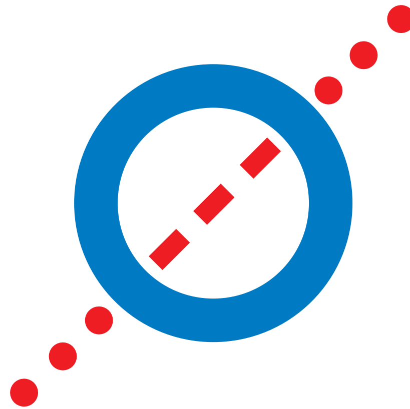
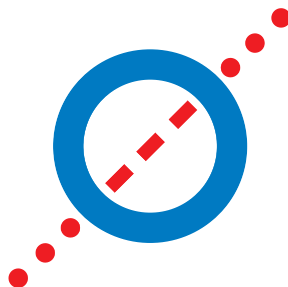


32nd Meeting of the International Narcotics Research Conference
Helsinki, Finland
July 14 -19, 2001

	Saturday 14.7.	Sunday 15.7.	Monday 16.7.	Tuesday 17.7.	Wednesday 18.7.	Thursday 19.7.
08.00		**Opening remarks**				
08.30		**Plenary lecture 1** *R. Huxtable*	**Plenary lecture 2** *A. Kastin*	**Plenary lecture 3** *M. Saarma*	**Plenary lecture 4** *P. Eriksson*	
09.00						
09.30						
10.00		**Symposium 1** *Chairs* *E. Kalso and* *M.-J.Kreek* **Opioids from clinic to hemistry**	**Symposium 3** *Chair P. Panula* **Opioid modulatory peptides**	**Symposium 5** *Chair E. Simon* **Progress in receptor biology**	**Symposium 6** *Chair L. Ahtee* **Opioid addiction behaviour**	**Symposium 8** *Chair C. Stein* **Opioids and immune function**
10.30						
11.00						
11.30						
12.00						**END OF THE MEETING**
12.30		LUNCH	LUNCH	LUNCH	LUNCH	
13.00						
13.30						
14.00	**REGISTRATION**	**Symposium 2** *Chair C. Chavkin* **G-protein receptors and pain**	**Symposium 4** *Chair B. Roques* **Synthetic ligands**	**FREE AFTERNOON**	**Symposium 7** *Chair F. Nyberg* **Opioids and pleasures of life**	
14.30						**Albert Herz Symposium**
15.00						
15.30						
16.00						
16.30		**Tea and posters**	**Tea and posters**		TEA	
17.00	**Opening reception at the University of Helsinki**				***Business meeting***	
17.30					**Founders´lecture**	
18.00				**Reception by the City of Helsinki**	*Chair C. Chavkin*	
18.30						
19.00						
19.30					**Banquet**	



32nd Meeting of the International Narcotics Research Conference Helsinki, Finland July 14 -19, 2001

Programme Committee

Eija Kalso, chair
Liisa Ahtee
Pirkko Paakkari
Pertti Panula
Raimo K. Tuominen
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General Information

Badges

Name badges must be worn at all times during the confrence. Badges are reguired for entrance into all meals, events, and scientific venues.

Registration Desk

The registration desk will assist in all conference needs. It will be open from Saturday, July 14 at the Scandic Hotel Grand Marina and from Sunday, July 15 to Thursday, July 19 at the Marina Congress Center. The registration desk will be open at the following times:

Saturday, July 14	14.00 - 16.00
Sunday, July 15	07.30 - 15.00
Monday, July 16	08.00 - 15.00
Tuesday, July 17	08.00 - 12.00
Wednesday, July 18	08.00 - 15.00
Thursday, July 19	09.30 - 12.00

Posters

All posters are on display from Sunday, July 15 to Wednesday, July 18. The posters should be mounted during lunch break on Sunday and taken down by lunch break on Wednesday. Poster sessions will take place on Sunday and Monday from 16.30 to 18.30. Posters have been organised under the topics of the symposia and the number refers to the respective poster board. Poster abstracts are in alphabetical order according to presenting author (*).

Meals

Buffet lunch is provided for all delegates from Sunday, July 15 to Wednesday, July 18 at 12.30 at the Marina Congress Center.

Social Programme

Saturday, July 14	Opening Reception at the University of Helsinki
Tuesday, July 17	Reception by the City of Helsinki
Wednesday, July 18	Banquet at the Marina Congress Center

Accommodations

Scandic Hotel Grand Marina
Katajanokanlaituri 7, FIN-00160 Helsinki
Tel: +358 9 16 661
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ACKNOWLEDGEMENTS

INRC 2001 is organised as a joint project between The University of Helsinki (Division of Pharmacology and Toxicology, Department of Pharmacy and The Pain Clinic, Department of Anaesthesia and Intensive Care) and The National Agency for Medicines. The meeting was made possible through generous grants from The Academy of Finland and The National Institute on Drug Abuse, NIH.

The Vice Rector of the University of Helsinki, Professor Thomas Wilhelmsson and the Lord Mayor of Helsinki, Eva-Riitta Siitonen are thanked for organising receptions for the delegates of the meeting.

The organisers wish to thank the following pharmaceutical companies that have supported INRC 2001 and thus enabled young researchers to attend the meeting:

GlaxoWellcome Oy
Janssen-Cilag Oy
Mundipharma Oy
Grünenthal GmbH
Pharmacia Oy
Schering-Plough Oy
Aventis Pharma Oy
Lääketeollisuus Oy

Conference Programme

Saturday 14.7.

14.00-16.00 **Registration at the Scandic Hotel Grand Marina**
17.00-19.00 **Opening reception at the University of Helsinki**

Sunday 15.7.

8.15-8.30 **Opening remarks: E. Kalso and C. Chavkin**

8.30-9.30 **R. Huxtable: The opium poppy and its alkaloids: A historical perspective**
Plenary lecture 1, Chair P. Paakkari

9.30 - 10.00 *Coffee break*

10.00-12.30 **OPIOIDS FROM CLINIC TO CHEMISTRY (SYMPOSIUM 1)**
10.00-10.15 **Chair E. Kalso and M. -J. Kreek: Introduction**

10.15-10.40 **J. Traynor**: Activation of G-protein by opioid receptors: effects of agonist properties

10.40-11.05 **G. Pasternak**: Alternative splicing as an explanation

11.05-11.30 **F. Porreca**: Mechanisms of opioid-induced pain and tolerance

Free papers (oral presentations of submitted abstracts)

11.30-11.50 **Zimmer A, Valjent E, Ksnig M, Zimmer AM, Clarke S, Robledo P, Chen C-C, Hahn H, Valverde O, Hill RG, Kitchen I, Maldonado R:** Absence of delta-9-tetrahydrocannabinol dysphoric effects in dynorphin-deficient mice

11.50-12.10 **Schulz S, Koch T, Pfeiffer M, Höllt V:**
Differential agonist-induced phosphorylation of four mouse μ-opioid receptor splice variants

12.10-12.30 **Wang D, Raehal KM, Bilsky E, Sadée W:** Inverse agonists and neutral antagonists at μ-opioid receptor (MOR): possible role of basal receptor signaling in narcotic dependence

12.30 -14.00 *Lunch*

14.00 -16.30 **G-PROTEIN RECEPTORS AND PAIN (SYMPOSIUM 2)**
14.00-14.15 **Chair C. Chavkin: Introduction**

14.15-14.40 **J.E. Zadina**: Endomorphins in chronic pain

14.40-15.05 **M. Scheinin** : α_2-adrenergic modulation of opioidergic systems

15.05-15.30 **R. Maldonado**: Involvement of the endogenous opioid system in cannabinoid responses

Free papers (oral presentations of submitted abstracts)

15.30-15.50 **Gomes I, Jordan BA, Ansnoff M, Pintar J, Devi LA:** A role for dimerization/oligomerization in opioid receptor cross-talk

15.50-16.10 **Schulz R, Wehmeyer A:** Confocal microscopy studies of GRK2 and GRK3 on sequestration of μ-, δ- and κ-opioid receptors

16.10-16.30 **Marinelli S, Schnell S, Wessendorf M, Vaughan CW, Christie MJ:** Opioid responses of spinally projecting rostral ventromedial medulla neurons

16.30 –18.30 **Tea and Posters**

Monday 16.7.

8.30-9.30 **A. Kastin: Peptides and the blood brain barrier**
Plenary lecture 2, Chair P. Panula

9.30-10.00 *Coffee break*

10.00-12.30 **OPIOID MODULATORY PEPTIDES (SYMPOSIUM 3)**
10.00-10.15 **Chair P. Panula: Introduction**

10.15-10.40 **P. Panula**: FRMF-related peptides

10.40-11.05 **H. Ueda**: Distinct roles of ppN/OFQ-derived peptides in pain control

11.05-11.30 **L. Negri**: A novel pronociceptive peptide

Free papers (oral presentations of submitted abstracts)

11.30-11.50 **Inoue M, Kawashima T, Matsunaga S, Sakurada T, Ueda H:** Potent nociceptive activity by nociceptin/orphanin FQ C-terminal fragments in nociceptors and spinal cord in mice

11.50-12.10 **N. T. Zaveri, C. J. Green, W. E. Polgar and L. Toll:** Transcriptional regulation of the human prepronociceptin gene

12.10-12.30 **Brandt A, Pietilä P, Östergård M, Panula P:** RF-amide peptides in the mouse CNS

12.30-14.00 *Lunch/INRC executive committee meeting*

14.00-16.30 **SYNTHETIC LIGANDS (SYMPOSIUM 4)**
14.00-14.15 **Chair B. Roques: Introduction**

14.15-14.40 **F. Noble:** New true inhibitors of enkephalin inactivity enzymes

14.40-15.05 **L.H. Lazarus:** Dmt, the opioid affair

15.05-15.30 **H. Schmidhammer:** HS 378 - a highly selective delta-opioid receptor antagonist with immunosuppressive properties

Free papers (oral presentations of submitted abstracts)

15.30-15.50 **Schiller PW, Lu Y, Weltrowska G, Berezowska I, Wilkes BC, Nguyen TM-D:** A general structural modification to transform opioid peptide agonists into potent and selective κ-, δ- or μ-antagonists

15.50-16.10 **Lengyel I, Biyashev D, Kocsis L, Al-Khrasani M, Rónai A, Fuesrt Zs, Tóth G, Orosz G, Borsodi A:** Changing the binding properties and biological activity of endomorphin 2 by structural modifications

16.10-16.30 **Holzgrabe U, Kuhl U, Campareri A, Brandt W:** Mechanism of action of diazabicyclononanone-type κ-agonists

16.30-18.30 **Tea and Posters**

Tuesday 17.7.

8.30-9.30 **Mart Saarma: Receptors for neurotrophic factors**
Plenary lecture 3, Chair L. Ahtee

9.30-10.00 *Coffee break*

10.00-12.00 **PROGRESS IN RECEPTOR BIOLOGY (SYMPOSIUM 5)**
10.00-10.15 **Chair E. Simon: Introduction**

10.15-10.40 **B. Kieffer:** New in sights into opioid pharmacology and physiology using triple μ/δ/κ-opiod receptor knockout mice

10.40-11.05 **V. Höllt:** Genetic variations on the theme of μ- and δ-opioid receptors

11.05-11.30 **A. Borsodi:** Endomorphins: Peculiar binding properties

Free papers (oral presentations of submitted abstracts)

11.30-11.50 **McLaughlin JP, Myers LC, Zarek PE, Mackie K, Chavkin C:** Phosphospecific antibody recognizes the desensitized form of the kappa opioid receptor (KOR)

11.50-12.10 **Xu N-J, Zheng R, Fan H-P, Pei G:** Proteome analysis of hippocampal protein expression following morphine treatment

12.10-12.30 **Yosef Sarne and Ma ánit Shapira:** Various protein kinases mediate delta opioid receptor down-regulation within the same cell

12.30-14.00 *Lunch*

14.00 **Free afternoon**
18.00 **Reception by the City of Helsinki**

Wednesday 18.7.

8.30-9.30 **P. Eriksson: CNS regeneration**
Plenary lecture 4, Chair F. Nyberg

9.30-10.00 *Coffee break*

10.00-12.00 **OPIOID ADDICTION/BEHAVIOUR (SYMPOSIUM 6)**
10.00-10.15 **Chair L. Ahtee: Introduction**

10.15-10.40 **T. Shippenberg:** Modulation of sensitization by endogenous opioid systems

10.40-11.05 **J. van Ree:** The dynamics of drug addiction: implication of endogenous opioids and dopamine

11.05-11.30 **N.T. Maidment:** OFQ/nociceptin and the mesolimbic dopamine system

Free papers (oral presentations of submitted abstracts)

11.30-11.50 **Bohn LM, Gainetdinov RR, Sotnikova TD, Lefkowitz RJ, Caron MG:** Morphine-induced locomotor activity is altered in beta-arrestin-2 knockout mice

11.50-12.10 **Yanai J, Metsuyanim S, Shahak H:** Neurobehavioral teratogenicity of heroin: cholinergic and non-cholinergic signaling defects

12.10-12.30 **Erdtmann-Vourliotis M, Mayer P, Riechert U, Ammon S, Höllt V:** A distressing environment sensitizes the brain's response to morphine

12.30-14.00 *Lunch*

14.00-16.30 **OPIOIDS AND PLEASURES OF LIFE (SYMPOSIUM 7)**
14.00-14.15 **Chair F. Nyberg: Introduction**

14.15-14.40 **P. Hyytiä:** Opioids and alcohol addiction

14.40-15.05 **A. S. Levine:** Opioid systems in food intake

15.05-15.30 **I. Kitchen:** Opioids and mother love

Free papers (oral presentations of submitted abstracts)

15.30-15.50 **Yoshikawa M, Takenaka Y, Nakamura F, Jinsmaa Y, Lipkowski AW:** Enterostatin as an antiopioid peptide

15.50-16.10 **Gerrits MAFM, van den Berg CL, Martens RJ, van Ree JM:** Long-term effects of early social isolation on adult social behavior and susceptibility for drug dependence: involvement of opioid systems

16.10-16.50 **Sinclair JD:** The use of pharmacological extinction in the treatment of drug addicition

16.30-17.00 *Tea*

17.00-17.30 *Business meeting*

17.30-18.15 **Founders' lecture**
Chair C. Chavkin: Introduction
P. Portoghese: From models to molecules in opioid research

19.30 **Banquet**

Thursday 19.7.

9.30-10.00 **J. Khalsa:** Medical consequenses of drug abuse: Research at NIDA
Chair: C. Chavkin

10.00-11.05 **OPIOIDS AND IMMUNE FUNCTION (SYMPOSIUM 8)**
10.00-10.15 **Chair C. Stein: Introduction**

10.15-10.40 **S. Chang**: Chronic exposure to morphine affects the neuroendocrine-immune axis

10.40-11.05 **R. Weber:** Immunopotentiating effects of non-peptide opioids on in vitro T-lymphocyte function

Free papers (oral presentations of submitted abstracts)

11.05-11.25 **Hauser KF, Gurwell J, Goody RJ, Turchan J, Nath A.:** Morphine exacerbates HIV TAT toxicity with differential effects in neurons and astroglia.

11.25-11.45 **Kraus J, Börner Ch, Giannini E, Hickfang K, Höllt V:** μ receptor expression in immune cells is induced by IL-4 and TNF-α

11.45 **End of the Meeting**

Albert Herz Symposium

Chairpersons V. Höllt (Magdeburg) and C. Stein (Berlin)

14.30-14.35 **V. Höllt (Magdeburg)**: Introduction

14.35-14.55 **E. Simon (New York)**: Historical overview

14.55-15.15 **P-Y Law & H. Loh (Minneapolis)**: Delta opioid receptor activation of Akt in cell survival

15.15-15.35 **V. Höllt (Magdeburg):** Polymorphisms of opioid systems

15.35-15.55 **R. Schulz (Munich):** Mechanisms of opioid tolerance

15.55-16.25 *Coffee break*

16.25-16.45 **R. Spanagel (Mannheim):** The effects of kappa-agonists on drug addiction

16.45-17.05 **R. Przewlocki (Cracow):** Opiates in chronic pain

17.05-17.25 **M. Millan (Paris):** Central analgesic mechanisms

17.25-17.55 **C. Stein (Berlin):** Peripheral opioid analgesia

Posters

The number after the title refers to the number of the respective poster board.

Opioids from clinic to chemistry

TOPICAL OPIOID AND CLONIDINE INTERACTION IN MICE (**1**)
Yuri Kolesnikov* and Gavril Pasternak

INTRATHECALLY ADMINISTERED SUBSTANCE P ENHANCES MORPHINE-INDUCED ANTINOCICEPTION THROUGH THE PRODUCTION OF SUBSTANCE P *N*-TERMINUS (**2**)
Tomoko Moriyama, Chikai Sakurada, Koichi Tan-no, Shinobu Sakurada, and Tsukasa Sakurada

NOCICEPTIVE BEHAVIOR PRODUCED BY INTRATHECAL DYNORPHIN PEPTIDES IN MICE (**3**)
Koichi Tan-No*, Akihisa Esashi, Osamu Nakagawasai, Fukie Niijima, Takeshi Tadano, Georgy Bakalkin, Lars Terenius and Kensuke Kisara

COMPARISON OF NOCISTATIN LEVELS IN CSF AMONG CHRONIC, ACUTE, NON-PAIN PATIENTS AND NORMAL VOLUNTEERS (**4**)
S Tachibana*, T-L Lee, M G Ricos, F M Y Fung, G Zhang, F-G Chen, N Chou, and E Okuda-Ashitaka, S Ito

TOLERANCE AND CROSS-TOLERANCE STUDIES WITH [DMT^1]DALDA (**5**)
Guo-Min Zhao, Dunli Wu, Yi Soong, Megumi Shimoyama and Hazel H. Szeto*

PEPTIDE SECRETION FROM THE BRAIN TO THE BLOOD VIA MULTIDRUG RESISTANCE ASSOCIATED PROTEIN (**6**)
W, Su,; M.A. King; G.W. Pasternak

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SPLICE VARIANT, MOR-1R, OF THE HUMAN MU OPIOID RECEPTOR GENE(OPRM) (**7**)
Ying-Xian Pan*, Jin Xu, Mingming Xu, Loriann Mahurter, Elizabeth Bolan and Gavril W. Pasternak

IN VITRO TRANSFER OF L-ACETYLMETHADOL (LAAM) ACROSS HUMAN PLACENTA (**8**)
T. Nanovskaya, S. Deshmukh and M.S. Ahmed*

DEXTROMETHORPHAN AND COLONIC TRANSIT - A PHARMACOLOGICAL ANALYSIS (**9**)
A. Cowan*, M.R. Pietras and S.F. Rittenhouse

COMPARISION OF THE INHIBITORY EFFECTS OF MU-OPIOID RECEPTOR AGONISTS ON MOUSE GASTROINTESTINAL TRANSIT (**10**)
F. Niijima*, K. Tan-No, O. Nakagawasai, T. Tadano and K. Kisara

CHARACTERISATION OF THE ACUTE ANTINOCICEPTIVE AND CONSTIPATORY PROPERTIES OF DIFFERENT OPIOIDS IN RATS AND GERBILS (**11**)
Vesa K. Kontinen*, Alexis K. Baker, Jef Vermeire, Theo F. Meert

FUNCTIONAL ROLES OF THE MU-, DELTA- AND KAPPA-OPIOID RECEPTORS AND THEIR SUBTYPES IN THE MEDIATION OF OPIOID-INDUCED HYPOTHERMIA IN MICE (**12**)
Alexis K. Baker*, Nancy Aerts, Theo F. Meert

INOTROPIC AND CARDIOPROTECTIVE ACTION OF [DMT^1]DALDA (**13**)
Dunli Wu, Yi Soong and Hazel H. Szeto*

NON-PEPTIDIC DELTA-OPIOID RECEPTOR AGONISTS REDUCE IMMOBILITY IN THE FORCED SWIM ASSAY IN RATS (**14**)
Emily M. Jutkiewicz*, Daniel C. Broom, John E. Folk, John R. Traynor, Kenner C. Rice, James H. Woods

CONVULSANT ACTIVITY OF NONPEPTIDIC DELTA-OPIOID RECEPTOR AGONISTS IS NOT REQUIRED FOR ANTIDEPRESSANT-LIKE EFFECTS (**15**)
Daniel C. Broom*, Emily M. Jutkiewicz, John E. Folk, John R. Traynor, Kenner C. Rice, James H. Woods

MORPHINE PROMOTES BREAST TUMOR PROGRESSION AND ANGIOGENESIS BY ACTIVATING PRO-ANGIOGENIC AND SURVIVAL-PROMOTING SIGNAL TRANSDUCTION PATHWAYS IN VASCULAR ENDOTHELIUM **(16)**
Kalpna Gupta*, Smita Kshirsagar, Gui-Hua Zhang, Robert Schwartz, Liming Chang, Ping Y. Law, Douglas Yee and Robert P. Hebbel

SHARED PROCESSING IN THE ROSTRAL ACC DURING OPIOID AND PLACEBO ANALGESIA **(17)**
P Petrovic*, E Kalso, K -M Petersson, M Ingvar

THE SIGNIFICANCE OF THE UGT2B7 HIS268TYR POLYMORPHISM IN THE FORMATION OF MORPHINE 3-GLUCURONIDE AND MORPHINE 6-GLUCURONIDE FROM MORPHINE IN HUMANS **(18)**
Monica Holthe*, Pål Klepstad, Petter Borchgrevink, Stein Kaasa, Jeffrey R. Idle, Hans E. Krokan and Frank Skorpen

IS THE PAIN RELIEF OBTAINED FROM METHADONE AND DEXTROMETHORPHAN IN A RAT MODEL OF MONONEUROPATHY DUE ONLY TO OPIOID AGONISM? **(19)**
Hyytiäinen A., Brugioni A., La Turraca A. and Malmberg-Aiello P.

