMGO binding than G/G in the cingulate, motor, and insular cortices, NAc core and shell, hypothalamus, thalamus, amygdala, PAG, superficial grey of superior colliculus, and VTA. No genotype differences were observed in somatosensory cortex, CPu, and hippocampus. In males A/A mice showed markedly higher [³H]DAMGO binding than G/G mice in substantial nigra and all the brain regions mentioned above except the motor cortex. In contrast, in female A/A mice displayed markedly higher [³H]DAMGO binding than G/G only in the thalamus, amygdala, PAG, superficial grey of superior colliculus, and VTA. Thus, the A112G SNP reduces MOPR expression in some, but not all, brain regions, and appears to be sex dependent. As this SNP eliminates one N-linked glycosylation site, there may be differences in the components of glycosylation machinery among brain regions and between sexes, leading to differential effects of the SNP on MOPR expression. (supported by NIDA grants)

Pathogenic activities of dynorphin a mutants that cause human neurodegenerative disorder spinocerebellar ataxia type 23: induction of nociceptive behaviors in mice through non-opioid mechanism

H. Watanabe (1), D.S. Verbeek (2), H. Mizoguchi (3), F. Nyberg (1), O. Krishtal (4), S. Sakurada (3), G. Bakalkin (1), (1) Dept. Pharmaceut. Biosci., Uppsala Univ., Sweden, (2) Dept. Genet., Univ. Groningen, The Netherlands, (3) Dept. Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (4) Inst. Physiology, Kiev, Ukraine

We previously identified four missense mutations in PDYN that cause human SCA23 (Bakalkin et al., Am J Hum Gen 2010). Three mutations were located in Dyn A1-17 substituting Leu5, Arg6, and Arg9 to Ser (L5S), Trp (R6W) and Cys (R9C). R6W and L5S mutations resulted in excessive Dyn A generation, while R6W- and R9C-Dyn A were highly neurotoxic. It had been previously reported that Dyn A administered intrathecally (i.t.) into mice produces a characteristic SBL responses, the hindlimb Scratching along with Biting and Licking of the hindpaw / tail (Tan-No et al., Pain, 2005). We here report that all mutants administered in femtomolar doses also produced SBL responses. Compared to wild type (wt)-peptide, mutant peptides were more potent in these responses 2-3 fold, while for L5S- and R6W-mutants effective doses were lower 10-30 – fold. R6W-mutant was the most potent peptide. The SBL-responses induced by R6W-Dyn A were dose dependently inhibited by morphine (i.p.; 0.1-1 mg/kg) or MK-801, an NMDA ion channel blocker (i.t. co-administration; 5-7.5 nmol). CP-99,994, a tachykinin NK1 receptor antagonist (i.t. co-administration; 2 nmol) and naloxone (i.p.; 5 mg/kg) failed to block effects of R6W-Dyn A. Thus, mutant-Dyn A peptides have elevated potential to produce nociceptive responses. Similarly to wt-Dyn A, these responses or at least those induced by R6W-Dyn A may be mediated through NMDA receptor but not opioid and tachykinin NK1 receptors. Enhanced non-opioid activities of mutant-Dyn A support the hypothesis on pathogenic role of these peptides in neurodegeneration in human brain. Supported by the Swedish Science Council, Uppsala Univ., Swedish Inst., a Rosalind Franklin Fellowship, Univ. Groningen, the Japan Society for the Promotion of Science.