G-protein receptors and pain

INVOLVEMENT OF MODALITY-SPECIFIC LOSS OF NOCICEPTOR SIGNALING IN THE MORPHINE-INSENSITIVE NEUROPATHIC PAIN **(20)**
Hiroshi Ueda*, Makoto Inoue, Takayuki Matsumoto and Toshiko Kawashima

PRONOCICEPTIN AND PRODYNORPHIN SYSTEMS IN NEUROPATHIC PAIN **(21)**
Przewlocka B.*, Mika J, Schafer M.K., Obara I., Sieja A., Przewlocki R.

DOES THE HUMAN MU OPIOID RECEPTOR COUPLE DIFFERENTLY TO Gi1a OR Gi2a ? **(22)**
D. Massotte* & G. Milligan

SPINAL ANALGESIC SYNERGY BETWEEN THE ENDOMORPHINS AND CLONIDINE, BUT NOT THE ENDOMORPHINS AND 2-METHYL-5-HT **(23)**
Dennis Paul*[1], Maria Sayah and James E. Zadina

VENLAFAXINE AND MIRTAZAPINE: COMMON OPIOID-MEDIATED ANTINOCICEPTIVE EFFECTS **(24)**
Shaul Schreiber, Tova Rigai·Chaim G. Pick*

MU AND ORL1 RECEPTOR CROSS TOLERANCE IS MEDIATED BY G PROTEIN-COUPLED RECEPTOR KINASE 2 (GRK2) UPREGULATION **(25)**
D. R. Thakker* and K. M. Standifer

THE INFLUENCE OF NOISE STRESS ON CENTRAL OPIOID SYSTEM **(26)**
Huei-Yann Tsai*, Hsiu-Mei Chiang , Jim-Shoung. Lai, Cheng-Chieh Lin and Yuh-Fung Chen

PKC-INVOLVEMENT IN THE ACUTE TOLERANCE TO PERIPHERAL MORPHINE ANALGESIA IN THE MOUSE CAPSAICIN TEST **(27)**
Takayuki Matsumoto, Rashid Harunor, Hiroshi Ueda*

ACTIVATION OF PERIPHERAL ORL1 RECEPTORS INHIBITS CAPSAICIN-INDUCED THERMAL NOCICEPTION IN RHESUS MONKEYS **(28)**
M.C. Holden Ko*, John R. Traynor, Norah N. Naughton, Alexander T. McKnight and James H. Woods

P-GLYCOPROTEIN INVOLVEMENT IN OPIOID ANALGESIA **(29)**
M.A. King*, W. Su, S.P. Milo and G.W. Pasternak

DIFFERENTIAL EFFECTS OF ENDOMORPHIN-1 AND ENDOMORPHIN-2 ON BEHAVIORAL ACTIVITY: MOR-1 ANTISENSE PROFILE **(30)**
G.C. Rossi*, Y-X. Pan, C.Abbadie, and G.W.Pasternak

EFFECTS OF ACUTE AND CHRONIC CLONIDINE AND PRAZOSIN ON MORPHINE TOLERANCE AND WITHDRAWAL IN MICE **(31)**
Umit Ozdogan*, Janne Lahdesmäki, Mika Scheinin

Opioid modulatory peptides

PEPTIDE AND ALKALOID AGONISTS DICTATE THE DIFFERENT FATE OF INTERNALIZED DELTA OPIOID RECEPTOR **(32)**
Allouche S., Marie N. and Jauzac Ph.

DESENSITIZATION OF HUMAN DELTA OPIOID RECEPTOR (HDOR) BY ALKALOID (ETORPHINE) AND PEPTIDE (DPDPE AND DELTORPHINE I) AGONISTS: IMPLICATION OF DIFFERENT KINASES **(33)**
Marie N., Allouche S., and Jauzac Ph.

PRE-TREATMENT WITH A GUINEA PIG ANTISERUM RAISED AGAINST AGMATINE INCREASES SENSITIVITY TO INDUCTION OF SPINAL OPIOID ANTINOCICEPTIVE TOLERANCE **(34)**
C.A. Fairbanks*, L.L. Kaminski, H.O. Nguyen, J. C. Roberts, K.F. Kitto, L., G.L. Wilcox

INTRAHYPOTHALAMIC INJECTION OF DELTORPHIN-II, A DELTA2 AGONIST, INDUCES HYPERTHERMIA VIA DELTA AND MU OPIOID RECEPTORS **(35)**
S.M. Rawls, E.B. Geller*, J. Cabassa, and M.W. Adler

LOCALIZATION OF NEUROPEPTIDE FF, SUBSTANCE P AND THE MU AND DELTA OPIATE RECEPTORS IN THE RAT SPINAL CORD **(36)**
K Kuokkanen*, A Brandt, J Korhonen, R Elde[2] and P Panula

IDENTIFICATION OF NOVEL PROTEINS INTERACTING WITH MU–OPIOID RECEPTOR **(37)**
Y.Liang, Z.Han, L.Brandenburg, T.Koch, T.Kroslak, and V.Höllt

BIOLOGICALLY ACTIVE PEPTIDE FROM HUMAN LEUKEMIA DIFFERENTIATION FACTOR IDENTIFICATION AND ITS PROTECTIVE PROPERTIES **(38)**
I.A. Kostanyan*, E.V. Surina, M.V. Astapova, A.P. Bogachuk, S.M. Dranitsyna, I.M. Molotkovskaja, T.N. Lepekhova, E.V. Navolotskaja, V.M. Lipkin

CORRECTION WITH THE HLDF-6 PEPTIDE OF ENDOGENOUS OPIOID SYSTEM PATHOLOGY OF MORPHINE-TOLERANT ANIMALS F POSTERITY **(39)**
Litvinova S.V., Kostanyan[1] I.A.*, Aristova V.V., Shulgovsky V.V., Bogachuk A.P.

[^{3}H]SUPER DALDA BINDING TO BRAIN MEMBRANES **(40)**
C.L. Neilan*, I. Berezowska, T.M-D. Nguyen, P.W. Schiller, and G.W. Pasternak

A TETRAPEPTIDE OF DERMORPHIN ANALOGUE PRODUCES AN EXTREMELY POTENT ANTINOCICEPTIVE EFFECT IN MICE **(41)**
K. Okuyama*, A. Yonezawa, S. Ogawa, M. Hagiwara, T. Morikawa, T. Sakurada and S. Sakurada

ANTINOCICEPTIVE AND ANTI-OPIOID EFFECTS OF SPINALLY ADMINISTERED NOCICEPTIN RELATED PEPTIDES IN THE CAPSAICIN TEST **(42)**
Tohru Orito*, Tomoko Moriyama, Chikai Sakurada, Koichi Tan-No Shinobu Sakurada and Tsukasa Sakurada

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Plenary and Founders′ Lectures

PLENARY LECTURE 1

THE OPIUM POPPY AND ITS ALKALOIDS: A HISTORICAL PERSPECTIVE

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Four topics will be discussed: (i) The botanical origins of Papaver somniferum; (ii) Evidence of prehistoric & historic knowledge; (iii) The isolation & structure determination of morphine; and (iv) The biosynthesis of morphine. Of around 250 species of poppies, only the subspecies of P. somniferum contain meaningful amounts of morphine. P. somniferum is a cultigen, a species created by humans. It appears to date to Neolithic times, some 10,000 years BP. Literary and artefactual evidence indicates that the opium poppy was known to the classical civilizations of the eastern Mediterranean and Near East. Examination of the chemistry of opium began in the early 19th century. In 1817, Sertürner reported the isolation of a base, which he called morphium, that exhibited the pharmacological effects of opium. His report precipitated an avalanche of investigations of alkaloid-containing materials. Despite intensive work by many chemists, the correct structure for morphine, a tetracyclic, rigid, T-shaped molecule with five asymmetric centers, was first proposed by Gulland and Robinson in 1925. The structure was co Along with consideration of structure went consideration of how the poppy made such a complex molecule. Winterstein and Trier proposed in 1910 that benzylisoquinolines could be formed from two molecules of dopamine and Robinson (1925) pointed out that cleavage of a bond in morphine yielded a benzylisoquinoline. With the advent of 14C in the 1950s, radiotracer experiments established that biosynthesis was rapid, the plant converting CO2 to morphine within 2 hr. Tracer studies also established the significan Almost 200 years of research on morphine led to the development of an enormous body of general chemical knowledge, and uncovered areas of biology apparently remote from the poppy and its secondary metabolites. This fruitful interaction of disciplines seems likely to continue, with continuing work on natural and synthetic peptide analogs and the pain pathways they modify.

PLENARY LECTURE 2

PEPTIDES AND THE BLOOD-BRAIN BARRIER

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No longer considered a static, impermeable barrier, the blood-brain barrier (BBB) is a dynamic player in the regulation of communication between the brain and the rest of the body. Some peptides (and polypeptides) enter the brain from the blood by saturable transport mechanisms whereas most enter by passive diffusion based on physicochemical properties such as lipophilicity and hydrogen bonding. Similarly, some peptides exit the brain to the blood by saturable transport systems and others leave together with the passive bulk reabsorption of CSF. For quantification of influx, the sensitive technique of multiple-time regression analysis is combined with HPLC to verify that the radioactivity remains on the intact peptide. The capillary depletion method with washout is used to ensure that the injected peptide reaches the brain parenchyma rather than being tightly bound to the lumen of the capillary endothelial cells that compose the BBB or loosely adherent to the vasculature. Size is not a major determinant of rate or type of entry, with the polypeptide leptin entering by a saturable transport system faster than the entry by passive diffusion of ingestive peptides such as NPY, orexin A, MCH, or AgRP(83-132). Similarly, the opiate modulating tripeptide MIF-1 (Pro-Leu-NH_2) enters brain faster than morphine or the tetrapeptide Tyr-MIF-1 (Tyr-Pro-Leu-NH_2), whereas Tyr-MIF-1 but not MIF-1 exits brain by a saturable transport system shared with the structurally dissimilar Met-enkephalin. Saturable transport systems into the brain exist for some cytokine polypeptides like IL-1, GMCSF, and LIF, but not for others like IL-2 or IFNg, and for some neurotrophin polypeptides like EGF but not for others like TGFa. Downregulation of transport can be seen with leptin after food restriction and upregulation after preinjection of glucose. Urocortin, like the related CRH, does not enter the brain any faster than the vascular control albumin, but its transport system can be activated by leptin and pretreatment with glucose. CRH, but not urocortin, leaves the brain by a saturable transport system in amounts sufficient to exert an effect in the periphery. In the spinal cord, transport of TNFa can be upregulated in experimental allergic encephalomyelitis as well as various forms of spinal cord injury. Thus, the BBB exerts can exert an important regulatory function in the passage of peptides and polypeptides between the blood and the brain.

PLENARY LECTURE 3

RECEPTORS FOR NEUROTROPHIC FACTORS

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Neurotrophic factors are a group of secreted polypeptide growth factors that are important for the intercellular communication during the development, maintenance and plasticity of the nervous system. Nerve growth factor (NGF)-family of neurotrophic factors, called neurotrophins, are best known for promoting neuronal survival, differentiation, neurite growth, and also as a regulator of synaptic development and plasticity. Among important neurotrophic factors is the glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFL). GDNF and other members of the family, neurturin (NRTN), artemin (ARTN) and persephin (PSPN) support several populations of neurons in the central nervous system, including midbrain dopamine neurons and motoneurons. Since GDNF and neurturin can rescue dopamine neurons in the animal models of Parkinson disease, as well as motoneurons in vivo, GFLs have raised hopes as new drugs for the treatment of neurodegenerative diseases. In addition, GDNF, NRTN and ARTN also support the survival and regulate the differentiation of many peripheral neurons, including sympathetic, parasympathetic, sensory and enteric neurons. GDNF also has important functions outside the nervous system, as a morphogenetic factor in kidney development and as a regulator of spermatogonia differentiation. GFLs promote their cellular effects through a unique receptor system, where a receptor tyrosine kinase c-Ret is shared by four GDNF family ligands. GDNF family ligands can not bind directly to c-Ret, but first form a high affinity complex with the glycosyl phosphatidylinositol (GPI)-anchored GDNF family receptor (GFR) α. The GFL-GFRα complex then binds to c-Ret thereby triggerring c-Ret dimerization, autophosphorylation, and intracellular signaling. GDNF specifically binds to GFRα1, NRTN to GFRα2, ARTN to GFRα3 and PSPN activates c-Ret via binding to GFRα4. The autophosphorylated c-Ret in turn activates several intracellular signaling proteins that regulate cell survival, differentiation, proliferation, migration, neurite growth and synaptic modulation. Characterisation of GDNF family signalling pathways is important for several reasons. Firstly, to develop small molecular drugs acting at this pathway for the treatment of several neurodegenerative diseases. Secondly, to understand the molecular mechanisms of pathogenesis of diseases caused by mutations in c-Ret. Activating mutations in RET cause the inherited cancer syndrome multiple endocrine neoplasia 2 (MEN2) characterised by medullary thyroid carcinoma. Inactivating mutations of the RET gene lead to the development of Hirschsprung's disease, characterised by the absence of intrinsic ganglionic cells in the distal gastrointestinal tract. Although significant progress has been made in the characterisation of RET mutations and changes in Ret downstream signalling in pathological conditions, several challenges still remain. Several new discoveries have further boosted interest towards the GDNF family of factors. Firstly, an elegant study demonstrated the potential of GDNF in the regeneration of sensory axons after spinal cord injury. Secondly, a rather unexpected role of GDNF in the regulation of biochemical and behavioral adaptations to chronic morphine and cocaine abuse was recently reported.

PLENARY LECTURE 4

CNS REGENERATION

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The generation of new neurons (neurogenesis) in the adult hippocampus is regulated in several steps. Two major events of significance for cell genesis from progenitor cells are proliferation and lineage determination. My laboratory focus on factors of importance for the regulation of proliferation and lineage determination, e.g. ciliary neurotrophic factor (CNTF), insulin-like growth factorI (IGF-I) and opioids. Recent studies suggest that CNTF may play a role in reactive gliosis, a hallmark of most CNS disorders. The CNTF receptor α-subunit (CNTFRα) was expressed in fibroblast growth factor-2 (FGF-2) expanded adult-derived rat hippocampal neural progenitors (AHPs). Binding of CNTF to CNTFRα resulted in activation of the Janus kinase - signal transducer and activator of transcription (Jak - STAT) pathway. It was demonstrated that CNTF is an instructive signal for astroglial type 2 cell fate in AHPs, and by electroporation of antisense oligonucleotides

against STAT3 it was shown that the astroglial lineage determination is specifically mediated via activation of STAT3. However, inhibition of the mitogen-activated protein kinase (MAPK) signalling pathway did not block CNTF-induced gliogenesis in progenitor cells. These experiments demonstrated that CNTF is an instructive signal mediating glial cell fate in undifferentiated progenitors. These results may have implications for future strategies aiming at using endogenous progenitors in brain repair. It has recently been demonstrated that running is a very potent inducer of neurogenesis in the dentate gyrus. Therefore we investigated the downstream mediators of growth hormone (GH) concerning effects on progenitor cells in the dentate gyrus. *In vivo* we investigated the effect of the peripheral administration of IGF-I on cellular proliferation in the adult rat dentate subgranular proliferative zone, and on the subsequent migration and differentiation of progenitor cells within the granule cell layer (GCL). Using bromodeoxyuridine (BrdU) labelling, we found a significant increase of BrdU-immunoreactive progenitors in the GCL after 6 days of peripheral IGF-I administration. BrdU-positive cells also increased significantly in animals treated with IGF-I for 20 days. Furthermore, the fraction of newly generated neurons in the GCL increased, as evaluated by the co-localisation of neuronal markers and BrdU after 20 days of IGF-I treatment. Thus, our results show that peripheral infusion of IGF-I increases progenitor cell proliferation and induces neurogenesis in progeny of adult neural progenitor cells. This corresponds to a 78±17% increase in the number of new neurons in IGF-I-treated animals compared to controls. *In vitro* we found that the IGF-I-receptor, is expressed in AHPs. IGF-I-treated cultures showed a dose-dependent increase in DNA synthesis and number of cells, well separated from the effects mediated by insulin, demonstrating a proliferative effect of IGF-I. We also demonstrated by means of inhibitors and dominant negative constructs that the MAPK signalling pathway was required for IGF-I-stimulated proliferation in AHPs. Interestingly we now have evidence that selective μ and δ opioid receptor agonists also modulate neurogenesis. These findings demonstrate the importance of extracellular signals and indicate that proliferation and cell fate determination are regulated by complex interactions between progenitors and extracellular cues like the CNTF, IGF-I and members of the opioid systems.

FOUNDERS´LECTURE

FROM MODELS TO MOLECULES IN OPIOID RESEARCH

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Over the years opioid ligands have been the most investigated class of compounds. The molecular diversity of opioids and the existence of subpopulations of opioid receptors have posed both challenges and opportunities for medicinal chemists. In this presentation, models for the design of opiate-derived ligands as probes are discussed together with their utilization to investigate the architecture of opioid recognition sites and organization of opioid receptors. The combination of structure-activity studies, site-directed mutagenesis, and molecular modeling have provided greater insight of molecular recognition from the perspective of both the ligand and opioid receptor.

Symposium Lectures

ACTIVATION OF G PROTEIN BY OPIOID RECEPTORS: EFFECTS OF AGONIST PROPERTIES

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Opioid receptors couple to pertussis-toxin sensitive Gi/o proteins. Agonist occupation of these receptors causes the dissociation of GDP from the Ga subunit and allows the binding of GTP. This activation of G protein may be measured by increased binding of the analog [^{35}S]GTPgS to determine agonist potencies and efficacies (including inverse agonists) and antagonist affinities. However, agonists can also facilitate the dissociation of pre-bound [^{35}S]GTPgS from Ga subunits. Efficacy measures gained in these assays are consistent with *in vivo* and clinical findings. For example with mu agonists: etorphine > fentanyl > morphine = methadone > buprenorphine > pentazocine > butorphanol > nalbuphine; potency varies in a different order with buprenorphine the most potent. The consequences of desensitization and co-expression of mu and delta receptors on [^{35}S]GTPgS binding will be considered. Finally, potential role(s) for RGS proteins (Regulators of G protein Signaling) in the cellular actions of opioids will be discussed. Supported by NIH grants DA 04087 and 00254.

ALTERNATIVE SPLICING AS AN EXPLANATION

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Unlike many areas of pharmacology, opioid research has long been directed by observations from the clinic. For example, multiple opioid receptors were proposed based upon the clinical interactions of morphine and nalorphine. Despite their common classificication as mu, most opioids used clinically have widely varying analgesic activities and side-effects among patients. Furthermore, they display incomplete cross tolerance. These observations are difficult to reconcile with a class of analgesics acting through a common receptor. Binding studies implied the existence of multiple mu receptors over 25 years ago, a concept supported by a series of selective mu antagonists. However, the definitive identification of multiple mu receptors required the cloning of the mu receptor. There are now at least 7 different mu opioid receptors. These variants display different regional distributions and are functionally distinct, despite their common, high affinity and selectivity for morphine and other mu ligands. Although the functional significance of many of these MOR-1 variants remains to be defined, their existence confirms, at a molecular level, the complexity of mu pharmacology, something clinicians have recognized for decades.

MECHANISMS OF OPIOID-INDUCED PAIN AND TOLERANCE

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Recent evidence suggests that, in addition to analgesia, opioids produce unexpected, paradoxical pain in both preclinical and clinical settings. Such opioid-induced pain limits not only acute antinociceptive efficacy but also appears to promote the decreased antinociception seen with sustained exposure (i.e., behavioral opioid antinociceptive tolerance). Manipulations which block opioid-induced pain also block the decrease in antinociceptive potency seen with sustained morphine, suggesting that opioid-induced pain may represent an important component of opioid antinociceptive tolerance. Among the manipulations which block opioid-induced pain and antinociceptive tolerance are rostral ventromedial medulla (RVM) injection of lidocaine and bilateral lesions of the dorsolateral funiculus (DLF) suggesting a tonically active descending pain modulatory (facilitatory) system which maintains opioid-induced pain and antinociceptive tolerance. Cells in the RVM which are known to mediate descending facilitation of pain are thought to express opioid mu receptors. Cytotoxic lesions of mu receptor expressing cells in the rostral ventromedial medulla using a dermorphin-saporin conjugate, also block opioid-induced pain and antinociceptive tolerance. Tonic activation of descending pain facilitation results in an upregulation of spinal dynorphin levels. Such abnormal levels of dynorphin are pronociceptive and act to maintain opioid-induced pain and apparent opioid antinociceptive tolerance. Opioid-induced pain, and antinociceptive tolerance, is not observed in prodynorphin knock-out mice. Spinal tissues from morphine pelleted mice show enhanced capsaicin-evoked CGRP release; enhanced release is blocked by antiserum to dynorphin and by prior lesions of the DLF. These findings suggest a novel physiological mechanisms of opioid tolerance which is related to enhanced pain mediated by activation of cells expressing opioid mu receptors in the RVM. Strategies which prevent opioid-induced pain also prevent antinociceptive tolerance. Thus, it seems possible that such approaches will allow for sustained opioid analgesic efficacy and their use in the treatment of chronic or prolonged pain states.

ENDOMORPHINS IN CHRONIC PAIN

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Chronic pain after nerve injury is associated with neuroplasticity in primary afferents and the spinal cord dorsal horn. Most studies of these syndromes have focused on increased excitatory processes, while an alternative, the loss of inhibitory processes, has received relatively little attention. Endomorphin 2 (Tyr-Pro-Phe-Phe-NH_2, EM2), an endogenous mu-selective opioid, is present in primary afferents and the dorsal horn. We tested whether EM-2-like immunoreactivity (EM2-LI) is altered after unilateral partial ligation of the sciatic nerve in mouse and rat. A chronic pain syndrome that included thermal hyperalgesia and mechanical allodynia was present within 24h and remained significant for at least 2 weeks. Dramatic decreases in EM2-LI in the spinal cord ipsilateral to the nerve injury were also observed during this time. The changes were restricted to lumbar regions innervated by the sciatic nerve. Consistent with earlier studies, a modest loss of Substance P-LI was also observed, but CGRP-LI was unaltered. By contrast, dynorphin-LI showed a significant increase. Thus, the profile of neuroplasticity after nerve injury includes decreases in EM-2-LI. Loss of the inhibitory influence of this endogenous opioid could contribute to the development of chronic pain.