Modulation of full-length mu opioid receptor (MOR-1) expression and function by truncated proteins with single transmembrane domain of the mu opioid receptor gene, OPRM1

J. Xu¹, M. Xu¹, G.C. Rossi², C.E. Inturrisi³, G.W. Pasternak¹, Y.-X. Pan¹ ¹Dept of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY. ²Dept of Psychology, CW Post College, Long Island University, Brookville, NY. ³ Dept of Pharmacology, Weill Medical College of Cornell University, New York, NY, USA

Mu opioids, widely used for pain management and also abused, act through mu opioid receptors. Clinical differences in the actions of many these drugs raised the possibility of mu receptor subtypes, a concept confirmed by the identification of a vast array of both 3' and 5' splice variants from the mu opioid receptor (OPRM1) gene. In the present study, we report the isolation of additional splice variants that encode truncated proteins containing single transmembrane domain (TM) from the OPRM1 genes. These single TM variants are conserved from mouse, rat and human. Expression level of these variants' mRNAs was quite abundant. We explored the function of the single TM variants in modulating full-length MOR-1 expression using stably transfected Tet-off CHO cell lines, in which the MOR-1 expression was constant whereas the expression of the single TM variants were under control of doxycycline. The results showed that the single TM variants can dimerize with MOR-1, which was initiated in endoplasmic reticulum (ER), leading to increased expression of MOR-1 protein determined by [³H] DAMGO binding and Western blot analysis. Furthermore, our studies suggested that the dimerization of the single TM variants with MOR-1 facilitated proper folding or conformation of MOR-1 in ER, promoting its escaping from ER-associated degradation pathway and increasing turnover rate of MOR-1 determined by pulse-chase studies. In vivo antisense studies suggested that the single TM variants play an important role in morphine analgesia. Our studies raise questions regarding the functional significance of truncated proteins from other GPCR families. (Supported by DA13997 & DA029244 (Y.-X.P), DA02615 & DA07242 (G.W.P) and DA01457, DA07274& DA05130 (C.E.I.) from NIDA)

Epigenetic mechanism of prodynorphin upregulation in the brain of human alcoholics: dependence on promoter methylation and USF2 transcription factor

T. Yakovleva, I. Bazov, M.M.H. Taqi, H. Watanabe, O. Kononenko and G. Bakalkin.

Dept. Pharmaceutical Biosciences, Uppsala University, Sweden.

Changes in the epigenetic landscape with DNA methylation / demethylation and histone modifications in gene regulatory elements have been proposed to underlie human neuropsychiatric diseases including substance addiction. Critical evidence for the hypothesis is still missing. Our study demonstrated that expression of the prodynorphin gene (*PDYN*) producing opioid peptides dynorphins is elevated in the prefrontal cortex (PFC) of human alcohol-dependent subjects. This upregulation may be a critical molecular event underlying development or maintenance of the addictive state. Molecular mechanisms of *PDYN* activation in human brain were addressed by analysis of alterations in DNA methylation in *PDYN* promoter. The 1.7 kB promoter area was analyzed in post-mortem specimens, the PFC (BA9) and motor cortex (MC; BA4) of human alcoholics and controls (n=14 subjects in each group) using bisulfite treatment / DNA pyrosequencing. A short CpG rich domain in the promoter was found to be strongly and significantly demethylated in the PFC in alcoholics. The differentially methylated region (DMR) encompassing approximately 150 nt, was apparently located within a single nucleosome. No differences were evident in the MC, which showed no *PDYN* activation in alcoholics. Search for transcription factor (TF) binding sites found canonical E-box in the DMR, and then Upstream Stimulatory Factor-2 (USF2) with strong histone-modifying activities was identified as a dominant E-box binding factor in the human PFC by EMSA. In this brain area, USF2 was found i) to be colocalized with PDYN protein using immunostaining; and ii) to be bound to the *PDYN* DMR using ChIP-qPCR. We hypothesize that the *PDYN* DMR functions as the brain-area specific "epigenetic switch" affected by alcohol; DMR demethylation may promote USF2-mediated recruitment of histone modifiers to the promoter resulting in PDYN activation in alcoholics. Supported by the Swedish Council for Working Life and Social Research, and the Swedish Science Research Council.

Sleep disturbances in a neuropathic pain-like condition are associated with altered GABAergic transmission in the cingulate cortex

A. Yamashita (1), S. Imai (1), H. Horiuchi (1), K. Niikura (1,2), M. T-Narita (1), N. Kuzumaki (1), T. Suzuki (1) and M. Narita (1), (1) Dept. Toxicol. Sch. Pharm. Pharmaceut. Sci., Hoshi Univ., Tokyo, Japan, (2) Lab. Biol. of Addict. Dis., Rockefeller Univ., New York, USA

Insomnia is a common problem for people with chronic pain. Cortical GABAergic neurons are part of the neurobiological substrate that underlies homeostatic sleep regulation. In the present study, we confirmed that sciatic nerve ligation caused thermal hyperalgesia and tactile allodynia in mice. Mild noxious heat stimuli caused a significant increase in the release of glutamate in the cingulate cortex of nerve-ligated mice compared to that of sham-operated mice. In this experimental model for neuropathic pain, we found an increase in wakefulness and a decrease in non-rapid eye movement (NREM) sleep under a neuropathic pain-like state. The membrane-bound GABA transporters (GATs) on activated glial fibrillary acidic protein (GFAP)-positive astrocytes were significantly increased in the cingulate cortex of mice with sciatic nerve ligation. In an experiment with primary cultured glial cells from the mouse cortex, treatment with glutamate led to the translocation of GATs from intracellular vesicles to the plasma membrane. Furthermore, extracellular GABA levels in the cingulate cortex after depolarization were rapidly decreased by nerve injury. Under this condition, sleep disturbance induced by sciatic nerve ligation was improved by the intracingle cortex injection of a GATs inhibitor. These findings provide novel evidence that sciatic nerve ligation decreases extracellular-released GABA along with an increase in membrane-bound GATs on activated astrocytes in the cingulate cortex of mice. These phenomena may, at least in part, explain the insomnia in patients with neuropathic pain.

Non-Medical Use of Prescription Narcotics in the Sequence of Adolescent Drug-Use Initiation and Epidemiology of Narcotic-Use by Indiana Adolescents

A. YoussefAgha, W. Jayawardene, M. Torabi, Dept. Of Applied Health Science, Indiana University Bloomington, Indiana, USA.

Non-medical use of prescription drugs, including narcotics, is the fourth most prevalent type of drug use engaged in adolescents. A habit of non-medical use of prescription drugs has the potential to develop into abuse and dependence on the medication. The objective is to study the epidemiology of using narcotics and other drugs by adolescents and to identify repercussions experienced by adolescents

due to the use of narcotics. Data from 1993 to 2009 Annual Surveys of Alcohol, Tobacco, and Other Drug Use by Indiana Children and Adolescents is used for the current study. A close-ended, self-administered, written, anonymous questionnaire was used to ask adolescents about their use of twenty different types of drugs. Market basket analysis was used to identify sequences of drug use. An index was created to evaluate consequences and risk behavior due to drug-use. Overall drug use among adolescents in Indiana is 45.3%, prescription drug use is 10.9% and annual use of narcotics is 10.9%, although only 0.04% uses narcotics alone. Overall, adolescent drug abuse has decreased in recent years while the use of narcotics has increased. A sequence exists between initial use of gateway drugs and nonmedical use of medications later in life. Combined drug use behavior is more common with increasing age. Rates of combined drug use are also higher among males and whites. Being female or white is associated with increased reporting of negative consequences due to narcotics use. Males and other races are more likely to report to personal-safety issues due to drug use. Combining narcotics with other prescription drugs is associated with increased reporting of negative consequences due to the drug use. Prevention programs should address issues related to gender, racial, and ethnic differences in drug abuse, prescription drug use including narcotics, sequences of drug-use initiation and negative consequences as well as risk behavior that result from adolescent drug use.