α_2-ADRENERGIC MODULATION OF OPIOIDERGIC SYSTEMS

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ABSTRACT NOT PROVIDED

INVOLVEMENT OF THE ENDOGENOUS OPIOID SYSTEM IN CANNABINOID RESPONSES

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Cross-interactions between opioid and cannabinoid systems in dependence and reward-related processes have been shown in many studies. We have recently established a model of tolerance and dependence to delta9-tetrahydrocannabinol (THC) in mice as well as a model of place conditioning to evaluate both rewarding and aversive properties of THC in mice. We have used these behavioral models in three strains of knockout mice lacking either mu, delta or kappa opioid receptors, and we have observed interesting cross-interactions between the two systems that may help to understand the mechanisms of action of cannabinoids. The functional interactions between the cannabinoid and opioid systems were also evaluated in pre-proenkephalin and pre-prodynorphin deficient mice. Antinociception induced in the tail-immersion test by acute THC was reduced in these two lines of mutant mice, whereas no difference between genotypes was observed in THC effects on body temperature and locomotion. Cannabinoid withdrawal syndrome was also significantly attenuated in pre-proenkephalin mutant mice. These results indicate that the endogenous enkephalinergic and dynorphinergic systems are involved in the antinociceptive responses of THC. Endogenous enkephalins also participate in the expression of the cannabinoid abstinence. The present study provides new data to clarify the involvement of the different components of the endogenous opioid system in the adaptive responses occuring during chronic THC exposure.

RF-AMIDE PEPTIDES IN MAMMALIAN BRAIN: NOVEL MULTIFUNCTIONAL SYSTEMS

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A mammalian RF-amide peptide neuropeptide FF recently cloned from several species acts in the brain as an antiopioid peptide but potentiates morphine analgesia in the spinal cord. The second RFamide peptide, reported as prolactin-releasing peptide PrRP, could also have important functions in sensory and autonomic regulation. PrRP peptide was not prominently expressed in a proper locus for prolactin release, but was abundant in the medulla oblongata, where it was expressed in a complementary manner with neuropeptide FF. Immunoreactivity was absent from the dorsal horn of the spinal cord. In accordance with this absence, no analgesic effect was found after inrathecal administration. PrRP20 in or adjacent to the nucleus of the solitary tract produced a significant, naloxone-reversible analgesia, whereas in the caudal ventrolateral medulla it had a weak hyperalgesic effect. Further identification of related peptides and their receptors in the CNS suggest that RF-amide peptides operate on different levels of the CNS to modulate multiple functions.

A NEW PRONOCICEPTIVE PEPTIDE

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From skin secretions of the frog Bombina variegata, we isolated a small protein termed Bv8. As deduced from cloned cDNAs, the murine and human Bv8 homologues have identical aminoterminal sequences. Bv8 mRNA is abundant both in human and in mouse testis and peripheral leukocytes. In situ hybridization shows that Bv8 RNA is widely expressed in the rodent CNS (olfactory bulb, pyramidal cells of the cortex, limbic regions, sensitive and motor nuclei of brain stem, Purkinje cells of cerebellum, posterior and anterior horns of spinal cord). I.c.v. and s.c. injections of Bv8 (30-600 pmol/rat) dose-dependently reduced the pain threshold to heat nociceptive stimulus (tail-flick test and plantar test) and to mechanical nociceptive stimulus (paw pressure test). Mechanical hyperalgesia induced by s.c.injection of Bv8 was reverted by pretreatment of rats with clonidine, L-732, MK 801, MPEP, L-NAME, indomethacin, quinacrine, U-73122, GF109203, PD98059. Bv8 mRNA from human leukocytes was 2.5 fold increased after 24 hours exposure of peripheral neutrophils to 50 nM GM-CSF. Moreover, the sequence of Bv8 precursor contains the typical signal peptide sequence for secretion.Thus, BV8 could be a new mediator of leukocyte-dependent hyperalgesia.

DISTINCT ROLES OF ppN/OFQ-DERIVED PEPTIDES IN PAIN CONTROL

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Prepro-nociceptin/orphanin FQ (ppN/OFQ) has various biologically active peptides in its sequence. N/OFQ is the first peptide, which has been introduced as an anti-opioid peptide. Nocistatin is the second peptide, which has been reported to inhibit the N/OFQ action. The third potential peptide is the ppN/OFQ (160-187) whose pharmacological and physiological actions are not well reported. In addition to these peptides, we recently found the fourth candidate, which is the C-terminal fragment of N/OFQ, N/OFQ(13-17). We report the pharmacological characterization of pronociceptive actions of these peptides, and the physiological roles of N/OFQ and ppN/OFQ (160-187) in the pain control at the level of spinal cord. N/OFQ, nocistatin and N/OFQ(13-17) activate capsaicin-sensitive C fibers through Gi and release substance P into the spinal cord, while ppN/OFQ (160-187) activates capsaicin-insensitive (A) fibers through Gs and release glutamate to activate NMDA receptors in the spinal cord. From the experiments using N/OFQ antagonists and anti-ppN/OFQ (160-187) IgG, we found that N/OFQ and ppN/OFQ (160-187) have different roles in the regulation of different modalities of pain.

TRUE DUAL INHIBITORS OF ENKEPHALIN-DEGRADING ENZYMES : DEVELOPMENT AND CLINICAL APPLICATIONS

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The discovery that the endogenous morphine-like peptides, Met- and Leu-enkephalins are inactivated by two metallopeptidases, which can be blocked by dual inhibitors represents a promising way to develop new analgesics, devoid of the side effects of morphine. A serie of recently designed aminophosphinic NEP/APN inhibitors leads to enhanced levels of Met-enkephalin-like immonureactivity in PAG and induces long-lasting antinociceptive effects. The analgesic responses mediated by these inhibitors was strongly improved by association with very low and inactive doses of morphine. Altogether, these results indicate that these compounds could be appropriate for treatment of chronic pain. Furthermore, these inhibitors modulate the functioning of the mesolimbic and nigrostriatal dopaminergic systems, implicated in mood control. At this time, the clinical trials require an improvement in oral bioavailability of these dual inhibitors.

DIMETHYLTYROSINE, THE OPIOID AFFAIR

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A remarkable alteration in the properties of opioids resides in the incorporation of 2',6'-dimethyl-L-tyrosine (Dmt) at the N-terminus of diverse opioidmimetics and underscores its applicability in the formation of new and potent opioid ligands. Introduction of Dmt into the Tyr-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid)-pharmacophore enhanced receptor affinity ($Ki\delta < 0.1$ nM) and functional bioactivty by orders of magnitude [Lazarus *et al.*, *Drug Disc. Today* **3**, 284-294 (1998)]; a similar augmentation was observed with endomorphin analogues, pyrazinone-based opioids and a unique class of synthetic opioidmimetic substances [Okada et al., unpublished data]. In the absence of Tic, Dmt facilitates high affinity interaction for μ receptors ($Ki\mu < 1$ nM). Our systematic analyses extends and verifies the hypothesis that Dmt is a universal residue for the acquisition of high affinity for both δ- and μ-opioid receptors, which suggest a similar binding site within these receptor types; Tic was considered responsible for antagonism, however, H-Dmt-Tic-NH_2 exhibited inverse agonism (EC_{50} = 2.66 nM). Evolution of the Dmt-Tic pharmacophore, a δ antagonist (pA_2, 8.2), into bifunctional/ heterofunctional ligands was associated with the nature of the spacer and its length between Tic and a C-terminally extended aromatic/hydrophobic substituent. The Dmt-Tic pharmacophore based on the formula Dmt-Tic-X-Y (X = aliphatic linkers to Y, a third aromatic center) coupled with 1H-benzimidazo-2-yl, phenyl or benzyl groups converts a δ antagonist into a ligand with potent δ-agonist activity (pEC_{50}, 9.90), which could serve as non-addictive analgesics during surgery, or mixed δ antagonism/μ agonism. Data verify that the μ receptor requires larger, hydrophobically-enhanced ligands lacking an anionic function, which discriminates between δ and μ receptors. Some hydrophobically extended analogues effectively inhibited the P-glycoprotein responsible for multidrug resistance in cancer.

HS 378 — A HIGHLY SELECTIVE DELTA-OPIOID RECEPTOR ANTAGONIST WITH IMMUNOSUPPRESSIVE PROPERTIES

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HS 378 – a 14-alkoxy-substituted indolomorphinan – is a potent pure delta opioid receptor antagonist with much higher delta selectivity than naltrindole. This compound exhibited an anti-proliferative activity against concanavalin A stimulated T-cells. HS 378 reduced significantly the degree of chronic inflammation and tissue damage associated with adjuvant arthritis in the rat. The attenuation of clinical symptoms of arthritis was accompanied by a ca. 60% decrease in paw volume. The mechanisms responsible for the anti-inflammatory actions of HS 378 are at least partly due to the modulation of cellular immune responses. Thus, HS 378 may be a potential anti-arthritic immunoregulatory drug for human inflammatory arthritic diseases.

NEW INSIGHTS INTO OPIOID PHYSIOLOGY AND PHARMACOLOGY USING TRIPLE MU/DELTA/ KAPPA OPIOID RECEPTOR KNOCKOUT MICE

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We have recently generated mice lacking either the m- (MOR), d- (DOR), or k- (KOR) opioid receptor gene. We now have interbred these mutant mice to generate mutant mice lacking all three genes. Binding studies confirmed the absence of m-, d-, and k-opioid receptor sites in the triple homozygous mutants. The mice are viable and fertile and the observation of spontaneous behavior shows modifications of locomotor activity and nociceptive thresholds in mutant mice. These mice allow now to investigate the genetic origin of non-classical opioid binding sites or activities. Here we will present data on our investigation of k2 receptor sites, defined by the pharmacology as non-k1 [^{3}H] bremazocine binding sites. Also we have examined possible mechanisms of the well-described immunosuppressive activity of naltrindole in the graft rejection process.

GENETIC VARIATIONS ON THE THEME OF μ- AND δ- OPIOID RECEPTORS

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Candidates for genetic predisposition to substance abuse are defects in genes of opioid receptors. A high number of polymorphisms has been detected in the μ- and δ- opioid receptors. Several of the mutations selectively alter physiological properties of the receptors. Examples are the phosphorylation site mutant of Ser268Pro in the μ opioid receptor which impairs down regulation and G-protein coupling or the absence of the complete third intracellular loop in a δ receptor variant which abolishes G-protein coupling but may preserve coupling to other pathways. In addition to coding regions several polymorphisms exist in the promoter regions of the μ opioid receptor gene. One of them impairs the binding of the Stat-6 binding factor which mediates transcriptional regulation of the μ opioid receptor by cytokines such as IL-4. Variants of the μ and the δ- opioid receptors showed postive associations with opiate and/or alcohol addiction in some studies. However, these association were weak indicating only a small, if any, contribution of the opioid receptor genes to these disorders.

ENDOMORPHINS - PECULIAR BINDING PROPERTIES

A. Borsodi, I. Lengyel, D. Biyashev, Cs. Tomboly, G. Toth, I. Szatmari, K. Monory. J. Hanoune* and S. Benyhe

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It is generally accepted, that endomorphins bind with high affinity and exclusively to the mu opioid receptor. However, our recent data indicate that tritiated endomorphin 1 with a specific radioactivity of 42 Ci/mmol - prepared by catalytic dehalogenation - is able to bind to a second, naloxone insensitive site. This site is not recognized by heterocyclic opiate ligands, neither agonists, nor antagonists. On the contrary, endomorphin 1 is displaced from its low affinity, high capacity site by several peptides, including methionine-enkephalin Arg-Phe, nociceptin, angiotensin, FMRF-amide, etc. This binding site seems to be present in tissues carrying low density or no mu receptors. The naloxone insensitive site is not coupled to G proteins, nor does affect adenylyl cyclase activity. Supported by OTKA T-032907 T-03086 and T-035211 grants and Janos Bolyai Fellowship (I.L.).

MODULATION OF SENSITIZATION BY ENDOGENOUS OPIOID SYSTEMS

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The repeated use of psychostimulants leads to a progressive enhancement of their behavioral effects. This process, referred to as sensitization, has been implicated in drug-craving and the paranoid psychosis that occurs in some individuals following the reinstatement of stimulant use. Marked alterations in the activity of endogenous opioid receptor systems are also observed in human addicts and experimental animals with a history of psychostimulant abuse. This talk will discuss neuroadaptations that occur in kappa opioid receptor systems following the repeated administration of cocaine and present evidence that alterations in the activity of these systems not only modulate the development of drug-induced sensitization but may also play an important role in mediating individual differences in vulnerability to the behavioral and neurochemical effects of at least one class of abused drugs. We will present evidence that pharmacological or genetic ablation of kappa opioid receptors exacerbates the behavioral effects of cocaine; an effect that is associated with an enhanced responsiveness of mesocorticolimbic dopamine neurons to cocaine and an increase in basal dopamine uptake and release. In contrast, an increase in the activity of kappa systems prevents the development of sensitization to cocaine as well as alterations in dopamine transporter function that occur as a consequence of repeated cocaine use. Anatomical, and biochemical evidence that these actions may result from the ability of kappa opioid receptor agonists to regulate the activity and cell surface expression of the dopamine transporter will be presented.

THE DYNAMICS OF DRUG ADDICTION: IMPLICATION OF ENDOGENOUS OPIOIDS AND DOPAMINE

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Different stages in the addiction course can be delineated: the initiation phase, the maintenance phase, the withdrawal phase and the relapse phase. Various psychological and biological mechanisms seem to be important for the drug use in these stages. The psychological mechanisms include liking the drug, which has been linked to reinforcement and euphoria, and wanting the drug, playing a role in the daily pattern of drug intake during maintenance, but also in the phenomenon of craving which may be important for relapse. There are indications that endogenous opioids play a role in modulating drug reinforcement, which may be pertinent to the individual susceptibility with respect to development of (psychic) dependence, in the dynamics of drug taking behaviour during the maintenance phase of drug dependence and in certain motivation effects induced by repeated drug (self)administration, which may be involved in craving and relapse. Another important substance may be dopamine. The role of this neurotransmitter in opioid reinforcement has been questioned. However, there are data suggesting that dopamine systems are involved in the daily intake of drugs of abuse. Dopamine has also been implicated in models of craving.

ORPHANIN FQ/NOCICEPTIN AND THE MESOLIMBIC DOPAMINE SYSTEM

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Accumulating evidence suggests that orphaninFQ/OFQ/N (OFQ/N) regulates mesolimbic dopamine (DA) neurotransmission. OFQ/N reduces extracellular DA in the nucleus accumbens, an action mediated in the VTA. This effect is also apparent in primary cultures of rat midbrain DA neurons, with an IC50 of 3nM. The behavioral significance of this action is suggested by OFQ's ability to block the acquisition of morphine place preference and to attenuate the acute locomotor response to cocaine. We have used ORL.1 knockout mice to determine if endogenous OFQ/N is an important modulator of the behavioral and neurochemical response to single or repeated heroin or cocaine administration. The extracellular DA elevation in response to acute heroin or cocaine was similar in WT and ORL.1 knockout mice. The locomotor response to acute cocaine, but not heroin, was attenuated in knockout mice. However, preliminary data indicate that behavioral sensitization to both these drugs may be accentuated in knockout mice.

OPIOIDS AND ALCOHOL ADDICTION

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Several lines of evidence suggest that the endogenous opioid system mediates partly the reinforcing effects of alcohol. A genetic predisposition towards alcohol drinking is accompanied by altered basal levels of opioid peptides in the brain and an increased sensitivity of the endogenous opioid system to alcohol. For example, the AA (Alko Alcohol) and ANA (Alko Non-Alcohol) rats lines, selected for high and low voluntary alcohol drinking, differ with respect to the amount of opioid peptides and their mRNA levels in distinct brain areas. Consistent with the role of the endogenous opioid system in alcohol reinforcement, both nonselective and selective opioid receptor antagonists suppress alcohol self-administration. The nucleus accumbens and amygdala may be important sites for the mediation of their suppressive effects that could result partly from an interaction between the mesolimbic dopamine pathway and the opioidergic system.

OPIOID SYSTEMS IN FOOD INTAKE

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There is a long history suggesting that opioids are involved in the rewarding aspects of feeding behavior. Our group has found that naloxone potently decreases intake of preferred foods and solutions. For example, naloxone decreases intake of a sucrose or polycose diet in food restricted rats, but has no effect on consumption of a less preferred starch based diet in restricted rats. Doses as low as a 0.01 mg/kg naloxone will decrease intake of a preferred diet, whereas doses as high as 3 mg/kg fail to affect intake of a less preferred diet in food deprived rats. While opioid receptor blockade decreases intake of sweet substances, it does not appear to do so by altering taste preference. Using a taste discrimination protocol, we found that naloxone had no affect on the ability of rats to recognize sweet tasting solutions. Human trials demonstrate a reduction in the pleasantness, but not recognition, of sweet foods/solutions after naltrexone administration. Thus, opioids may be involved in the reinforcing qualities of foods and may contribute to the development of hyperphagia leading to obesity.

OPIOIDS AND MOTHER LOVE

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Opioid peptides have been shown to play a role in developmental processes including cell proliferation and in the activation of behavioural responses such as suckling. Maternal stimuli have also been shown to regulate the development of opioid peptides and receptors and these effects may have an important bearing on how opioid systems function in the adult. We have previously shown that removal of the mother from her pups activates a population of delta opioid receptors in the brain which are involved in mediating stress-induced analgesia. By studying these behavioural responses in lactating and non-lactating surrogates we have been able to show that the key stimulus for the development of this delta receptor population is the removal of the mother's milk. Moreover, by carrying out substitution experiments with casein-rich and casein-free milk we have now identified casein as the key constituent of maternal milk that is responsible for regulating opioid receptor development. The story thus returns to the question of the importance of the beta-casomorphins (milk-derived proteins with opioid receptor activity) isolated more than twenty years ago. Is mother's milk simply the best?

CHRONIC EXPOSURE TO MORPHINE AFFECTS THE NEURO-IMMUNE AXIS

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Our studies focus on morphine's ability to disrupt the hypothalamic-pituitary-adrenal (HPA) axis, and the subsequent consequences of that disruption on the body's defense mechanisms. A pathological stimulus can affect both the nervous and immune systems. Activation of the HPA axis serves to modulate immune responses through a negative feedback mechanism. We have shown that chronic morphine exposure attenuates the activation of the hypothalamic paraventricular nucleus (PVN) which initiates this cascade of modulatory events, thus, indirectly suppressing the inhibitory feedback from the HPA axis. Morphine also potentiates the detrimental inflammatory response by increasing the secretion of pro-inflammatory cytokines. Additionally, treatment with morphine appears to directly enhance the permeability of vascular endothelial cell (VEC) barriers. Thus, our data indicate that chronic morphine can severely compromise the body's defense system through both direct and indirect mechanisms (supported in part by DA 07058).

MEDICAL CONSEQUENCES OF DRUG ABUSE: RESEARCH AT NIDA

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The US National Institute on Drug Abuse (NIDA), a part of the National Institutes of Health (NIH) supports about 85% of the worlds research on drug addiction and associated consequences. Drug abuse is associated with serious medical and health consequences affecting almost every physiological system in the body. Currently NIDA's Basic Division supports research on basic mechanisms of drug action in the brain including the role of genetics in drug action; the Epidemiologic division supports studies of incidence, prevalence, study of etiological factors of drug abuse, and intervention strategies; and the Center on AIDS and Other Medical Consequences of Drug Abuse supports research on medical consequences, including developmental effects of pre and postnatal exposure to drugs; and of co-occurring infections including HIV, hepatitis, TB, STDs, and others. It manages and coordinates NIDA's AIDS budget of about $240 million. Some of the most recent programs it has supported include: research on drug abuse and AIDS; metabolic and endocrine complications of drug abuse and HIV/AIDS; pharmacokinetic/pharmacodynamic drug interactions among drugs of abuse and pharmacotherapeutics used in the treatment of addiction, infections including HIV/AIDS (antiretrovirals) and mental disorders (e.g., benzodiazepines), and "natural remedies". New initiatives that are in development will be discussed.

IMMUNOPOTENTIATING EFFECTS OF NON-PEPTIDE OPIOIDS ON IN VITRO T-LYMPHOCYTE FUNCTION

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ABSTRACT NOT PROVIDED

Oral Presentations

ABSENCE OF DELTA-9-TETRAHYDROCANNABINOL DYSPHORIC EFFECTS IN DYNORPHIN-DEFICIENT MICE

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The involvement of dynorphin on THC and morphine responses has been investigated by using mice with a targeted inactivation of the prodynorphin (Pdyn) gene. The absence of dynorphine leads to a substantial reduction of late-phase nociceptive responses in the formalin test, while acute pain responses are unaltered. Dynorphin deficient mice also show specific changes in the behavioral effects of Delta9-tetrahydrocannabinol (THC), including a reduction of spinal THC analgesia and the complete.

INVERSE AGONISTS AND NEUTRAL ANTAGONISTS AT ? OPIOID RECEPTOR (MOR): POSSIBLE ROLE OF BASAL RECEPTOR SIGNALING IN NARCOTIC DEPENDENCE.