Expression of *OPRK1*, *PDYN* and *CXCR4* in the caudate in postmortem brain of HIV infected and HIV negative subjects

V. Yufarov (1), A. Ho (1), S. Morgello (2), M.J. Kreek (1), (1) Laboratory of Addictive Diseases, The Rockefeller University, New York, NY, USA, (2) Pathology, Mount Sinai Medical Center, New York, NY, USA

Opioid and chemokine receptors are implicated in neuronal functions, immune responses, and co-receptor-dependent HIV-1 infection. Recent studies have demonstrated cross-desensitization between opioid and chemokine receptors, including bi-directional cross-talk between CXCR4 and KOR receptors in both *in vitro* and in nociceptive tests in rats (Finley et al, 2008). The aims of the study were to examine (1) levels of the *CXCR4*, *OPRK1* and *PDYN* mRNAs in the caudate of HIV positive and HIV negative subjects; (2) the correlation of the *CXCR4*, *OPRK1*, and *PDYN* mRNA levels with mRNA levels of markers of glial and neuronal cells. Tissues from postmortem brain of 24 HIV+ and 14 HIV- subjects were obtained from the Manhattan HIV Brain Bank (The Mount Sinai Medical Center, New York, NY). Total RNA was isolated from the caudate, and used for synthesis of cDNA.

Quantification of mRNA of *OPRK1*, *PDYN*, *CXCR4*, *GFAP*, *CD163* and *CD68* was carried out using SYBR Green RT-PCR. Copy number of cDNA transcripts was expressed normalized to GAPDH cDNA. There was no difference in the *OPRK1*, *CXCR4*, or *PDYN* expression in the caudate between HIV+ and HIV- subjects. There were significantly higher levels of *GFAP*, *CD163* and *CD68* mRNAs in HIV+ subjects compared to HIV- ($p < 0.05$). No correlation was found between levels of *OPRK1* and *CXCR4* mRNA in either HIV+ or HIV- subjects. Regression analyses showed significant correlation in expression of *OPRK1* with *CD163* in HIV+ subjects, and of *PDYN* expression with *CD68* in both HIV- and HIV+ brains. The increased expression of glial/macrophage markers reflects a pronounced gliosis and inflammation in brain of HIV infected subjects. Our results suggest a relationship of *OPRK1* and *PDYN* with specific markers of immune cells. Support: NIDA-P60-05130 (MJK), NIMH-R01-79880 (MJK), NIMH -U01-MH083501

Duration of withdrawal from chronic escalating-dose binge cocaine: effects on cocaine-induced conditioned place preference and expression of selective components of the opioid system

Y. Zhang, S. D. Schlussman, E. R. Butelman, A. Ho and M. J. Kreek, The Laboratory of the Biology of Addictive Diseases The Rockefeller University New York, NY, USA

Relapse to cocaine is a serious problem in the treatment of cocaine addiction. Understanding cocaine re-exposure-induced behavioral and neurobiological alterations following chronic escalating-dose binge cocaine administration and withdrawal may provide insight into the neurobiological basis of cocaine relapse. To investigate how exposure to chronic escalating-doses of cocaine affects development of cocaine-induced conditioned place preference (CPP) and changes in components of the endogenous opioid system, mice were injected with either escalating-dose binge cocaine (15-30 mg/kg/injection x 3/day) or saline for 14 days. Place preference conditioning with 15 mg/kg of cocaine commenced either 1 or 14 days after the last day of binge cocaine or saline injections. After 1 or 14 days' withdrawal, CPP was studied in three groups of mice: 1. Mice had received escalating-dose cocaine, then conditioned with cocaine. 2. Mice had received saline, then conditioned with cocaine. 3. Mice had received saline, then conditioned with saline. Cocaine induced CPP in mice previously exposed only to saline following 1 or 14 days' withdrawal. Mice did not form CPP to cocaine following 1 day withdrawal from escalating-dose binge cocaine, but mice did develop CPP to cocaine following 14 days' withdrawal from escalating-dose binge cocaine.