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The mu opioid receptor, MOR, displays spontaneous (basal) G-coupling in vitro. Antagonists were tested for intrinsic basal MOR signaling in vitro before and after morphine-pretreatment measuring GTP?S binding to cell membranes and cAMP levels in intact cells. beta-CNA, C-CAM, BNTX, and nalmefene were identified as inverse agonists (suppressing basal MOR signaling). Naloxone and naltrexone were neutral antagonists (not affecting basal signaling) in untreated cells whereas inverse agonistic effects became apparent only after morphine pretreatment. In contrast, 6alpha- and 6beta-naltrexol and –naloxol, and 6beta naltrexamine were neutral antagonists regardless of morphine pretreatment. In an acute and chronic mouse model of morphine-induced dependence, 6beta-naltrexol caused significantly reduced withdrawal jumping compared to naloxone and naltrexone, at doses effective in blocking morphine antinociception. This supports the hypothesis that naloxone-induced withdrawal symptoms result at least in part from suppression of basal signaling activity of MOR in morphine –dependent animals. Supported by DA04166 from NIDA

DIFFERENTIAL AGONIST-INDUCED PHOSPHORYLATION OF FOUR MOUSE MU-OPIOID RECEPTOR SPLICE VARIANTS

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The C-terminal splice variants (MOR1C, MOR1D, MOR1E) of the mouse μ-opioid receptor (MOR1) differ substantially in their agonist-selective membrane trafficking. These variants add various lengths of sequence to the tail including serines and threonines. In the present study, we performed whole cell phosphorylation assays, to test the hypothesis that these additional serines and threonines are phosphoacceptor sites for G protein-coupled receptor kinases. We show that the mouse μ-opioid receptor is rapidly (20 min) phosphorylated in the presence of the opioid peptide [D-Ala^2, Me Phe^4, Glyol^5]enkephalin (DAMGO) but not in response to morphine. In contrast, the μ-receptor splice variants MOR1C, MOR1D and MOR1E are phosphorylated in the presence of both DAMGO and morphine. Thus, these findings may provide a mechanistic basis for the observed differences in morphine-induced internalization and downregulation of the mouse μ-opioid receptor splice variants.

A ROLE FOR DIMERIZATION/OLIGOMERIZATION IN OPIOID RECEPTOR CROSS-TALK.

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A number of G-protein coupled receptors (GPCR) have recently been shown to physically associate with each other to form homo- or hetero-oligomers. We have previously shown that delta and kappa receptors heterodimerize with each other and that heterodimerization alters the ligand binding and signaling properties of the receptors. Recently we have found that co-expression of mu and delta receptors in heterologous cells results in heterooligomerization. Treatment of these cells with lotor function in the presence of TIPP(psi) is due to delta receptors. In a related study we examined the ability of opioid receptors to interact with other members of the GPCR family. When co-expressed in heterologous cells, delta and kappa receptors are able to oligomerize with beta2-adrenergic receptors. Although the ligand binding properties of the receptors are not altered, agonist induced trafficking properties are significantly altered as a result of heteromerization. Taken together these results support a role for dimerization/oligomerization in the modulation.

CONFOCAL MICROSCOPY STUDIES OF GRK2 AND GRK3 ON SEQUESTRATION OF μ-, d- AND k-OPIOID RECEPTORS

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Stimulation of opioid receptors triggers their phosphorylation by G protein-coupled receptor kinases (GRK) which may be followed by receptor sequestration. We examined the effect of GRK2 and GRK3 on μ-, d- and human-k-opioid receptor internalization by means of confocal microscopy. Each receptor type was fused with Green Fluorescence Protein (GFP), and the GRKs were fused with Red Fluorescence Protein (DsRed). HEK 293 cells stably expressing the μ-, d- and khuman-receptor, respectively, were μ-receptor failed to trigger translocation of GRK2 or 3, and internalization of the “green” μ-receptor was never accompanied by one of the kinases tested. Thus, the function of GRK2 and 3 was found to be linked to d- and k-receptors, while μ-opioid receptors failed to interact with these GRKs .

OPIOID RESPONSES OF SPINALLY PROJECTING ROSTRAL VENTROMEDIAL MEDULLA NEURONS

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Previous models based on opioid actions in the rostral ventromedial medulla (RVM) in brain slices (eg. Pan, Nature 389:382-5 1997) have suggested that mu-opioids directly inhibit GABAergic neurons to disinhibit spinally projecting serotonergic antinociceptive neurons expressing k-receptors. This model is at odds with immunohistochemical and in vivo studies. Rhodamine beads were injected into rat dorsal horn to investigate properties of identified RVM projection neurons using whole cell patchd 9% unresponsive. A subset of each cell type also responded to selective delta-ligands. Opioid sensitivity of spinally projecting RVM neurons in vitro suggests a new model of organization of opioid responses of the RVM.

POTENT NOCICEPTIVE ACTIVITY BY NOCICEPTIN/ ORPHANIN FQ C-TERMINAL FRAGMENTS IN NOCICEPTORS AND SPINAL CORD IN MICE

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Nociceptin/orphanin FQ (N/OFQ), heptadecapeptide is reported to be metabolized by aminopeptidase N and endopeptidase 24.15. Here we report the potent algogenic activity by its C-terminal fragments, but not by the N-terminal ones. In the present experiments, various N/OFQ fragments (metabolites) were intraplantarly injected to see if they induce nociceptive flexor responses in mice. N/OFQ (13-17) showed the most potent nociception among these peptides. The pharmacological characterization revealed that this C-terminal fragment causes a nociception through Gi activation and substance P-release from nociceptor endings, just like in the case with N/OFQ-induced nociception. However, there was no significant difference in the potency between wild-type and N/OFQ receptor (NOR) gene knock-out mice. These results suggest that N/OFQ (13-17) could potentially mediate the nociception through a novel mechanism independent of NOR activation in the nociceptors. [Supported by HFSP]

TRANSCRIPTIONAL REGULATION OF THE HUMAN PREPRONOCICEPTIN GENE

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We have previously sequenced and characterized the promoter region of the human prepronociceptin (ppN/OFQ) gene. Inclusion of 120 bp DNA adjacent to the first intron 23 bp ahead of the ATG start codon causes a significant enhancement in transcriptional activation of the 1.2 kb promoter, as determined by stimulation of luciferase activity when transfected into a pGL3 reporter vector. This 120 bp region is well conserved in mouse and human ppN/OFQ genes and contains putative Sp1 and Etf transcription factor binding sites. Electrophoretic mobility shift assays (EMSAs) using a 30 bp oligo containing the putative Sp1 site caused a significant retardation in the EMSA, although Etf-containing and control oligos were ineffective. When we used Sp1 antibody in supershift assays, there was a partial supershifting of the bound oligo. To determine if the Sp1-containing 30 bp DNA was important in transcription, we deleted this region. Transient transfection experiments using 1.2 kb of promoter with this 30 bp deletion mutation showed very poor transcriptional activity compared to full length constructs, but greater transcriptional activity than the mutation deleting the full 120 bp.

RF-AMIDE PEPTDES IN THE MOUSE CNS

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The expression of RFamide peptide precursor mRNAs of neuropeptide FF (NPFF), prolactin releasing peptide (PrRP) mRNA and RFamide related peptide (RFRP) mRNA was analysed by in situ hybridisation, immunohistochemistry and receptor autoradiography in ICR, Balb/c and C57BL/6 mouse strains. Each type of peptide precursor was observed to have a specific but essentially nonoverlapping, neuroanatomical origin in the mouse CNS. Immunohistochemical analysis revealed closely associated neuronal netwd. Further studies using a recently developed NPFF-deficient mouse model are likely to reveal possible compensatory changes in the other two peptide gene products. This information is useful in evaluating the roles of these related peptides in e.g. hormonal regulation and autonomic functions.

CHANGING THE BINDING PROPERTIES AND BIOLOGICAL ACTIVITY OF ENDOMORPHIN 2 BY STRUCTURAL MODIFICATIONS

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We investigated whether C-terminal modifications and N-methylation of Phe[3] could improve the binding affinity and the biological activity of endomorphin 2 (E2). A number of C-terminal modifications resulted in peptides with decreased binding affinity to the mu opioid receptor (MOR) (determined by displacement of tritiated E2; Spetea et al., BBRC, 1998) and decreased level of [^{35}S]GTPγS binding. However, YPF-Pheol (Pheol=phenylalanilol) showed increased binding affinity to MOR and higher potency in stimulating [^{35}S]GTPγS binding, but its maximal stimulation was lover than that induced by E2. N-methylation of Phe[3] (MePhe) attenuated the binding affinity and produced a rightward shift in the stimulation of [^{35}S]GTPγS binding. Some of the modified peptides partially, while YPF-phenyl-ethyl-N-allyl amide fully inhibited the E2 or DAMGO stimulated [^{35}S]GTPγS binding. In contrasts, YP-MePhe-Pheol had no inhibitory effect. Experiments on mouse vas deferens indicated that the observed changes are related to the agonist properties of E2. [Supported by János Bolyai Fellowship (I.L.) and Grants from OTKA (T032736, T03086, T03841, T032907) and ETT (15/2000)]

A GENERAL STRUCTURAL MODIFICATION TO TRANSFORM OPIOID PEPTIDE AGONISTS INTO POTENT AND SELECTIVE κ-, δ- OR μ-ANTAGONISTS

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To re-examine the role of the positively charged N-terminal amino group of κ-, δ- or μ-opioid peptides in the interaction with their receptors, we prepared analogs containing a 2',6'-dimethyltyrosine residue in place of Tyr[1], in which the amino group was either deleted or replaced with an alkyl, phenyl, hydroxy or halogen group. Antagonists were obtained in all cases, indicating that positive charge deletion in combination with 2',6'-dimethyl substitution at the Tyr[1] aromatic ring promoted binding to inactive conformations of κ-, δ- and μ-receptors. Importantly, the receptor selectivity was often maintained or even improved upon agonist to antagonist conversion. For example, replacement of Tyr[1] with (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(2S)-Mdp] (methyl substitution) in dynorphin A-(1-11)-NH_2 resulted in the first highly potent dynorphin A-derived κ antagonist (*dynantin*) which showed significantly higher κ selectivity than norbinaltorphimine. Other [(2*S*)-Mdp[1]]-analogs of opioid peptides turned out to be the first potent and selective opioid peptide derived μ antagonists and the first cyclic δ opioid peptide antagonists.

MECHANISM OF ACTION OF DIAZABICYCLONONANONE-TYPE KAPPA-AGONISTS

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HZ2, 2,4-di-2-pyridyl-3,7-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one-1,5-diester, was found to exhibit high affinity and selectivity to the kappa-opioid receptor. In addition, HZ2 showed a unusual long duration of action which cannot be explained by the affinity to the receptor calculated from molecular modelling (Score). However, the keto group in position 9 was found to have a high reactivity. In presence of protons the keto function can form a hemiaminal using a pyridine[1] nitrogen. Correspondingly, the diazabicyclononanone may react with OH, NH or SH groups of the receptor protein. Docking studies of HZ2 to the kappa-opioid receptor offer the chance that the keto function may react either with the side chain of a cysteine or a serine residue. Semiempirical calculations were carried out to investigate the mechanism of the intra- and intermolcular hemiaminal formation in comparison. These calculations confirm the principal possibility of the hemiaminal formation from thermodynamic as well kinetic point of view. The covalent hemiaminal binding can explain the long-lasting activity of the compound. Since a hemiaminal formation is reversible the diazabicyclononane is still able to dissociate from the receptor. U. Kuhl, A. Cambareri, U. Holzgrabe et al., J. Chem. Soc. Perkin 2, 2083-2088 (1999)

PHOSPHOSPECIFIC ANTIBODY RECOGNIZES THE DESENSITIZED FORM OF THE KAPPA OPIOID RECEPTOR (KOR)

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Phosphorylation of serine 369 of the KOR is required for G-protein receptor kinase 3 (GRK)-induced desensitization (Appleyard, 1999). An affinity purified rabbit polyclonal antibody (anti-KOR-P) generated to a phosphopeptide based on this region displayed selectivity for the phosphopeptide in ELISA assays. Moreover, the anti-KOR-P showed a higher reactivity in Western blots to phosphorylated KOR. When solubilized mouse brain membrane (MBM) protein was incubated with purified GRK3, anti-KOR-P labeling increased 67±13% over untreated KOR. Conversely, when MBM protein was pretreated with protein phosphatase-1, anti-KOR-P labeling decreased by 56±4%. Anti-KOR-P labeling in Western blots increased 59±22% over controls in mice pretreated with a single acute dose of U50,488 (30 mg/kg, i.p.), and 110±29% in mice made behaviorally tolerant following 5 days of U50,488 (escalating dose, i.p.). Acute administration of U50,488 to GRK3 knockout mice did not increase anti-KOR-P labeling in Western blots, despite evidence of behavioral analgesia. Finally, using confocal microscopy, the intensity of anti-KOR-P labeling of 293 cells transfected with KOR-GFP was dramatically increased after treatment with 1 µM U50,488.

VARIOUS PROTEIN KINASES MEDIATE DELTA OPIOID RECEPTOR DOWN-REGULATION WITHIN THE SAME CELL

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Various protein kinases (PKs) have been proposed to mediate opioid receptor (OR) down- regulation in different experimental preparations. The present study was aimed to test whether different PKs can mediate OR down-regulation within the same cell. For this purpose we studied HEK-293 cells transfected with rat dOR as well as N18TG2 cells that endogenously express dOR. We exposed the cells to the opioid agonist etorhine in the absence or presence of various PKs inhibitors, and measured the binding of the opioid ligand [^{3}H]diprenorphine. We found that at least two tyrosine-kinases (TKs) mediate dOR down-regulation in parallel routes in HEK-293 cells. G protein-coupled receptor-kinase (GRK) had only a minor role in these cells. On the other hand, GRK was the predominant kinase mediating dOR down-regulation in N18TG2 cells, with a minor role for TKs. We conclude that down-regulation can involve divers protein kinases within the same cell, and that, in different cells, down-regulation is mediated by different PKs depending on the kinase profileof the cells.

PROTEOME ANALYSIS OF HIPPOCAMPAL PROTEIN EXPRESSION FOLLOWING MORPHINE TREATMENT IN MICE

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Our previous study has shown that chronic morphine treatment significantly affect the neuronal function (such as LTP) in hippocampus. We continued in the present study to conduct the proteome analysis of hippocampal protein expression following acute or chronic morphine treatment in mice. The hippocampal extracts were separated by high resolution two-dimensional electrophoresis (2-DE), and over 1200 protein spots were able to be visualized by sliver staining in narrow pH range (4-7) gel. As compared with the saline control mice, the significant changes in protein expression were observed in mice following the acute treatment with 100 mg/kg (but not with 10 mg/kg) morphine. There were 141 protein spots (more than 10%) showing quantitative and qualitative variations by image analysis (>2 fold increase or decrease in quantification). Some of the variation seemed to result from the shift of the adjacent spots, suggesting the morphine treatment may alter the post-translational modification or/and isoform expression of these proteins. Protein spots excised from 2-D gels were further subjected to in-gel digestion with trypsin, and the resulting peptides were characterized by MALDI-TOF-MS and database searching. Among six proteins with most significant differentiation identified so far, the three down-regulated ones were synaptosomal-associated protein 25 (SNAP-25), actin, and one unknown protein. The three up-regulated spots were calregulin and two others that did not match any protein in the database. The preliminary results from the 2DE revealed that the variations of protein expression were less dramatic and with different pattern in the chronic morphine treatment (from 10 mg/kg to 100 mg/kg in 6 days) when compared to those in the acute treatment (100 mg/kg). Our data from proteome analysis clearly indicate that the morphine treatment would induce significant alteration of protein expression in hippocampus and the expression of some of those proteins might adapt to the chronic morphine treatment.

MORPHINE-INDUCED LOCOMOTOR ACTIVITY IS ALTERED IN BETA-ARRESTIN-2 KNOCKOUT MICE

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The GPCR regulatory element, Barrestin-2, is involved in mu opioid receptor desensitization in vivo. Mice lacking Barrestin-2, experience enhanced morphine analgesia, do not develop morphine antinociceptive tolerance, yet still undergo naloxone-precipitated withdrawal. We have evaluated other morphine-induced behaviors in these mice. Surprisingly, the Barr2-KO mice displayed much less locomotor activation following morphine treatment than their WT littermates. The dose-response curve for morphine (5 - 40 mg/kg, s.c.) was significantly shifted rightward for locomotor activity. In *in vivo* microdialysis experiments, morphine induced a comparable elevation in striatal dopamine release in both genotypes. In addition, cocaine induced the same degree of locomotor activity in both groups of mice; suggesting that the dopaminergic system is unaltered. Since dopamine is presumably a central component of both locomotor activity and reward, it will be interesting to evaluate the rewarding effects of morphine in these mice which lack morphine antinociceptive tolerance yet still experience morphine dependence. *DA006023 (L.M.B.), HL16037 (R.J.L.), and NS19576 (M.G.C.).*

A DISTRESSING ENVIRONMENT SENSITIZES THE BRAIN'S RESPONSE TO MORPHINE

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Commencing spontaneous opiate intake is favored by stress, in humans as well as in experimental animals. To elucidate the neuronal background, we exposed rats to moderate stress and measured morphine-induced c-fos expression in the brain. The rats were either briefly immobilized or, more akin to the situation of human addicts, exposed to a distressing environment. In each case, the morphine-induced c-fos synthesis in the dorsal striatum doubled as compared to unstressed animals. Further links between morphine and stress were established by the observations that a moderate dose of morphine counteracted the stress-induced c-fos synthesis and that the c-fos response to stress was very similar to the response to morphine withdrawal. These findings provide a connection between distressing factors and gene expression response to opiates.

NEUROBEHAVIORAL TERATOGENICITY OF HEROIN: CHOLINERGIC AND NON CHOLINERGIC SIGNALING DEFECTS

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Mice were exposed transplacentally to heroin by injecting dams with 10 mg/kg daily on gestational days 9-18. At adulthood, they showed behavioral deficits related to septohippocampal cholinergic synaptic function, concomitant with both pre- and postsynaptic cholinergic hyperactivity. Most significant was the increase in basal membrane-bound protein kinase C (PKC) activity, and a consequent desensitization of PKC to cholinergic input, both of which were highly correlated with behavioral perfasal adenylyl cyclase activity and caused suppression of receptor-mediated stimulatory responses, in this case involving beta-adrenergic input. (Supported by the NC-Israel Partnership and The Israeli Anti-Drug Authority.)

ENTEROSTATIN AS AN ANTIOPIOID PEPTIDE

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Enterostatin (VPDPR) is released from the amino terminus of procolipase on its activation. Enterostatin-immunoreactive material has been also found in brain. Enterostatin has been reported to suppress food intake after icv administration in animals fed high-fat diet. Antiopioid mechanism has been suggested for the inhibition of food intake induced by enterostatin. Here, we examined the effect of enterostatin on opioid-induced analgesia. Analgesic activity of morphin measured by tail pinch test in mice was suppressed by VPDPR given by icv administration. However, those by delta and kappa opioids were unaffected by enterostatin. Although enterostatin has been reported to have affinity for opioid receptors, we could not detect any measurable affinity. The antianalgesic activity of enterostatin was inhibited by RU486, an antagonist for glucocorticoid receptor. It has been reported that enterostatin elevated corticosterone level and that dexamethasone inhibited morphin analgesia. These results suggest that antianalgesic activity of enterostatin is mediated by corticosterone. The antianalgesic activity of corticosterone might be not mediated by nuclear glucocorticoid receptor because it was observed immediately after the administration. Among enterostatin fragment peptides, DPR exhibited higher antianalgesic activity than VPDPR. However, DPR did not suppress food intake suggesting that antianalgesic and anorectic activities of enterostatin are mediated by different mechanisms.

LONG-TERM EFFECTS OF EARLY SOCIAL ISOLATION ON ADULT SOCIAL BEHAVIOR AND SUSCEPTIBILITY FOR DRUG DEPENDENCE; INVOLVEMENT OF OPIOID SYSTEMS

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Clinical research has demonstrated an important association between substance dependence and 'early onset' psychiatric disorders, such as antisocial personality disorder and attention deficit hyperactivity disorder. These disorders are usually present before the addiction habit starts, indicating that preceding abnormal social behavior may coincide with the susceptibility to become dependent on drugs. Furthermore, preclinical research has shown that early social attachment is important for In the present study the effects of early social isolation (week 4-5 of life) in rats on adult social behavior and on the sensitivity for intravenous cocaine self-administration were assessed. As opioid systems are implicated a variety of rewarding behaviors, opioid systems are also suggested to be involved in the long-term effects of early social isolation. To study this the consequences of early social isolation on mu-, delta-, and kappa-opioid receptors in the adult brain were investigated using in vi Early social isolation reduced social exploration in adult rats. Morphine treatment counteracted this reduction in isolated rats, but decreased social exploration in socially housed rats. With respect to the opioid receptors, early social isolation resulted in regiospecific increases in mu-opioid binding sites with a 58% increase in the basolateral amygdala and a 33% increase in the bed nucleus stria terminalis. Morphine treatment in isolated reversed this upregulation in both areas. The number of delsed a leftward shift in the dose-response curve for cocaine intake, indicating that the animals have become more sensitive for cocaine reward after early social isolation. The results show that early social isolation causes long term effects on social behavior, self-administration behavior and on the number of mu-, delta-, and kappa-opioid receptors in distinct brain areas. The data seem to confirm the suggestion that disruption of early social relationships produces abnormal (social) behavior which, in turn, may coincide with the susceptibility to become a drug addict later in life. In addition, endogenous opioid systems may have an important modulatory role in this.

THE USE OF PHARMACOLOGICAL EXTINCTION IN THE TREATMENT OF DRUG ADDICTION

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The key feature in the development of addiction is learning reinforced by the pharmacological effects of the drugs. It has long been known, however, that learned behaviors can be removed by extinction, a mechanism triggered by the failure to obtain reinforcement after the learned response has been made. Therefore, Wikler in 1976 proposed that opiate addiction could be treated with extinction induced pharmacologically by blocking reinforcement with opioid antagonists such as naltrexone. His hypothesis has been supported by both the preclinical and clinical tests. Preclinical studies demonstrated that opiate self-administration by animals is extinguished when the behavior is made while an opioid antagonist is present. A large NIDA double-blind, placebo-controlled clinical trial showed that patients taking naltrexone before self-administering heroin or methadone had significantly better results than those on placebo, but there were no significant benefits from naltrexone under conditions precluding extinction. Reinforcement from the opioidergic system is also important for the development of alcoholism. Consequently, it was proposed that pharmacological extinction with opioid antagonists would be effective in the treatment of alcohol dependence (Sinclair, 1989). A long series of preclinical studies demonstrated extinction of drinking and lever pressing for ethanol. Data from the recent Finnish factorial double-blind, placebo-controlled trial plus data from earlier clinical trials have now shown that opioid antagonists are effective in treating alcoholism when used with protocols favorable for extinction but not when instructions prevent extinction (e.g., taking naltrexone only during abstinence). Theoretically, pharmacological extinction is a new form of medical treatment with wide possibilities. With naltrexone or nalmefene, it should be effective in treating addiction to all drugs for which the reinforcement is mediated by the opioid system, and for treating other opioidergically reinforced compulsive behaviors, such as gambling, kleptomania, self-injurious behavior, and bulimia.