Penk mRNA levels in the caudate putamen and nucleus accumbens core were higher whereas MOP-r densities were lower, in mice that developed CPP to cocaine after 14 days' withdrawal from escalating-dose binge cocaine. Thus, duration of withdrawal plays an important role in the rewarding effect of cocaine. Elevation in **Penk** mRNA levels may explain, in part, why the longer withdrawal interval induced CPP to cocaine re-exposure. Acknowledgements: Cocaine-HCl was generously provided by NIH-NIDA Division of Drug Supply and Analytical Services. This work was supported by NIH-NIDA DA05130 to MJK

Chronic morphine tolerance upregulated spinal proinflammatory cytokines which are revised by HSV vector expressing interleukin 4 in rats

X. Zheng^{1,2}, J. Sun¹, S. Liu^{1,2}, M. Mata¹, D. Fink¹ and S. Hao^{1,2}, ¹Dept. of Neurol, Univ. Michigan, Ann Arbor, MI, USA ²Dept. of Anesthesiology, Univ. Miami Miller Medical School, Miami, FL, USA

Morphine is very effective analgesics used to treat moderate to severe pain in clinic, however, the repeated use of morphine is limited by the off-target effects (e.g., tolerance and dependence). Recent studies show that chronic morphine activates glial cells in the central nervous system to release proinflammatory cytokines during the tolerance. In the current study, we confirmed that chronic morphine (IP, once a day) for 7 days induced antinociceptive tolerance and increased spinal glia marker, TNF α and IL-1 β . Then, we examined the neuroimmune response in the rodent model of morphine tolerance using the Herpes Simplex Virus (HSV) vector expressing interleukin-4 (IL-4, one of anti-inflammatory cytokines). Subcutaneous inoculation of HSV expressing IL-4 into the hind paw delayed the development of morphine tolerance behavior response. Inoculation of the HSV vector expressing IL-4 prevented the increases in the spinal TNF α and IL-1 β in rats with morphine tolerance. The study suggested that proinflammatory cytokines involved the development of morphine tolerance, and that gene therapy expressing anti-inflammatory cytokine may provide a novel approach to treating morphine tolerance. This study was supported by grants from the NIDA, NINDS.

Chronic voluntary alcohol drinking enhances proopiomelanocortin (POMC) gene expression in nucleus accumbens (NAc) and hypothalamus of alcohol-preferring rats

Y. Zhou¹, G. Columbo², K. Niikura¹, M.A.M. Carai², A. Ho¹, G.L. Gessa², M.J. Kreek¹

¹Lab of Biology of Addictive Diseases, Rockefeller University, New York, USA; ²CNR Neuroscience Institute, Monserrato (CA), Italy

Evidence obtained in humans and rodents indicates that beta-endorphin is critical in the regulation of alcohol-drinking behavior. However, the alcohol effect on POMC gene expression has not been studied in rodent mesolimbic regions, like NAc. In the present study, we first utilized POMC-EGFP promoter transgenic mice to visualize POMC neurons, and found that POMC-EGFP cells were scattered through NAc shell and core, in addition to hypothalamic arcuate nucleus. We then examined whether there are genetically determined differences in basal mRNA levels of POMC and mu opioid receptor (MOP-r) between selectively bred Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats, and if mRNA levels are altered by chronic alcohol drinking in sP rats. Rats of both lines were offered either water or a free choice of 10% (v/v) alcohol and water with unlimited access for 17 days. POMC and MOP-r mRNA levels were measured in NAc core and shell, caudate-putamen (CPu), medial/basolateral amygdala (MeBLA) and hypothalamus. sP rats had higher basal POMC mRNA levels only in hypothalamus compared with sNP rats, indicating genetically determined differences between these two lines. Alcohol drinking increased POMC mRNA levels in both hypothalamus and NAc shell of sP rats. sP rats had lower basal MOP-r mRNA levels in NAc shell, MeBLA and CPu than sNP rats, and alcohol had no effect on MOP-r mRNA levels in these regions. Our results for the first time show the POMC-expressing neuron distribution in NAc of POMC-EGFP mice, and suggest that increased POMC gene expression at basal levels in hypothalamus (genetically determined) and after alcohol drinking in both hypothalamus and NAc (drug-induced) are associated with high alcohol preference and consumption in sP rats. NIH-DA-P60-05130 (MJK); CNR grant (GC).