MORPHINE EXACERBATES HIV TAT TOXICITY WITH DIFFERENTIAL EFFECTS IN NEURONS AND ASTROGLIA

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Opiate drug abuse may increase the incidence of AIDS associated dementia complex in human immunodeficiency virus (HIV) exposed individuals. To assess potential toxic interactions, mouse neuron or astroglia-enriched striatal, or human forebrain neuronal, cultures were treated with opioids and/or HIV-1 Tat1-72 (Tat) protein. Subpopulations of neurons and astroglia possessed μ, d, or ? opioid receptors (OR). Morphine significantly increased caspase activation and cell death in Tat exposed human and/or murine neurons at 24 h, while morphine plus immunoneutralized Tat or Tat?31-61 (inactive) was nontoxic. In astroglia, morphine plus Tat caused synergistic increases in calcium, but cell survival was unaffected. All effects of morphine were markedly attenuated by naloxone. Thus, opiates potentially exacerbate HIV neurotoxicity through mechanisms that target both OR-expressing neurons and astroglia. Support: NIH NS39253, DA13559 & DA13728.

MU RECEPTOR EXPRESSION IN IMMUNE CELLS IS INDUCED BY IL-4 AND TNF-ALPHA

Jürgen Kraus, Christine Börner, Elisa Giannini, Kathrin Hickfang and Volker Höllt

The aim of this study was to investigate regulation of mu opioid receptor expression in immune cells by cytokines. Under unstimulated conditions no mu receptor transcripts were found by RT PCR in immune cells (cell lines of B cells (Raji), monocytes (U937) and endothelium (HMEC-1); primary T cells and granulocytes). A dramatic induction of transcription was observed in all these cells after stimulation with either IL-4 or TNF-alpha. Transient expression of reporter gene constructs in combination with promoter deletion and mutation analysis in Raji, HMEC-1 and SH SY5Y cells revealed a single cis-active promoter element in the human gene at nt -997 and the rat gene at nt -727 conferring IL-4 regulation of mu transcription. Gel shift assays (EMSA) identified the elements as binding sites for transcription factor Stat-6. Furthermore, reporter gene analysis and EMSA revealed that TNF-alpha regulates mu receptor transcription via NFkappaB, for which several binding sites on the gene promoters were found. Interestingly, a polymorphism has been reported in humans within the Stat-6 binding site which decreases transcription factor binding and trans-activation of reporter gene transcription.

Poster Abstracts

are in alphabetical order according to presenting author or first author if presenting author has not been indicated (*). The number after the title refers to the respective poster board number.
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IN VITRO TRANSFER OF L-ACETYLMETHADOL (LAAM) ACROSS HUMAN PLACENTA (8)

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LAAM is being investigated as an alternative for treatment of the pregnant opiate addict. We report on the kinetics for transplacental transfer (TPT) of LAAM under steady state conditions and its effects on the tissue (T) utilizing dual perfusion of placental lobule. LAAM (35ng/ml) was perfused with antipyrine (AP, as a marker) in the maternal (M) circuit (C). LAAM appeared in the fetal (F) C with a lag time of 7.45 min, twice that for AP. The % F transfer rate of LAAM was 16.9 and its clearance 0.54 ml/min., 1/2 that for AP. The concentration of LAAM in the T was 185.5 ng/g vs. 7.5 for AP. The distribution ratios for LAAM were T/F, 36.6 and T/M 5.6 respectively and its elimination t1/2 was 6 hrs due to its slow release from the T. These data indicate that LAAM is highly retained by the T and its TPT to FC is low. The concentration of LAAM sequestered by T did not adversely affect its viability parameters namely, oxygen consumption, glucose utilization and lactate production. Our data suggest that the tested concentration of LAAM may have neither an indirect (via placenta) nor direct effect on the fetus.

HETEROLOGOUS BLOCKADE OF δ-OPIOID RECEPTOR DESENSITIZATION BY INVERSE β2-ADRENOCEPTOR AGONISTS (67)

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Receptor sensitivity changes are important in modulating the cell's responsiveness to extracellular stimuli. Whereas chronic agonist treatment results in receptor desensitization, internalization and down-regulation, exposure of the cells to "inverse agonists" produces opposite effects, *i.e.* sensitization and up-regulation of the persistently inactivated receptor. Using stably β 2-adrenoceptor (β 2-AR) transfected NG108-15 hybrid cells, the present study investigated whether inverse β 2-AR agonists could possibly interfere with agonist-induced desensitization of δ-opioid receptor (δ-OR) function. Although the inverse β 2-AR agonist ICI-118,551 had any effect on acute δ-OR activities, inactivation of spontaneously active β 2-ARs during the course of chronic [D-Ala^2, D-Leu^5]enkephalin (DADLE; 1 μM) treatment completely prevented agonist-induced δ-OR desensitization, internalization and down-regulation. Pretreatment of the cells with monensin (prevents receptor recycling) and ocadaic acid (protein phosphatase blocker) both reversed the inverse β 2-AR agonist effect. Thus, inverse β 2-AR agonists appear to overcome agonist-induced δ-OR desensitization possibly by accelerating receptor recycling and resensitization.

PEPTIDE AND ALKALOID AGONISTS DICTATE THE DIFFERENT FATE OF INTERNALIZED DELTA OPIOID RECEPTOR (32)

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In a previous study, we observed a more robust desensitization of the human delta opioid receptors induced by peptide agonists (DPDPE and deltorphin I) after a 30 min pretreatment when compared to an alkaloid agonist, etorphine (Allouche et al., 1999). We investigated whether this faster desensitization could be linked to a different fate of the agonist-induced receptor internalization. Using binding experiments, we observed that peptides induced an important receptor internalization to lysosomal compartments while etorphine produced a sequestration to a lesser extent and that these receptors were probably targeted to endosomes. These results suggest a different fate of internalized opioid receptor depending on the chemical nature, peptide or alkaloid, agonists. Allouche et al. (1999), Eur. J. Pharmacol., 371, 235-240.

MORPHINE WITHDRAWAL ALTERS EXPRESSION OF NEUROTROPHIC FACTORS IN THE RAT BRAIN (110)

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Recent evidence suggests that various psychotropic drugs alter gene expression in the brain. Strong induction of immediate early genes has been reported under conditions of morphine withdrawal. Alterations in late response genes which may play a role in neuroplasticity such as neurotrophic factors are less well documented. Thus we now report the regulation of two neurotrophins in response to precipitated morphine withdrawal. Rats were treated with ascending doses of morphine for 10 days. Withdrawal was induced by application of naloxone. Animals were decapitated 180 min after drug application. As revealed by in situ hybridization BDNF (Brain Derived Neurotrophic Factor) mRNA expression and FGF-9 (Fibroblast Growth Factor-9) mRNA expression was downregulated in the hippocampus (BDNF mRNA decreased by 34% in the dentate gyrus ($p<0.01$), FGF-9 mRNA decreased in CA1 (-42%, $p<0.05$)) and FGF-9 mRNA expression decreased additionally in the red nucleus (-44%, $p < 0.05$). In contrast, mRNA expression of the receptor for BDNF, TrkB, remained unchanged. We conclude that neurotrophins are regulated in a similar way in the hippocampus in opiate withdrawal, this response could be related to withdrawal induced stress. Downregulation of neurotrophic factor expression in the hippocampus as a part of the limbic system could be involved in adaptive changes ultimately leading to opiate addiction.

EXPRESSION OF NOVEL IMMUNE DERIVED ORPHANIN FQ/NOCICEPTIN TRANSCRIPTS IN HUMAN B CELLS (136)

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Previous studies reported that the novel opioid like receptor (NOP) was expressed in various immune tissues and human cell lines. In order to determine if NOP signaling could be induced by immune derived peptide agonist, we sought to determine if human peripheral blood mononuclear (PBM) cells could also express Orphanin FQ/Nociceptin (OFQ/N). Previously, we demonstrated that following mitogen activation of mixed lymphocytes, full-length OFQ/N transcripts were upregulated. Here, we report the isolation and characterization of shorter transcripts, which appear to be ubiquitously expressed in both resting and activated PBM. Using 5' RACE and cDNA library screens, we isolated OFQ/N transcripts containing a novel 5' exon, 108 bp in length. Expression of these transcripts is likely to be regulated by a novel promoter and seems to be limited to immune tissues. Cell sorting experiments demonstrate that the immune derived OFQ/N messages are limited to CD19+ PBMs or B lymphocytes.

FUNCTIONAL ROLES OF THE MU-, DELTA- AND KAPPA-OPIOID RECEPTORS AND THEIR SUBTYPES IN THE MEDIATION OF OPIOID-INDUCED HYPOTHERMIA IN MICE (12)

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We have investigated the roles of the mu-, delta- and kappa-opioid receptors and their subtypes in the mediation of opioid-induced hypothermia in mice. Measuring rectal temperature after i.p. injection, we tested opioid agonists (morphine, fentanyl, SNC80, U50,488H and loperamide) alone and combined with opioid antagonists (naloxone, beta-funaltrexamine, naloxonazine, naltrindol, BNTX, naltriben, nor-binaltorphimine, DIPPA and methyl-naltrexone). All agonists produced hypothermia. The effects of mu-agonists were antagonised by naloxone and by the mu-1-antagonist naloxonazine. The delta-2-antagonist naltriben potentiated the effects of mu-agonists. SNC80-induced hypothermia was blocked by the delta-antagonist naltrindol but not by the delta-1-antagonist BNTX. U50,488H-induced hypothermia was antagonised by naltriben and the kappa-antagonist nor-binaltorphimine. The peripherally acting opioid loperamide produced hypothermia which was blocked by several antagonists including the peripherally acting antagonist methyl-naltrexone. In summary, mouse body temperature can be used to study interactions between receptor-selective opioid agonists and antagonists acting centrally as well as peripherally

QUANTITATIVE AUTORADIOGRAPHY OF MU, DELTA, KAPPA, ORL-1 AND A1 RECEPTORS IN THE BRAINS AND SPINAL CORDS OF A2A ADENOSINE RECEPTOR KNOCKOUT MICE (68)

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There is evidence for interactions between adenosine and opioids and mice deficient in adenosine A2A gene are hyperalgesic. To further study these interactions we have carried out quantitative receptor autoradiography to determine any changes in the binding of mu, delta, kappa and ORL-1 opioid as well as A1 adenosine receptors in A2A receptor knockout mice. Adjacent coronal sections were cut from the brains and spinal cords of +/+ and -/- mice and binding of [3H]DAMGO, [3H]DELT-I, [3H]CI-971 receptors in the brain and of delta and kappa opioid receptors in the spinal cord but not in the brain of A2A knockout mice.

DELINEATION OF THE MECHANISM OF ERK REGULATION BY MU OPIOIDS (69)

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In previous studies on the neurotrophic actions of opioids, we reported on the mechanism involved in kappa opioid activation and chronic mu inhibition of MAP kinase phosphorylation in rat C6 glioma cells that were grown under conditions in which they express an astrocytic phenotype. Here we demonstrate that acute (1-5 min) mu opioid agonists, endomorphin and morphine, stimulate phosphorylation of the MAP kinase, ERK. Selective inhibitors of L-type calcium channels, intracellular Ca^{2+} release, clathrin-mediated endocytosis, PKC, FGF receptor and matrix metalloproteases attenuated acute mu agonist stimulation of ERK phosphorylation. Taken together with previous findings, the data suggest that acute mu and kappa opioid signaling to ERK is initiated via a similar Ca^{2+} and phosphatidylinositide dependent mechanism. However, mu and kappa signaling appear to differ in their requirement for receptor-endocytosis. Finally, evidence is presented to suggest that opioid signaling converges with the growth factor receptor pathway by transactivation of a receptor tyrosine kinase in C6 cells. Supported by NIH grant DA05412.

KAPPA-BINDING PROPERTIES AND G-PROTEIN ACTIVATION OF DYNORPHIN-A-1-17 (53)

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The aim of this study was to develop and characterise novel radiolabelled probe for kappa-opioid receptor based on the structure of endogenously occurring dynorphin peptides. Dynorphin-A-1-17 was prepared by solid phase peptide synthesis both in unlabelled and in tritiated form (40 Ci/mmol). Receptor binding and G-protein activation of the ligands were evaluated in membrane fractions of rat brain and chinese hamster ovary cells expressing κ-opioid receptors. Dynorphin-A-1-17 dose-dependently stimulated the incorporation of 35S-labelled GTPgammaS into brain and cell membranes and this effect was inhibited by naloxone. The tritiated compound bound to kappa-receptors in specific manner with high affinity. Equilibrium Kd values were below or around 1 nM depending on the tissue used and the assay type. Chemical integrity of the radioligand under incubation conditions was proven by HPLC. Rank order potencies of ligands in competition assays are consistent with a kappa-binding profile. It is concluded that the radiolabelled dynorphin derivative is a valuable tool in studying κ-receptor function at cellular and molecular level. Supported by OTKA T-25711 T-03086, T-30841 and T-35211 grants.

THE OPIOID-INDUCED REGULATION OF TRANSCRIPTION AT THE CELLULAR LEVEL (70)

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Opiates, acting on opioid receptors (coupled to G_i/G_o classes of the G proteins) inhibit cyclic AMP (cAMP) formation, Ca^{2+} conductance and activate potassium conductance, leading to the suppression of neuronal excitability. However, opioids can also cause an increase in intracellular Ca^{2+} and activate mitogen-activated protein kinases (MAPK). Therefore it appears that opioids, at the cellular level, exert both inhibitory and stimulatory effects. The adaptations in cAMP and Ca^{2+} levels result in alterations in the activity of several transcription factors. Especially *Ca^{2+}/cAMP Responsive Element Binding Protein* (CREB) and *Activated Protein 1* (AP-1) can establish a direct link between opioid-regulated signal transduction pathways and the modulation of gene expression. It was found that acute administration of opioids increased CREB phosphorylation and binding to consensus CRE and AP-1 elements without affecting total CREB protein level. In contrast, prolonged opioid treatment normalized back to basal the levels of CRE and AP-1 DNA binding activity and slightly decreased the levels of phoshorylated CREB. Withdrawal from the drug elicited an increase in phosphorylated CREB levels and induced CRE and AP-1 DNA binding activity. Consequently, opioids regulated CRE- and AP-1- directed transcription of luciferase reporter gene as well as the expression of target genes (e.g. proenkephalin). Our findings provide evidence that the regulation of gene expression may contribute to development of tolerance and addiction. Our results also highlight the role of transcription factors in the adaptations to opioids at the cellular level.
This study was supported by KBN grant P05A.107.20

DEFECTIVE OPIOID PEPTIDE PROCESSING CORRELATES WITH DECREASE IN OPIOID RECEPTOR SYSTEM IN CPE ^{FAT}/CPE ^{FAT} MICE (106)

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Carboxypeptidase E (CPE) is involved in the biosynthesis of a number of neuropeptides including opioid peptides. A point mutation in the carboxypeptidase E (CPE) gene results in a loss of CPE activity and development of late onset obesity. We found alterations in the processing of peptides derived from Prodynorphin (ProDyn) and proopiomelanocortin (POMC) in discrete brain regions of *Cpe* ^{fat} mice. This precedes the development of late onset obesity. We also examined how changes in the level of opioid peptides affect the receptor system. We found that the functional activity of μ and κ opioid receptors (evaluated by [35S] GTP gamma S binding) is reduced in the brain of *Cpe* ^{fat} mice. These changes are in good agreement with increased level of β-endorphin1-31 and Dynorphin A-17 in *Cpe* ^{fat} mice. In a related study, we found that Cocaine –and amphetamine regulated transcript (CART 55-102) levels decrease only after 10-12 weeks, correlating with the development of obesity. It is possible that the changes in the level of the anorectic peptide CART 55-102 may promote obesity in *Cpe* ^{fat} mice through a novel pathway, however the involvement of the opioid system is evident also.

A NEW MODEL OF THE KAPPA OPIOID RECEPTOR: DOCKING OF SELECTIVE AGONISTS AND ANTAGONISTS (54)

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Using homology modelling, we have developed a model for the kappa opioid receptor based on the recently reported high resolution structure of bovine rhodopsin. The residues which we have identified to form part of the active site, are similar to those proposed in other models and are consistent with site-directed mutagenesis and chimera studies. Firstly, we tested our receptor model against a pharmacophore model proposed by Brandt et al. in which the orientation of the basic NH group in the kappa agonist, ketazocine (KCZ), is equitorial. The message portion of the kappa selective ligands under study, bind to a pocket formed by extracellular loop (EL) 2 and transmembrane regions (TM) 3 and 6. The address portion of the kappa antagonists, a second basic nitrogen, interacts with the negatively charged Glu 297 residue as previously reported. A new finding is that the hydrophobic portion of the address is able to occupy either of two lipophilic pockets, formed by EL 2 and 3 or TM 6 and 7 respectively. For norBNI, these pockets are occupied by the cpm and phenolic moeities of the 2nd pharmacophore, respectively. This has allowed us to identify new synthetic targets for evaluation as kappa selective ligands. Funded by NIDA grant DA00254

DOCKING OF DELTA SELECTIVE AGONISTS AND ANTAGONISTS TO TWO BINDING SITES OF THE DELTA OPIOID RECEPTOR (55)

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Based on the recently reported high resolution structure of bovine rhodopsin, we have developed a molecular model of the delta opioid receptor. An initial model was formed by homology modelling and loop search algorithms. Refinement of the model was performed using the AMBER force field. Inspection of the model with PROCHECK showed a high quality model with regard to dihedral angle distribution. We then performed docking studies using a multitude of delta selective opioid agonists and antagonists, including 14-alkoxy substituted indolo- and benzofuromorphinans, beta-casomorphins and others. We detected two possible binding sites, one formed by the extracellular loops and another further inside the transmembrane helix bundle. These results are consistent with site-directed mutagenesis and chimera studies. The docking studies help to understand structure activity relationships which are in agreement with earlier proposed models. Aspects of the differing modes of action of agonists and antagonists will also be discussed.

DOCKING OF MU SELECTIVE AGONISTS AND ANTAGONISTS TO TWO BINDING SITES OF THE MU OPIOID RECEPTOR (56)

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We have developed a molecular model of the mu opioid receptor based on the recently reported high resolution structure of bovine rhodopsin. Firstly, a crude model was formed by homology modelling and loop search algorithms. Refinement of the model was performed using the AMBER force field. Inspection of the model with PROCHECK showed a high quality model with regard to dihedral angle distribution.Subsequently, docking studies were performed using a multitude of mu selective opioid agonists and antagonists including morphine derivatives, fentanyls and several mu selective peptides. We detected two possible binding sites, one formed by the extracellular loops while the other was inside the transmembrane helix bundle. The docking studies help to understand structure activity relationships of mu selective ligands belonging to different classes of compounds. The results are in agreement with models of structure activity relationships we proposed earlier. Aspects of the different modes of action of agonists and antagonists will also be discussed.

DOCKING OF KAPPA SELECTIVE AGONISTS AND ANTAGONISTS TO TWO BINDING SITES OF THE KAPPA OPIOID RECEPTOR (57)

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We have developed a molecular model of the kappa opioid receptor based on the recently reported high resolution structure of bovine rhodopsin. Homology modelling and loop search algorithms were used to create a crude initial model. The AMBER force field was used to refine the model. Inspection of the model with PROCHECK showed a high quality model with regard to dihedral angle distribution. Docking studies were then performed using a multitude of kappa selective opioid agonists and antagonists, including 2,4-diaryl-substituted 3,7-diazabicylononanones, several aryl-acetamides, KCZ, norBNI and others. Two possible binding sites were detected, one formed by the extracellular loops and another inside the transmembrane helix bundle. The docking studies help to understand the structure activity relationships of different classes of kappa selective ligands. The results are in agreement with a model of structure activity relationships which we proposed earlier. Further aspects of the different modes of action of agonists and antagonists will also be discussed.

NEW ASPECTS OF A SIGNAL TRANSDUCTION PATHWAYS BASED ON DOCKING STUDIES OF MU, DELTA AND KAPPA OPIOIDS TO THEIR CORRESPONDING RECEPTORS (58)

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Here we present a summary of our docking studies of mu, delta and kappa selective opioid ligands and their corresponding receptor models. In all three cases, different possible modes of binding could be detected. From the analysis and comparison of the binding sites, certain areas could be detected which are highly similar among the three receptor types, while others which are not as similar might be responsible for selectivity. These results also allow for discussion of possible modes of activation or pathways of signal transduction within the receptors when occupied by a ligand.

CONVULSANT ACTIVITY OF NONPEPTIDIC DELTA-OPIOID RECEPTOR AGONISTS IS NOT REQUIRED FOR ANTIDEPRESSANT-LIKE EFFECTS (15)

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Recent studies have shown that nonpeptidic delta-opioid receptor agonists possess antidepressant-like activity when tested in the rat forced swim assay. As these compounds have previously been shown to also possess convulsant properties in mice, the aim of the present study was to observe such convulsions in rats and to assess the relevance of convulsive activity to antidepressant-like activity. The nonpeptidic delta-opioid receptor agonists SNC80 and (+)BW373U86 both produced dose-dependent convulsant activity in rats similar to that seen in mice. The delta-opioid receptor antagonist naltrindole prevented the convulsant activity of (+)BW373U86 and the effects of this drug in the forced swim assay. This indicated a delta-opioid mechanism of action for both effects. The anticonvulsant, midazolam prevented convulsions but did not prevent the antidepressant-like activity of (+)BW373U86. In addition, animals tolerant to the convulsant effects of (+)BW373U86 still displayed antidepressant-like effects. In summary, delta-mediated convulsions do occur in rats but are not required for the antidepressant-like activity of these drugs. Research supported by USPHS grants DA00254, GM07767 and DA07267.

ACUTE AND CHRONIC MORPHINE AFFECT PROOFQ/N GENE EXPRESSION IN DIFFERENT RAT BRAIN AREAS (111)

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Nociceptin or orphanin FQ (OFQ/N) has been suggested to play a functional antiopioid role at cerebral level. We investigated the possible effects of acute and chronic morphine treatments upon pronociceptin (proOFQ/N) gene expression in the rat CNS. Morphine (10 mg/kg i.p.) was administered acutely or twice daily for seven days and proOFQ/N mRNA levels were evaluated in rat brain areas by Northern analysis. Acute morphine caused significant ($p<0.05$) changes of proOFQ/N mRNA levels in the striatum (145% vs controls), hippocampus (242%), nucleus accumbens (158%), temporal-parietal cortex (149%) and midbrain (47%). Chronic morphine induced a significant increase of proOFQ/N mRNA levels in the brainstem (174%), midbrain (172%), hippocampus (265%) and ventral tegmental area (148%) whereas a decrease was observed in the striatum (59%) and nucleus accumbens (51%). These data show that morphine can modulate proOFQ/N gene expression in several brain areas including those related to pain transmission, thus supporting the interplay between OFQ/N and opioid systems and suggesting the involvement of OFQ/N in the mechanisms underlying the development of morphine tolerance.

INDUCTION OF N/OFQ LEVELS BY TRAUMATIC BRAIN INJURY (47)

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Nociceptin/Orphanin FQ (N/OFQ) is an opioid-related neuropeptide involved in stress, anxiety, and inflammatory responses. We have reported earlier that N/OFQ gene expression is dramatically increased in vitro by factors induced in the CNS after ischemic, excitotoxic and traumatic injuries. Here we describe the induction of N/OFQ peptide levels in vivo after traumatic brain injury. We have detected a profound increase in N/OFQ immunoreactivity in the cerebral cortex in the vicinity of a stabade in the elevation of N/OFQ immunoreactivity by traumatic brain injury. We have administered SB202190, a selective p38 inhibitor, through the Hamilton syringe used to inflict the stab wound injury. Control animals received vehicle during injury. SB202190 significantly reduced the increase of N/OFQ levels in the lesion area. Thus p38 MAP kinase pathway appears to mediate N/OFQ induction after stab wound injury. The function of N/OFQ in injury-induced responses in the brain is currently being investigated.

EFFECTS INDUCED BY CB-1 RECEPTOR AGONISTS ON VISUAL EVOKED RESPONSES AND OSCILLATORY POTENTIALS IN THE MOUSE (71)

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Oscillatory potentials (OPs) consist of short lasting (15-30 ms) low voltage (5-30 microV) bursts of sinusoidal waves, which are embedded inside traditional visual evoked response waveforms, and can be extracted through off-line high-pass filtering. In view of the diffusion of cannabinoids as drugs of abuse, and the presumed physiological influence exerted by OP in the synchronization of some electrical signals along visual pathways, we started a series of studies in order to verify: 1) whether CB-1 agonists induce excitatory (or vice-versa depressant) effects on visual evoked responses induced by flash stimuli (F-VER) and onOP in the free-moving, unanesthetized mouse. 2) whether CB-1 agonists may influence cerebral excitability in a model of inherited epilepsy in DBA/2J mice. Preliminary results show that anandamide at lower dose (5 mg/kg i.p.) induce decrease of amplitude (from an average of 75 microV ES 9, to 52, ES 8) and of latency (from 31ms, ES 4 to 26.5, ES 4) of F-VEPs, with quite analogous changes of OPs, while higher doses (15 and 45 mg/kg) induce dose-dependent decrease of amplitude and increase of latency. Inherited spike-and-wave epileptiform pattern, which are recorded in the electroencephalogram of DBA mice was not modified by single administration of the drug. These data suggest that some CB-1 agonists induce an excitatory effect on the visual pathways, without influencing general brain excitability. Further studies are in progress to compare anandamide effects with methanandamide, with WIN 55,212-2 and with delta-9 THC, and to have information on effects induced by repeated doses on the visual sistem.

EXPRESSION OF THE CLONED MU OPIOID RECEPTOR MOR-1 AND ITS SPLICE VARIANTS IN LUNG (72)

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Opioid receptors have well documented actions within the central nervous system. However, evidence has been accumulating for perpipheral actions as well. Topical opioids are analgesic through activation of peripheral nerves and evidence has been accumulating for opioid receptors in immune cells. Opioid receptors also have been proposed with the lung (for review, see Life Sci. 66:2221, 2000), perhaps explaining the utility of nebulized morphine for dyspnea. Since the initial description of the cloned mu opioid receptor (MOR-1, also described as MOP1), a number of splice variants have been reported. In the current studies, we now report the presence of MOR-1 and several additional splice variants with in the lung using RT-PCR. Using primers defined by the variants previously reported, we obtained evidence for the expression of MOR-1, as well as MOR-1C, MOR-1G and MOR-1K. These variants have been cloned and sequenced and correspond to the same splice variants previously reported in mouse brain. Additional work exploring the significance of these variants will hopefully extend our understanding of the clinical utility of morphine for non-analgesic uses.

CO-EXPRESSION OF DELTA OPIOID RECEPTORS WITH MU RECEPTORS IN GH3 CELLS CHANGES THE FUNCTIONAL RESPONSE TO MU AGONISTS (73)

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GH3 cells show spontaneous activity characterized by bursts of action potentials and oscillations in [Ca2+]i. In GH3 cells expressing only mu receptors (GH3MOR cells), the mu receptor-specific ligand DAMGO inhibits spontaneous Ca2+ signaling. By contrast, in cells expressing both mu and delta receptors (GH3MORDOR cells) DAMGO has an excitatory effect on Ca2+ signaling that is mediated by thapsigargin-sensitive intracellular Ca2+ stores. The electrophysiological effects of mu or delta recept formation of a mu/delta heterodimer with distinct functional properties. Supported by NSF IBN-9982585 (A.C.) and NIDA DA05010 (T.H. and A.C.)

THE EFFECT OF PAEONIFLORIN ON DRUG-INDUCED BITING AND SCRATCHING BEHAVIOR (114)

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Paeony roots (*Paeonia lactifloria* PALL) has been used in gynecological disorders as an spasmolytic and analgesic in Chinese literature. From our preliminary studies, we found that paeoniflorin, a major component of paeony roots, has antinociceptive effect on both writhing response and formalin test. In this study, it showed that paeoniflorin attentuated the seizure-like excitation induced by high dose morphine (80 mg/5ml) which was also inhibited by MK-801(18 ng/5mL). This inhibitory effect of paeoniflorin was potentiated by treatment with MK-801. In addition, paeoniflorin did inhibit drug-induced biting and scratching behavior. Drugs, such as glutamate, NMDA, AMPA and trans-ACPD etc were used in this study. Moreover, we investigated the inhibitory effects of paeoniflorin and antisense oligodeoxynucleotide (ODN) of NMDA receptor subunits (NR1, 2A, 2B, 2C) on NMDA-induced biting and scratching behavior. The results showed that paeoniflorin potentiated the inhibitory effect of antisense ODNs on NMDA-induced biting and scratching behavior. In conclusion, it was suggested that the inhibitory effect of paeoniflorin on drug-induced biting and scratching behavior might be via the inactivation of NMDA receptor.

CROSS DESENSITIZATION BETWEEN MU OPIOID RECEPTOR AND CHEMOKINE RECEPTOR CCR5 CO-EXPRESSED IN CHO CELLS (137)

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It has been shown that chemokines and opioids cause chemotactic responses in immune cells and opioids desensitize chemotactic responses to some chemokines and *vice versa*. We established CHO cell clones stably co-transfected with the mu opioid receptor and the chemokine receptor CCR5 to examine biochemical mechanisms underlying cross-desensitization between the two receptors. DAMGO and RANTES exhibited profound inhibitory effects on chemotaxis caused by the other, validating the use of the CHO cells as a model. DAMGO pretreatment enhanced phosphorylation of the CCR5 receptor and profoundly reduced RANTES-promoted [^{35}S]GTPgS binding and p44/42 MAP kinase phosphorylation without changing the K_d and B_{max} values of [^{125}I]MIP-1b binding for the CCR5. Conversely, RANTES preincubation slightly increased phosphorylation of the mu opioid receptor, significantly reduced DAMGO-induced [^{35}S]GTPgS binding and slightly decreased DAMGO-caused p44/42 MAP kinase phosphorylation; however, it did not alter the K_d and B_{max} values of [^{3}H]diprenorphine binding for the mu opioid receptor or cause internalization of the mu opioid receptor. Thus, activation of either receptor enhanced phosphorylation of the other and affected receptor-G protein coupling and downstream effector response. (Supported by NIH grants DA 04745, DA06650, DA11263, DA13429)

COMPLETE LOSS OF [3H] NALOXONE AND [3H] BREMAZOCINE BINDING IN THE BRAINS OF MU, DELTA, KAPPA TRIPLE KNOCKOUT MICE (74)

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Mice deficient in the mu, delta and kappa opioid receptor gene have been generated by a number of groups and their phenotype characterised. More recently mice lacking all three opioid receptors have been generated. Here we have carried out autoradiography of the opioid receptors using [3H] naloxone in the brains of mu, delta and kappa triple knockout mice to determine if there are any further opioid receptors encoded for by other genes. [3H] bremazocine binding was also carried out to detygous animals indicating that there are no further genes which encode for opioid receptors and there are no kappa sub-types encoded for by anothergene.

UPREGULATION OF THE MU, DELTA AND KAPPA RECEPTORS BUT NO CHANGE IN THE ORL1 RECEPTOR IN THE BRAINS OF DYNORPHIN/ ENKEPHALIN DOUBLE KNOCKOUT MICE (75)

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The cloning of the opioid peptides and receptors has led to the development of opioid receptor and opioid peptide knockout mice. Previous peptide knockout studies in enkephalin and nociceptin/orphanin FQ knockout mice have revealed a region specific up regulation of the mu and delta receptors in the enkephalin deficient mice and of the ORL1 receptors in the nociceptin/orphanin FQ mutant mice. Dynorphin/Enkephalin double knockout mice have recently been generated by homologous recombination. To further investigate compensatory mechanisms that exist within and between the opioid and ORL1 systems we have carried out detailed quantitative autoradiographic mapping of the opioid and ORL1 receptors in these animals. Sections were cut from brains 20um using a mapping interval of 300um. [^{3}H] DAMGO (4nM), [^{3}H] deltorphin-I (7nM), [^{3}H] CI-977 (2.5nM) and [^{3}H] *leucyl* nociceptin (0.4nM) were used to label the mu, delta, kappa and ORL1 receptors respectively. Here we report a region specific up regulation of the opioid receptors which reached approximately 15% for mu and delta and 30% for kappa. No change was seen in the ORL1 receptor expression in homozygous animals.

THE CELLULAR DYNAMICS OF THE ORL1 RECEPTOR IN LIVING CELLS: INTERNALISATION AND DIMERISATION (76)

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The pharmacology of internalisation of the nociceptin (Noc) receptor ORL1 was studied in living cells. ORL1 carrying at the C terminus one EGFP molecule was stably expressed in HEK293 cells and checked for its pharmacological properties. Using confocal microscopy, the kinetics of internalisation was determined at various temperatures under saturating concentrations of Noc. ORL1 was not internalise at 4°C, even after 60 min, whereas more than 80% of receptor was internalised at 22°C ($t_{1/2}$= 20 min) and 37°C ($t_{1/2}$= 8 min). The agonist lofentanil was similarly efficacious but not the Houghten hexapeptide Ac-RRYKWR-NH_2. The Banyu antagonist J-113397 but not the III-BTD was able to completely block the internalisation induced by Noc or lofentanil. The latter could also be prevented by treating the cells with 0.45 M sucrose, implicating the involvement of clathrin-dependent pathways in the ORL1 internalisation process. These results suggest that receptor regions necessary for internalisation include the transmembrane cavity of ORL1 and that surface exposed regions alone seem not to be sufficient. Receptors oligomerisation was monitored by FRET between ORL1-EBFP and ORL1-EGFP transiently co-expressed in HEK cells. The addition of 10^{-7} Noc induced within 3 min a shift in the l emission from 460 nm (blue) to 520 nm (green) when excited at 360 nm (EBFP maximal excitatory l), indicating a neighbouring of less than 100 A between the ORL1 receptors. We are currently testing other ligands to compare their relative efficacy on internalisation and oligomerisation.

MU OPIOID RECEPTOR-MEDIATED ERK-ACTIVATION INVOLVES CALMODULIN-DEPENDENT EGF RECEPTOR TRANSACTIVATION (102)

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Phosphorylation of the MAP kinase, ERK, by G protein-coupled receptors (GPCRs) involves multiple signaling pathways. One pathway entails EGF receptor (EGFR) transactivation followed by ERK activation. This study demonstrates that a similar signaling pathway is used by the mu opioid receptor (MOR) expressed in HEK293 cells and involves calmodulin (CaM). Stimulation of MOR resulted in both EGFR and ERK phosphorylation. An intermediate role of EGFR activation, involving endogenous EGF release, is supported by using inhibitors of EGFR Tyr kinase and of membrane metalloproteases. To test whether CaM contributes to EGFR transactivation and ERK phosphorylation by MOR, we compared wild type MOR with mutant (K273A-MOR), which binds CaM poorly but couples normally to G proteins. Stimulation of K273A-MOR with DAMGO attenuated ERK phosphorylation. Wild type MOR stimulated EGFR Tyr-phosphorylation 3-fold more than K273A-MOR, indicating that direct CaM-MOR interaction plays a key role in transactivation. This novel pathway of EGFR transactivation may be shared by other GPCRs shown to interact with CaM. Supported by NIDA grant DA05412.

DEXTROMETHORPHAN AND COLONIC TRANSIT – A PHARMACOLOGICAL ANALYSIS

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We have identified a new action of dextromethorphan (Dex) – slowing colonic transit in the mouse glass bead test – and now present additional pharmacological data. Male Swiss mice (25-30 g; n=8-10) were pretreated with Dex 20 min (s.c.) or 30 min (p.o.) before a 3-mm glass bead was inserted 2 cm into the rectum of each animal. Expulsion of the bead was timed over 30 min. Antitransit-50 (A50) values obtained with Dex were 20 (13-27) mg/kg s.c. and 88 (55-121) mg/kg p.o. The A50 value obtained for dextrorphan, the primary metabolite, was 32 (23-42) mg/kg s.c. Tolerance developed to the slowing effect of Dex (50 mg/kg s.c. daily) on day 5. (-)-Naloxone (0.001-1 mg/kg s.c.) antagonized the antitransit action of Dex (50 mg/kg s.c.) in a dose-related manner whereas this antagonism did not occur with (+)-naloxone (1 mg/kg s.c.), naloxone methiodide (1 and 3 mg/kg s.c.) or rimcazole (3 mg/kg s.c.). These results with Dex, the non-competitive NMDA antagonist and antitussive, implicate a centrally located opioid link in the mediation of delayed colonic transit. Our findings may have importance for future drug development in the treatment of colonic motor dysfunction.

AFFINITY AND SELECTIVITY OF N-SUBSTITUTED CYCLORPHAN DERIVATIVES (60)

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Mixed kappa agonists and mu agonists/antagonists are potential pharmacotherapeutics for cocaine abuse. Cyclorphan possesses these properties and is a candidate for therapeutic use to reduce cocaine abuse. This current study evaluated the affinity and selectivity of four cyclorphan derivatives for the multiple opioid receptors. The parent compound (-)cyclorphan had a Ki value of less than 0.1 nM for both the kappa and mu receptors, with a slightly higher affinity for the kappa receptor thass for the mu receptor. Thus, unlike the parent compound cyclorphan or the MCL-119 and -120 derivatives, MCL-121 and -122 had a higher affinity for the mu receptor than the kappa receptor. Also, the functional efficacy of these compounds, as measured by the [35S]GTPgammaS binding assay will be presented. (Supported by NIDA grants K05-DA00360, DA03742, U-19-DA11007, and K05-DA00101.)

COMPARISON OF G PROTEIN-COUPLED RECEPTOR DIMER MODELS (66)

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Burgeoning evidence suggests that G protein-coupled receptors can dimerize to form either hetero- or homodimers. The role of dimerization remains to be clarified, but some possibilities include the modulation of function and trafficking of receptors. GPCR dimerization may involve contact or domain swapped dimers, but neither model has been confirmed. Recently, we have modeled dimeric opioid receptors that have the dimer interface between either transmembrane helices 5,6 or 4,5. The models were constructed using x-ray data for rhodopsin as an initial design template. Interactive manipulation of the geometry using Insight II, followed by minimization and molecular dynamics using Discover, led to refined models. We have docked bivalent ligands with known mu opioid agonist activity to the recognition sites of these dimerizied receptor models. Comparison of our models with data from earlier studies containing bivalent ligands will be discussed.

MOTIVATIONAL EFFECTS OF N/OFQ ADMINISTERED INTO THE NUCLEUS ACCUMBENS AND VENTRAL TEGMENTAL AREA (115)

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We have previously examined motivational effects of N/OFQ using an unbiased conditioned place preference procedure with intracerebroventricular (i.c.v.) administration. Surprisingly, repeated conditioning with N/OFQ did not produce place preference or aversion in that study. We have now examined the motivational effects of N/OFQ with microinjections into limbic regions of the rats' brains. N/OFQ (1.0 nmoles) produced conditioned place aversion after microinjections into the ventral tegmental area, and conditioned place preference after microinjections into the nucleus accumbens. Injections into surrounding sites were ineffective. These findings concur with previous reports that N/OFQ decreases mesolimbic dopamine neurotransmission through actions in the ventral tegmental area, and increases dopamine neurotransmission through actions in the accumbens. The lack of apparent motivational effects after i.c.v. administration (previous study) may have resulted from motivationally-unrelated actions of N/OFQ on sensory processing, or learning and memory – actions that could have interfered with the formation of conditioned associations.

δ-OPIOID RECEPTOR CONSTITUTIVELY INTERNALIZED VIA A DYNAMIN-DEPENDENT PATHWAY (77)

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The ability of GPCR such as the opioid receptor to modulate the growth response has been well documented. Previously we demonstrated that pertussis toxin (PTX) pretreatment of fibroblasts in the quiescent phase of the cell cycle could attenuate serum-induced growth. A probable mechanism for such an observation is that PTX blocks the constitutive activity of the GPCRs such as the opioid receptor. In order to demonstrate a robust constitutive activity for the opioid receptor, the ability of the receptor to internalize in the absence of agonist was examined. A δ-opioid receptor-green fluorescent protein chimera was constructed and stably expressed in an NIH3T3 fibroblast cell line. When these cells were cultured under a sub-confluent proliferative condition, opioid agonists could inhibit the forskolin-stimulated adenylyl cyclase activity, could activate the Erk1/2 and could induce internalization of the receptor. However, when these cells were growth arrested either by contact inhibition or by serum withdrawal, these activities were not observed under the same agonist concentrations. The absence of agonist activities could be traced to a time-dependent decrease of the δ-opioid receptor expressed at the cell surface, as determined by ^{3}H-diprenorphine binding with whole cells or by FACS analysis of the total cell fluorescence. The disappearance of the δ-opioid receptor could be detected also by confocal microscopy. The loss of the cell surface δ-opioid receptor was mediated by the dynamin-dependent pathway. Co-expression of the dominant negative mutant of dynamin, K44A, in NIH3T3 cells expressing the δ-opioid receptor could attenuate the magnitude of the receptor decrease when the cells were made quiescent. These data suggest that δ-opioid receptor has robust constitutive activity, and that the ability of the receptor to constitutively internalize could have great impact on the receptor function both extracellularly and intracellularly. (*This research is supported in parts by DA07339)*

INTRAHYPOTHALAMIC INJECTION OF DELTORPHIN-II, A DELTA2 AGONIST, INDUCES HYPERTHERMIA VIA DELTA AND MU OPIOID RECEPTORS (35)

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It is well established that central mu and kappa receptors mediate opioid-induced hyperthermia and hypothermia, respectively. In contrast, the role of delta receptors in thermoregulation is unclear, although several lines of evidence suggest an involvement. First, delta opioid receptor binding and mRNA expression are present in hypothalamic nuclei that regulate body temperature. Second, delta agonists have been reported to alter the thermosensitivity of hypothalamic neurons. Therefore, the present study investigated the effect of deltorphin (DELT), a selective delta$_2$ agonist, on the body temperature of male Sprague-Dawley rats (250-350 grams). Injected into the preoptic area of the anterior hypothalamus (POAH), DELT (0.1-20 mg/mL) produced hyperthermia in a dose-dependent manner. To determine whether the hyperthermia was delta or mu opioid receptor-sensitive, either the selective delta$_2$ antagonist, naltriben (NTB), or the selective mu antagonist, CTAP, was injected into the POAH 30 min prior to the administration of DELT (1 mg/mL). NTB (1 mg/mL) attenuated the DELT-induced hyperthermia. CTAP (0.5 mg/mL) delayed the onset of the hyperthermia. When CTAP and NTB were coadministered, DELT-induced hyperthermia was completely blocked. The present data support the hypothesis that the POAH is the primary functional site in thermoregulation and suggest that intra-POAH delta$_2$, as well mu, opioid receptors mediate the hyperthermic response to opioids. (Supported by Grant DA 00376 and T32 DA 07237 from NIDA).

PRE-TREATMENT WITH A GUINEA PIG ANTISERUM RAISED AGAINST AGMATINE INCREASES SENSITIVITY TO INDUCTION OF SPINAL OPIOID ANTINOCICEPTIVE TOLERANCE (34)

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Agmatine (AG) is present in the mammalian central nervous system. Exogenously adminstered AG has since been shown to modify opioid-induced tolerance, and reverse pain due to inflammation, neuropathy, and spinal cord injury. AG inhibits NOS and antagonizes the NMDA receptor. The role of endogenous agmatine remains to be assessed. Anti-AG IgG (but not normal IgG) reversed exogenous AG-mediated (but not MK801-mediated) inhibition of NMDA-evoked behavior in mice, suggesting specificity of the IG also modulates other plasticity-related events remains to be determined. (Supported by NIH/R01-DA-11236 to GLW and NCCAM grant P50AT00009-02 supports CAF.)

MUTATIONAL ANALYSIS OF CONSERVED RESIDUES OF THE DELTA- OPIOID RECEPTOR RESPONSIBLE FOR RECEPTOR ACTIVATION AND FUNCTION (99)

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A remarkably conserved sequence that is shared among most G protein-coupled receptors is the aspartate-arginine-tyrosine (DRY) motif that is located at the amino terminus of the putative second intracellular loop, considered to be critical in G protein recognition and activation. Using site-directed mutagenesis, we investigated the possibility of an identical role for the aspartate of this DRY motif in the mouse d-opioid receptor. Mutation of the aspartate 145 into alanine, resulted in reduced expression and loss of antagonist binding in membranes of transiently transfected COS-7 cells, abolished DSLET mediated-inhibition of forskolin-stimulated cAMP accumulation and phosphatidylinositol formation in cells expressing similar levels of receptor. Moreover, mutation of the asparagine 67, located at the first transmembrane domain of the d-opioid receptor, considered to be critical in the formation of the receptor binding polar pocket to alanine, improves DSLET mediated inhibition of cAMP accumulation and retains phosphatidylinositol hydrolysis at the same levels as the wild type. Taken together, our data demonstrate that both highly conserved amino acids, aspartate-145 of the DRY motif and asparagine-67 of the first transmembrane domain, play important roles in d-opioid receptor function and activation. (Supported by YPER6 and EPETII grants to Z.G).

DIPSOGENIC ACTION OF A NOVEL PEPTIDE: BV8 (130)

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The small protein Bv8 has been isolated from Bombina frog. BV8 mammalian homologue is expressed in testis, brain, dorsal and ventral horns of spinal cord, cardiac muscle, skeletal muscle, gastro-intestinal tract, kidney, liver and peripheral leukocytes. Bv8 dose-dependently (0.05-10 mcg/rat, icv) enhanced drinking in rats (maximum cumulative effect = 11.9±0.9 ml). Comparable dipsogenic effect was obtained with 40-100 times higher s.c.doses. Angiotensin II receptor antagonists losartan (5 mcg, icv) and EXP73174 (0.5 mcg, icv) did not antagonize BV8 dipsogenic effect. Pretreatment with atropine (2 and 15 nmol, icv) or with the beta-adrenergic antagonist propranolol (10 and 20 mg/kg, s,c,) did not significantly reduce Bv8 dipsogenic effect. Icv Bv8-induced drinking was blocked by the alpha2-adrenergic agonist clonidine (30 mcg/kg, i.p.; 1 mcg/rat, icv), by the NMDA receptor antagonist MK801 (1 and 30 nmol/rat icv) and by intragastric administration of 10 ml water. Arterial blood pressure (130±10 mmHg) or plasma osmolality (303±11 mmol/kg) were unchanged. Dipsogenic doses of BV8 activated c-fos expression in the sub-fornical organ, a brain area reach in osmoresponsive neurons subserving drinking.

MORPHINE PROMOTES BREAST TUMOR PROGRESSION AND ANGIOGENESIS BY ACTIVATING PRO-ANGIOGENIC AND SURVIVAL-PROMOTING SIGNAL TRANSDUCTION PATHWAYS IN VASCULAR ENDOTHELIUM (16)

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Opiates are used to treat pain in patients with cancer. Cancer progression depends upon angiogenesis. Here we show that morphine promotes breast tumor growth and elucidate a role for morphine in tumor angiogenesis. Morphine and the mu selective agonist, DAMGO, stimulate human microvascular endothelial cell (HMEC) proliferation and angiogenesis, in vitro and in vivo. Morphine induced HMEC tube formation on Matrigel (45+15%) in vitro. In mice, Matrigel implants admixed with 10 UM morphine showed a comparatively dense network of blood vessels, qualitatively and quantitatively, compared to controls ($p<0.05$). Morphine effects were mediated by phosphorylation of MAPK/ERK via a Gi coupled G-protein receptor and NOS, Ras and P13-Kinase signaling pathway. Morphine prevented apoptosis in serum starved HMEC by 35%, preserved a significantly higher number of cells in ' S' phase ($p<0.01$, vs. untreated) and promoted cell cycling in G2/M phase. Pro-angiogenic activity of morphine may be clinically significant because morphine (in clinical doses) promoted breast tumor growth of human MCF7 cell xenografts in mice. We speculate that opiate analgesics may promote tumor progression in cancer patients.

DYNORPHIN: NON-OPIOID MECHANISMS OF TOXICITY (51)

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Dynorphin A (dyn) (1-17) has demonstrated toxicity in striatal neurons via NMDA and non-opioid/non-NMDA mechanisms. We studied the mechanisms of dyn toxicity in neuron-enriched cultures derived from embryonic mouse striata using time-lapse photography. After 48 hr, dyn (1-17) was significantly neurotoxic, and this was unaffected by either naloxone or MK-801, but was attenuated by CNQX. The (13-17) dyn fragment also produced marked neurotoxicity that was not prevented by naloxone, MK-801 or CNQX, while (3-13) and (6-17) fragment toxicity was significantly attenuated by MK-801. Dyn may activate AMPA/kainate receptors directly or indirectly via glutamate release, although our culture conditions do not support glutamate retention or accumulation. Our findings suggest that, in addition to actions at ?- and NMDA receptors, dyn can be toxic to striatal neurons via an AMPA/kainate receptor mechanism. NIDA 13559 & DA13728.

INFLUENCE OF THE NOVEL PEPTIDE HLDF6 ON MORPHINE TOLERANCE AND DEPENDENCE (117)

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The novel hexapeptide HLDF6 is a fragment of an 8.2 kDa cell differentiation factor derived from the HL-60 cell line (Kostanyan et al., 1994, FEBS Lett., 356: 327-9). Initially, we used a conditioned place preference (CPP) schedule in male Wistar rats to determine whether this peptide possessed dose-related motivational properties in comparison with morphine. It was revealed that morphine (1.5 mg/kg) produced CPP by the third cycle of a 7-day repeated conditioning schedule ($P<0.05$, one-factor ANOVA), but no effect was observed with either vehicle or HLDF6 (1-100 mcg/kg), suggesting that HLDF6 lacked positive motivational properties as assessed by this CPP schedule. In subsequent experiments, chronic subcutaneous administration of increasing doses of morphine (20-60 mg/kg) twice daily over 14 days in adult male C57Bl/6 mice caused both dependence, as indicated by observed signs of naloxone-precipitated withdrawal abstinence, and also tolerance, as evidenced by a decrease in morphine antinociception in the hot plate test. The HLDF6 peptide (4 mg/kg) was administered 24 hours prior to testing and it was noted that it restored up to 96% of the antinociceptive effect of morphine in tolerant subjects. It was also devoid of inherent behavioral effects, but it altered certain features of naloxone-induced morphine withdrawal (jumping, sniffing, aggression and shaking). Moreover, in 25 % of cases, HLDF6 prevented the manifestation of abstinence hyperalgesia suggesting that this peptide modifies the expression of not only tolerance but also dependence to morphine. Supported by INTC grant # 1554 and Wellcome Trust BRC Grant # 059823.

OPIOID WITHDRAWAL DECREASES BASAL MAP KINASE LEVELS THROUGH PROTEIN KINASE A (PKA) (108)

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It has been established that opioid receptors, like many other G protein-coupled receptors (GPCRs) can signal to downstream effectors through the activation of extracellular signal-regulated protein kinases (ERK 1 and 2). Repeated exposure to opioid agonists leads to a progressive loss of opioid receptor function, and two separate phenomena – receptor desensitization and down-regulation – appear to contribute to this decrease. Studies have shown that ERK activation may contribute to the development of opioid receptor desensitization and down-regulation, although no clear mechanism of action has been established. During opioid withdrawal, several biochemical and molecular events occur including a super-activation of adenylyl cyclase, an increase in protein kinase A (PKA) activity, and a rise in cAMP response-element binding protein phosphorylation (pCREB). We now have evidence that the withdrawal of opioids from chronically exposed C6-μOR-expressing cells has a profound impact on phospho-ERK levels. Naloxone-precipitated withdrawal produces a decrease in phospho-ERK to near undetectable levels. This phenomenon appears to be totally dependent on an increase in PKA activity, as it was abolished by the presence of H89. Furthermore, an increase in pCREB levels occurs simultaneously with the loss of ERK activity, suggesting that an imbalance in ERK- and pCREB-regulated gene transcription may exist during withdrawal. Lastly, we will present evidence on the putative mechanisms by which PKA abolishes cellular ERK 1 and 2 levels during opioid withdrawal. (Support from NIDA in the form of RO1-DA00017 to EJS and K01-DA00437 to HKK).

DELTA AND KAPPA OPIOIDS OPPOSITELY REGULATES GROWTH HORMONE RECEPTOR MRNA LEVELS IN HUMAN IM-9 LYMPHOBLASTS (138)

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The immune system and the neuroendocrine system have been shown to be functionally interactive. Lymphocytes possess opioid receptors as well as growth hormone receptors (GHR) by which opioids and growth hormone (GH) may modulate immune functions. We have previously demonstrated that morphine up-regulate levels of GHR mRNA as well as GH binding in human IM-9 lymphoblasts in a time- and dose-dependent manner. Changes that was abolished by pretreatment with naloxone, indicating involvement of classical opioid receptors. The present study was undertaken to investigate the effects of subtype selective opioid ligands on the regulation of GHR gene expression in the IM-9 cell line. Treatment with DADLE, a delta selective opioid agonist, at a concentration of 0.01 mM for 3 h significantly increased the expression of GHR mRNA in the IM-9 cells. An effect that was completely abolished when the cells were pretreated with 0.05 mM naltrindole. Converse to the effects of DADLE, three hours of treatment with the kappa selective opioid agonist U69, 593 at 0.1 μM for 3 hours significantly decreased the expression of GHR mRNA. The effect of U69, 593 was blocked by pretreatment with nor-binaltorphimine, a kappa selective opioid antagonist.

OPIATES ACTIVE AT SELECTIVELY LOCATED MU-OPIOID RECEPTORS INHIBIT GABAergic VENTRAL TEGMENTAL AREA NEURONS (118)

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Opiate attenuation of GABAergic neurotransmission in the ventral tegmental area (VTA) plays a major role in disinhibiting mesocorticolimbic dopamine neurons. We combined in vivo extracellular recording with intracellular neurobiotin filling and immunocytochemistry to identify the 1) effects of mu-opioid receptor (MOR) activation, and 2) cellular distribution of MOR in VTA GABAergic neurons. The effects of heroin and [D-Ala2, N-Me-Phe4, Gly-ol]-Enkephalin (DAMGO) administration on the firing rates of VTA GABA neurons in anesthetized rats were evaluated. Heroin administration (0.06 - 0.6 mg/kg, i.p.) inhibited the firing rates of all VTA GABA neurons studied. Naloxone (1.0 mg/kg, i.p.) reversed the effects of heroin and produced a marked rebound excitation on the firing rate of these neurons. The inhibitory responses of VTA GABA neurons to microelectrophoretic DAMGO was less pronounced. Electron microscopic examination of neurobiotin-filled VTA GABA neurons showed MOR gold-silver particles that often were associated with intracellular organelles in large-sized dendrites. In contrast, small neurobiotin-labeled dendrites showed MOR labeling along nonsynaptic portions of dendritic plasma membranes. These findings indicate that this population of VTA GABAergic neurons are markedly sensitive to opiates that activate MOR strategically located on distal dendrites. This suggests that opiate facilitation of VTA dopamine transmission results from disinhibition produced by a primary inhibitory effect of heroin on this homogeneous population of MOR-containing GABA neurons. This work is supported by PHS grants DA11768 to ALS and DA08301 to SJH.

THE SIGNIFICANCE OF THE UGT2B7 HIS268TYR POLYMORPHISM IN THE FORMATION OF MORPHINE 3-GLUCURONIDE AND MORPHINE 6-GLUCURONIDE FROM MORPHINE IN HUMANS (18)

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UGT2B7 is the major UGT isoform responsible for the formation of morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G) from morphine in humans. The ability to generate M3G and M6G varies substantially between different individuals, which may be related to genetic variation in *UGT2B7*. In the present study the significance of the only known polymorphism in *UGT2B7*, His268Tyr, with respect to morphine glucuronidation was investigated by restriction enzyme analysis in 77 cancer patients on chronic morphine therapy. M3G/morphine and M6G/morphine plasma ratios were 38.7±17.6 (18.0-82.2) and 7.5±3.3 (2.4-15.4), respectively, in individuals with the His/His genotype (n=20), 42.7±30.0 (7.7-244.4) and 7.4±6.4 (0.7-50.0) in individuals with the His/Tyr genotype (n=35), and 36.0±20.2 (11.4-87.6) and 6.1±3.6 (1.3-14.4) in individuals with the Tyr/Tyr genotype (n=22). These results indicate that the *UGT2B7* His268Tyr polymorphism does not account for the considerable variability in morphine glucuronidation in humans.

IS THE PAIN RELIEF OBTAINED FROM METHADONE AND DEXTROMETHORPHAN IN A RAT MODEL OF MONONEUROPATHY DUE ONLY TO OPIOID AGONISM? (19)

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Neuropathy is characterised by hyperalgesia in which the effectiveness of opioid drugs is controversial. Indeed, a tricyclic antidepressant, amitriptyline, is clinically the one most used. It has been reported that the known opioid agonist, methadone, and a widely-used antitussive, dextromethorphan, also have ionotropic glutamate NMDA-receptor antagonistic properties. We studied these drugs in a model of mononeuropathy (Bennett and Xie, Pain 33:87-107,1988). The nociceptive threshold was determined 14 days after nerve injury by using the mechanical Randall-Selitto method. Methadone (1 and 5mg/kg i.p.) dose-dependently and significantly decreased the hyperalgesia and, at higher doses, had a strong, long-lasting antinoceptive effect. The reference drugs, morphine (5 mg/kg i.p.) and amitiptyline (15 mg/kg i.p.) had only antihyperalgesic effects. Dextromethorphan (45mg/kg s.c.) elevated the pain threshold significantly to the level of the non-operated, naïve rats. Although naloxone (2 mg/kg s.c.) was found to be hyperalgic in the non-operated paw, the methadone and dextromethorphan effects were not completely antagonized by this drug, thus suggesting their action on the NMDA receptor and, therefore, their incomplete opioid nature .

STRUCTURE-ACTIVITY RELATIONSHIPS IN THE SERIES OF DIAZABICYCLONONANONE-TYPE KAPPA-AGONISTS (59)

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The 2,4-di-2-pyridyl-3,7-dimethyl-3,7-diazabicyclo [3.3.1]nonan-9-one-1,5-diester, HZ2, is characterised by a high affinity and selectivity to the kappa-opioid receptor, in addition to a favourable pharmacological profile, long duration of action and good oral bioavailability. In order to study the influence of the substituents on the k-affinity the pyridine rings in 2- and 4- position were replaced with various different substituted arene rings, and the methyl groups in 3 and 7 position with alkyl, cycloalkyl, and substituted alkyl chains. Applying a double Mannich reaction 80 different diazabicylononanes could be synthesised. Pharmacological evaluation revealed the pyridine rings in position 2 and 4 to be replaceable with m-fluoro-, m-hydroxy- and p-methoxyphenyl rings without a loss of affinity to the kappa-receptor. Substituents of increasing size in position 3 and 7 considerably diminished the affinity to the k-receptor. The HZ2-methoiodide (N7), was found to exhibit the same pharmacological profile as the base HZ2. Molecular modelling studies using the most active diazabicyclononanes in comparison with various arylacetamides gave rise to a binding site close to the outer part of helix 5 and 7 of the kappa-receptor.

INHIBITORY EFFECTS OF J-113397, A NEWLY DEVELOPED ANTAGONIST OF NOCICEPTIN RECEPTOR (ORL1), ON [^{35}S]GTPgS BINDING TO ORL1 RECEPTOR IN MOUSE BRAIN (61)

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We determined in vitro functional profile of J-113397, the most potent and selective antagonist of nociceptin receptor (ORL1), on [^{35}S]GTPgS binding to mouse brain, and compared it with that of [Phe^{1}psi(CH$_2$-NH)Gly^2]nociceptin(1-13)NH$_2$ and naloxone benzoylhydrazone. J-113397 antagonized nociceptin-stimulated [^{35}S]GTPgS binding to mouse brain with an IC$_{50}$ value of 7.6 nM, but had no effect on basal [^{35}S]GTPgS binding by itself. Schild plot analysis demonstrated competitive antagonism of J-113397. The selective antagonism of J-113397 on ORL1 receptor was confirmed by a [^{35}S]GTPgS binding study using ORL1 receptor-deficient mice. [Phe^{1}psi(CH$_2$-NH)Gly^2]nociceptin(1-13)NH$_2$ and naloxone benzoylhydrazone showed partial agonistic activity on ORL1 receptor and strong agonistic activity on other opioid receptor, respectively, suggesting that these are unsuitable for characterization of ORL1 receptor. We conclude that J-113397 is the most potent and selective antagonist of ORL1 receptor in mouse brain, and therefore will be a useful tool for characterization of ORL1 receptors in the brain.

CHANGES IN NNOS IMMUNOREACTIVITY ARE COMPANIED BY THE DOPAMINERGIC ACTIVATION INDUCED BY APOMORPHINE (112)

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It is well known that dopaminergic activation plays an important role in opioid-induced psychological dependence and behavioral sensitization. An administration of dopamine agonist, apomorphine 2 mg/kg (s.c.), produces the cage climbing behavior in mice, showing a typical dopaminergic activation. Previous studies indicated that NOS inhibitors attenuate the climbing behavior induced by apomorphine. The present study investigates the expression of neuronal NOS (nNOS) within the striatum and hhippocampus of the mouse brain at 7h after apomorphine treatment. These findings suggest that the activation of dopamine receptor is companied by increase in the expression of nNOS in the striatum and hippocampus of the mouse. (Supported by the Korea Research Foundation Grant, KRF-2000-003-F00091).

THE EFFECT ON OPIOID PEPTIDES IN THE RAT BRAIN, FOLLOWING CHRONIC TREATMENT WITH THE ANABOLIC ANDROGENIC STEROID NANDROLONE DECANOATE (119)

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Clinical evidence suggest that abuse of anabolic androgenic steroids (AAS) may result in profound changes in personality, depressive symptoms, irritability and increased aggression. It is still unknown whether these alterations are related to changes in any particular transmitter system or if they are persistent or reversible. In this study we have focused on AAS effect on the endogenous dynorphin and enkephalin system in the brain. Male rats were given im injections of the AAS nandrolone decanoate (15 mg/kg), once daily for two weeks. Opioid peptide immunoreactivities (ir) were assessed by radioimmunoassay in two groups immediately after the treatment and in two other groups after additional three weeks without any drug treatment. Chronic AAS treatment increased the activity in the dynorphin B-ir as well as MEAP-ir in the hypothalamus, striatum and periaqueductal gray (PAG). In addition, the steroid induced an imbalance between the dynorphin and enkephalin opioid system in the nucleus accumbens, hypothalamus and PAG. This imbalance remained after the recovery period. Since increased peptide activity was found in brain regions regulating emotions and aggression it was suggested that the actual endogenous opioid systems are involved in AAS-induced changes in these behaviours.

THE ROLE OF AGONIST EFFICACY IN MU OPIOID DESENSITIZATION RATES FOR RECEPTORS EXPRESSED IN XENOPUS OOCYTES AND IN MOUSE HIPPOCAMPAL SLICES (78)

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Desensitization by mu agonists of different efficacies was studied in *Xenopus* oocytes and mouse dentate gyrus. At low levels of MOR expression (no spare receptors) in oocytes coexpressing Kir3.1 and Kir3.4, DAMGO evoked a larger maximal response than Methadone which produced a larger response than Morphine (this efficacy ranking is different than others have reported). When MOR was over-expressed (spare receptors), the agonists produced the same maximal effect. Treatment with DAMGO produced a robust GRK3/arrestin3-dependent reduction in response, whereas treatment with the partial agonists, methadone and morphine produced lesser desensitization consistent with their lower agonist efficacies. We next examined the desensitization rates in hippocampal slices. The maximal effects of DAMGO, fentanyl, morphine and methadone were not significantly different, indicating spare MOR in the dentate gyrus. Prolonged treatment (60 min) with saturating doses of either morphine or methadone produced a significant desensitization that was not different from fentanyl or DAMGO. Thus with receptor saturation, efficacy did not control opioid receptor desensitization rate in hippocampus. Supported by DA11672

NON-PEPTIDIC DELTA-OPIOID RECEPTOR AGONISTS REDUCE IMMOBILITY IN THE FORCED SWIM ASSAY IN RATS (14)

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The present study examined the effect of opioid receptor agonists on the rat forced swim assay. The delta-opioid receptor agonists SNC80 and (+)BW373U86 produced a decrease in immobility indicating an antidepressant-like effect. The kappa-opioid selective agonist CI977 showed a significant change in immobility that was not identifiable by dose. No changes were seen with the mu-selective agonist morphine. A delta-opioid mechanism of action in the forced swim assay was likely since naltritivity and potential tolerance. Taken together, the delta-agonists in the forced swim assay differ from typical antidepressants. Research supported by USPHS grants DA00254, GM07767 and DA07267.

NOCICEPTIN/ORPHANIN FQ INCREASES ANP SECRETION IN NEONATAL CARDIAC MYOCYTES (50)

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Nociceptin/orphanin FQ (N/OFQ) is a novel heptadecapeptide with an amino acid sequence similar to that of endogenous opioid peptide dynorphin A. The present study was designed to investigate the direct effect of N/OFQ on the ANP secretion in cultured neonatal cardiac myocytes via N/OFQ receptor (NOP) activation. The secretion of ANP from cultured neonatal rat cardiac myocytes was increased in terms of incubation time. N/OFQ, at a dose of 0.3, 1, 3, and 10 mM, caused increases in ANP secretibranes revealed the presence of two distinct sites. The high affinity site (10.9 ¡¾ 1.6 nM) is far less abundant than the low affinity site. Therefore, these results suggest that N/OFQ causes an increase in ANP secretion in cultured neonatal cardiac myocytes by decreasing cAMP through its binding sites.

ACTIVATION OF PERIPHERAL ORL1 RECEPTORS INHIBITS CAPSAICIN-INDUCED THERMAL NOCICEPTION IN RHESUS MONKEYS (28)

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The aim of the study was to investigate the potential nociceptive or antinociceptive function of peripheral ORL1 receptors in non-human primates. An experimental pain model has been established in rhesus monkeys by using capsaicin. Capsaicin 100 ug injected subcutaneously into the tail evoked a nociceptive response manifested as a reduced tail-withdrawal latency in 46C water, from a maximum of 20 sec to 2-3 sec. Co-administration of orphanin FQ (1-30 ug) with capsaicin dose-dependently inhist-induced local antinociception in the tail. These results provide the first functional evidence that activation of peripheral ORL1 receptors can diminish thermal nociception in primates (Supported by USPHS Grant DA00254).

KAPPA AND DELTA HETEROMERIC RECEPTORS IN RAT SPINAL CORD: EVIDENCE FOR KAPPA-2 OPIOID RECEPTORS (79)

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A long-standing debate exists between pharmacological and genetic studies of opioid receptors. Pharmacological investigations have identified a relatively large number of opioid receptor subtypes, whereas genetic studies have only identified three receptor genes corresponding to mu, delta, and kappa. One recent explanation for the discrepancy is the formation of heteromers by opioid receptors. In vitro studies demonstrated that kappa- and delta-opioid receptors form heterodimers when co-expressed in cell lines. The ligand binding profile of the heterodimers suggest that the receptors are the kappa-2 receptor subtype. However, the ability of these receptors to dimerize in vivo is still uncertain. The current study examined the existence of kappa-delta heteromers in rat spinal cord. Rat spinal cords were removed, and kappa and delta receptors were immunoprecipitated. Western blot analysis of the spinal preparations suggests the presence of heteromers composed of the kappa and delta receptors. In a [3H]-naloxone displacement assay U69,593 displaced the radioligand from a single site in spinal cord membranes with a Ki of approximately 3 micromolar. When the membranes were reduced with dithiothreitol a second site was exposed. The Ki for U69,593 at this second site was approximately 1 nM. These findings suggest that heteromeric opioid receptors exist in vivo and that these heteromers add to the diversity of opioid function.

P-GLYCOPROTEIN INVOLVEMENT IN OPIOID ANALGESIA (29)

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Interactions between the brain and the body are complex. The ability of the Pgp transport system to pump functionally active compounds from the brain to periphery defines a potentially important mechanism for the central nervous system to modulate peripheral systems. In Pgp1 antisense treated mice, lowering Pgp1 expression significantly enhanced systemic morphine analgesia and prevented tolerance, but diminished the analgesic activity of centrally administered morphine, implying that suprase Pgp1's role in the production of opioid analgesia.

MOUSE MU-OPIOID RECEPTOR SPLICE VARIANTS MOR1, MOR1C, MOR1D, AND MOR1E DIFFER IN THEIR MORPHINE-INDUCED INTERNALIZATION, DESENSITIZATION AND RECEPTOR RESENSITIZATION (107)

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The μ-opioid receptor is rapidly internalized after activation by DAMGO, but not after activation by morphine. Replacement of the μ-receptor C-terminus with the C-tail of the δ-receptor led to a receptor chimera which is internalized after morphine treatment, indicating that the C-terminus of the μ-opioid receptor can influence the agonist selectivity of endocytosis. Recently three new C-terminal splice variants (MOR1C, MOR1D, and MOR1E) of the mouse mu-opioid receptor (MOR1) were identified. We stably expressed these splice variants in HEK 293 cells. As detected by confocal microscopy all splice variants were internalized after DAMGO treatment, but only MOR1D and MOR1E were internalized after morphine treatment. In addition cAMP measurements revealed no difference for the DAMGO-induced desensitization and receptor resensitization of all receptor types, whereas morphine treatment led to a slower desensitization and a faster resensitization of the MOR1D and MOR1E receptor type compared to the MOR1-wildtype. These results reinforce the hypothesis that receptor internalization is an important mechanism for receptor recycling conferring resistance to agonist-induced desensitization.

TOPICAL OPIOID AND CLONIDINE INTERACTION IN MICE (1)

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It is well known that spinal morphine and clonidine produce synergistic effects. The aim of the current study was to explore the use of topical opioid and clonidine. Male CD-1 mice were used in all studies. To examine peripheral analgesic mechanisms, we used topical opioid and clonidine paradigm in which the tail was immersed either in dimetyl sulfoxide (DMSO) or propylene glycol, containing various drugs. Analgesia was determined in thermal tail-flick assay. DMSO solutions containing clonidine produced potd the effects of combination. Synergistic potentiation of analgesia through topical administration opioid/clonidine composition provides a new and improved approach to peripheral pain management.

CHARACTERISATION OF THE ACUTE ANTINOCICEPTIVE AND CONSTIPATORY PROPERTIES OF DIFFERENT OPIOIDS IN RATS AND GERBILS (11)

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Although opioids have been extensively studied in animal models overt the last decades, there are relatively few direct comparisons where several different, clinically used reference compounds have been tested for both analgesic and adverse effects. We have investigated the antinociceptive and constipatory properties of six opioid analgesics: morphine, fentanyl, codeine, pentazocine, tramadol and buprenorphine in rats and gerbils (*Meriones unguiculatus)* using both oral and subcutaneous administration routes. These opioids produced dose-related antinociceptive effects in thermal (tail-withdrawal reaction and hot plate tests) and chemical (formalin and writhing tests) pain models. Opioids also produced dose-related inhibition of castor oil-induced diarrhoea, and reduced intestinal propulsion, measured by gastrointestinal transit of a charcoal test meal. Different potency ratios between the antinociceptive and the constipatory effects of the opioids studied seem to reflect clinically recognised differences. This would indicate that these models are valid for studying novel opioid compounds.

BIOLOGICALLY ACTIVE PEPTIDE FROM HUMAN LEUKEMIA DIFFERENTIATION FACTOR. IDENTIFICATION AND ITS PROTECTIVE PROPERTIES (38)

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Human Leukemia Differentiation Factor (HLDF), isolated from culture medium of human promyelocytic HL-60 line cells, treated with retinoic acid, has been studied. During investigation of the HLDF the six-membered peptide (HLDF6) was identified, which kept the ability of the whole factor to cause differentiation of HL-60 cells. We failed to detect the receptors to HLDF6 peptide on the surface of HL-60 cells. HLDF6 was shown to influence on binding of a number of cytokines participating in proliferation processes. Besides the peptide revealed the expressed antiapoptotic properties. It protects as the HL-60 cells so primary culture of cerebellar granule neurones from alcohol, sodium azide, C2 ceramide and cold shock.

CORRECTION WITH THE HLDF-6 PEPTIDE OF ENDOGENOUS OPIOID SYSTEM PATHOLOGY OF MORPHINE -TOLERANT ANIMALS F_1 POSTERITY (39)

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In recent years in the context of drug abuse widespread occurrence the investigators attention has been attracted to the study of different types of pathologies in functioning of morphinized animals posterity organisms and also to elaboration of precaution and correction modes. We revealed the pathology in endogenous opioid system of these animals posterity, expressed in the rise of nociceptive reactions threshold, when analgesic doses of morphine were used to form the parents' tolerance. At the same time it is known that dynamics of nociceptive reactions thresholds are the basic marker of endogenous opioid system state. We studied the possibility to correct the revealed form of endogenous opioid system functioning pathology at morphine-tolerant animals posterity with natural biologically active HLDF-6 peptide. The peptide was intraperitoneally injected to young rats from the age of 4 to the age of 10 weeks daily at dose 0,2 mg per kg of animal weight. The thresholds of thermonociceptive reactions were measured with tail-flick test on automatic analgesimeter. The peptide was injected after nociception measurement to exclude the influence of the procedure on thresh olds magnitudes indicators. Two series of control animals were used: 1 – F1 posterity from "pure" not morphinized rats; 2 – F1 posterity from morphinized rats. The following picture was observed, when the peptide was not injected: the threshold of nociceptive reactions at pure rats posterity was stable, the threshold dynamics at morphine-tolerant animals posterity sharply increased from 6-weeked age. The rats of experimental group after peptide injection demonstrated some increase in threshold at the age of 6 weeks, however from 7-weeked to 9-weeked age the thresholds correction, i.e. not the increase, but the decrease to the level of the background and control animals from pure parents magnitudes took place. Therefore the systematic HLDF-6 peptide injection was shown to reduce essentially the thermonociceptive reactions thresholds dynamics. The mechanism of peptide action is under investigation.

INSULIN REGULATES OPIOID RECEPTOR FUNCTION AND NUMBER THROUGH DIRECT TYROSINE PHOSPHORYLATION (101)

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Recent studies have demonstrated that significant cross talk occurs between single-transmembrane receptor tyrosine kinases (RTKs) and seven transmembrane G protein-coupled receptors (GPCRs). Both receptor types are capable of undergoing agonist-dependent tyrosine phosphorylation, which appears to be crucial for their coupling to certain signal transduction cascades like the activation of extracellular signal-regulated protein kinases (ERK 1 and 2). Insulin, which binds to and activates its own RTK, was shown to affect mu opioid-mediated analgesia, in vivo, in a process that was inhibited by a tyrosine kinase inhibitor. Previously, our laboratory has reported that tyrosine phosphorylation of cloned delta ORs via the protein tyrosine kinase, Src, facilitates agonist-stimulated receptor desensitization, internalization, and down-regulation. Our present study examined whether insulin regulates mu- or delta OR function, in vivo and in vitro, via tyrosine phosphorylation. A single exposure to insulin (1 mg/ml) induces a rapid and dose-dependent increase in the appearance of phosphorylated tyrosine residues in both mu- and delta opioid receptors expressed in C6 glioma cells. Insulin-mediated tyrosine phosphorylation of ORs occurred in the absence of opioid agonists, but was significantly increased by the presence of DAMGO or DSLET. PP1, a selective Src inhibitor, prevented this response under all conditions. Hyper-phosphorylation of opioid receptors was accompanied by a decrease in agonist-mediated ^{35}SGTP-g-S binding, in the absence of changes to receptor number, suggesting receptor desensitization had occurred. Chronic exposure to insulin (1 mg/ml x 4 days) produced the opposite effect, and potentiated opioid-mediated ^{35}SGTP-g-S binding, both in vitro and in vivo, by increasing opioid receptor density. These results suggest that the insulin RTKs modulate the basal number and function of GPCRs via alterations in tyrosine phosphorylation (Supported by NIDA grants R01-DA00017 and K05-DA00364 to EJS and K01-00437 to KK).

LOCALIZATION OF NEUROPEPTIDE FF, SUBSTANCE P AND THE MU AND DELTA OPIATE RECEPTORS IN THE RAT SPINAL CORD (36)

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Neuropeptide FF (NPFF) is involved in opiate dependence, tolerance and abstinence. High levels of NPFF immunoreactivity, mRNA and receptor binding sites are found in the spinal cord. In this study coexistence of NPFF, substance P and the delta and mu opiate receptors was studied on different levels of the rat spinal cord. A high density of immunoreactive fibers containing the mu and delta opiate receptors, substance P and NPFF were located in the superficial laminae. The immunoreactive fibers for the opiate receptors and substance P extended deeper into lamina II than the ones immunoreactive for NPFF. Fibers and terminals immunoreactive for substance P and the mu and delta opiate receptors were more numerous than the ones for NPFF. Very little or no colocalization could be seen in the nerve terminals, suggesting that spinal and afferent neurons which possess mu and delta opiate receptors or substance P do not produce NPFF, which is thus generated in distinct neurons.

DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF THE HUMAN MU OPIOID RECEPTOR GENE USING MULTIPLEX PCR AND SINGLE-NUCLEOTIDE EXTENSION ON OLIGONUCLEOTIDE GELPAD MICROARRAYS (80)

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The recently completed sequencing of the human genome has led to an increased appreciation of the importance of defining specific differences or polymorphisms in the genomes of individuals and understanding the potential contribution of these polymorphisms to specific aspects of human physiology and disease. Of particularly importance is the identification of single nucleotide polymorphisms (SNPs), which, because of their high density within the genome, will enable fine resolution linkage mapping and which have particular utility in haplotype analyses. Methods that allow rapid, inexpensive, and reliable detection of SNP markers are increasingly important for genetic and genomic studies. We report here on the development of custom oligonucleotide gelpad microarrays as a matrix for single nucleotide extension or "minisequencing" reactions that allow detection of specific SNPs of the human mu opioid receptor gene. In combination with multiplex PCR of DNA isolated from individual blood samples, this method can identify multiple SNPs from multiple exons of the gene. Supported by NIH grants DA12848, D05130, DA00049, and RR00102.

NOCICEPTIN RECEPTOR LIGANDS AS POTENT MODULATORS OF THE REWARDING AND CRAVING EFFECTS OF ETHANOL (131)

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Nociceptin, an endogenous ligand of the opioid receptor-like 1 receptor, has been shown to inhibit hypothalamic ß-endorphinergic neurons, which and are known to mediate the rewarding properties of ethanol. It has been reported that nociceptine can modify ethanol intake in rats and prevent the acquisition of ethanol-induced conditioned place preference in rats. Our experiments also have shown, that i.c.v. administration of nociceptine (5 nM) prevented the acquisition of ethanol-induced place preference in NMRI mice. Moreover, nociceptine was also found to block the expression of the conditioned place preference and the ethanol-induced reinstatement of the conditioned place preference after prolonged extinction. In experiments with nociceptin-peptide knockout mice (development of ethanol-induced conditioned place preference) we failed to observe robust differences incomparison with wild type mice, although a tendency for increased sensitivity to ethanol rewarding effects was found in KO mice. It is concluded, that stimulation of nociceptine receptors can inhibit the rewarding, motivational and craving effects of ethanol. It is, therefore, proposed that synthetic analogues of nociceptine (eg. Ro 64-619) might have high therapeutic potential for the treatment of alcohol-seeking behavior and relapse.

EXTRACELLULAR SIGNAL-REGULATED PROTEIN KINASE (ERK) ACTIVATION IN RAT VENTRAL TEGMENTAL AREA BY THE μ-OPIOID AGONIST FENTANYL: AN *EX VIVO* STUDY (109)

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Although the primary sites and mechanisms of action for different drugs of abuse are diverse, the mesolimbic dopamine system appears to mediate reinforcing effects of psychoactive drugs. Endogenous opioid systems are also thought to play a significant role in drug reinforcement. For example, opioid antagonists have been shown to modulate initiation of cocaine self-administration (SA), i.e. acute naltrexone (NTX) inhibited acquisition of cocaine SA, primarily through the VTA. Chronic NTX caused a shift in the cocaine SA dose-response curve, suggesting enhanced reinforcing effects of cocaine. Taken together, μ-opioid receptors in the VTA may mediate individual differences in sensitivity to drugs of abuse. We aim to use signal transduction proteins, which are activated by the μ-opioid agonist fentanyl, to monitor μ-opioid receptor activation in rat VTA with respect to addiction proneness. VTA slices were *ex vivo* treated with fentanyl and stained with phospho-specific antibodies for different signal transduction proteins. A dose-dependent and naloxone reversible activation of ERK was observed. Currently the possibility to use ERK as a tool to monitor sensitivity of the VTA μ-opioid receptor system is explored.

EFFECTS OF EBP50/NHERF ON REGULATION AND SIGNAL TRANSDUCTION OF THE HUMAN KAPPA OPIOID RECEPTOR (81)

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EBP50/NHERF is a PDZ-domain-containing phosphoprotein. We have investigated whether EBP50/NHERF is associated with the human kappa opioid receptor (HKOR) and whether it plays a role in regulation and signaling of HKOR. When expressed in CHO cells stably transfected with HKOR, EBP50/NHERF co-immunoprecipitated with HKOR. Expression of EBP50/NHERF, but not a truncated form which lacks the ERM-binding domain, abolished U50,488H-induced down-regulation of HKOR. However, EBP50/NHERF did not affect U50,488H-stimulated [^{35}S]GTPgS binding and p42/p44 MAP kinase activation, nor did it affect U50,488H-induced desensitization and internalization of HKOR. To determine the motif of HKOR involved in EBP50/NHERF binding, we generated two HKOR mutants, HKOR-A and HKOR-EE, in which one Ala or two Glu residues were added to the C-terminus, respectively. Neither HKOR-A nor HKOR-EE co-immunoprecipitated with EBP50/NHERF. In addition, U50,488H-induced down-regulation of HKOR-A and HKOR-EE were not significantly blocked by EBP50/NHERF. Thus, EBP50/NHERF binds to the C-terminus of HKOR and plays an important role in the trafficking of HKOR. (Supported by NIH grants DA04745 and DA11263).

IDENTIFICATION OF NOVEL PROTEINS INTERACTING WITH MU–OPIOID RECEPTOR (37)

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Our earlier experiments have demonstrated that the carboxyl terminus of μ-opioid receptor play an important role in the agonist-induced receptor internalization, desensitization and membrane retargeting. In the present study we used the yeast two-hybrid system to search for proteins that act directly with the rat μ-opioid receptor. We used full length of μ-opioid receptor, c-terminus of μ-opioid receptor, and the c-terminus plus the third intracellular loop of μ-opioid receptor as m-opioid receptor and three proteins modulating the opioid receptor activity, as 23PEBP, beta-arrestin1 and beta-arrestin2.

MOLECULAR BASIS OF DIFFERENCES IN U50,488H-INDUCED PHOSPHORYLATION AND DESENSITIZATION BETWEEN HUMAN AND RAT KAPPA OPIOID RECEPTORS (82)

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The human kappa opioid receptor (hkor) and Flag-tagged hkor (Flag-hkor), but not the rat kappa opioid receptor (rkor) and Flag-tagged rkor (Flag-rkor), stably expressed in CHO cells, were desensitized by U50,488H pretreatment as determined by [^{35}S]GTPγS binding. In addition, Flag-hkor, but not Flag-rkor, was phosphorylated by U50,488H stimulation. Over-expression of GRK2 plus arrestin2 or GRK3 plus arrestin3 did not result in desensitization or phosphorylation of the rkor by U50,488H. We constructed two chimeric receptors, Flag-h/rkor and Flag-r/hkor, in which the C-terminal domains of Flag-hkor and Flag-rkor were swapped. Flag-r/hkor, but not Flag-h/rkor, was desensitized and phosphorylated by U50,488H, indicating that the C-terminal domain plays a critical role in the difference. To further characterize, we generated two mutants, Flag-hkorS358N and Flag-rkorN358S. Flag-rkorN358S, but not Flag-hkorS358N, underwent U50,488H-induced desensitization. However, neither Flag-rkorN358S nor Flag-hkorS358N was phosphorylated by U50,488H. These results indicate that the C-terminal domain, particularly the 358 locus of kor, plays a crucial role in the species difference in U50,488H-induced desensitization and phosphorylation. Our findings suggest that one should be cautious in extrapolating studies on kor regulation from rats to humans. (Supported by NIH grants DA04745 and DA11263)

U50,488H-INDUCED INTERNALIZATION AND DOWN-REGULATION OF KAPPA OPIOID RECEPTORS: MOLECULAR BASIS OF DIFFERENCES BETWEEN RAT AND HUMAN RECEPTORS (96)

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We showed previously that prolonged activation by U50,488H led to internalization and down-regulation of the human kappa opioid receptor (hkor), but not the rat kappa receptor (rkor). Here we investigated molecular basis underlying these differences using receptor constructs epitope-tagged with Flag and stably expressed in CHO cells. Flag-hkor, but not Flag-rkor underwent internalization and down-regulation after exposure to U50,488H. Two chimeric receptors, Flag-h/rkor and Flag-r/hkor, were generated, in which the C-terminal domains of hkor and rkor were swapped. Flag-r/hkor displayed significant U50,488H-induced internalization and down-regulation, whereas Flag-h/rkor did not, indicating that the C-terminal domain of the hkor is essential for the processes. To further characterize, we generated two mutants, Flag-hkorS358N and Flag-rkorN358S. Flag-hkorS358N displayed greatly reduced U50,488H-induced internalization compared with Flag-hkor, but was not down-regulated by U50,488H. However, Flag-rkorN358S was internalized, but not down-regulated. Thus, Ser358 in the hkor is critical for U50,488H-induced internalization and down-regulation and the trafficking of Flag-rkorN358S appears to be more complex than the wildtypes. (Supported by DA04745 and DA11263)

L-NAME PREVENTS EEG AND BEHAVIORAL ALTERATIONS INDUCED BY DELTORPHIN II IN THE RABBIT (113)

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Central administration of deltorphin II (selective d opioid receptor agonist) produces EEG seizure activity in rats and in rabbits, associated with wet-dog shakes, myoclonic twitches and convulsive activity. These deltorphin II-induced effects were antagonized in rats by pretreatment with naltrexone. Opioid peptide naloxone-reversible EEG seizures are reported after i.c.v. peptide administration in rats and rabbits. In rabbits, EEG seizures induced by opioid peptide were unaccompained by convulsive motor activity and wet-dog shakes were also present. Recently, we have demonstrated that L-N^G-nitro arginine methyl ester (L-NAME) was able to reduce EEG and behavioural alterations induced by morphine (less selective opioid receptor agonist) in the rabbit indicating an important interaction between nitric oxide (NO) and opioid system. The present study was undertaken to verify whether L-NAME was able to influence deltorphin II-induced EEG seizure in rabbits. I.c.v. administered (20 ml/toto) did not induce EEG or behavioral alterations. Also, power spectral EEG recorded from cortex and hippocampus were similar to those observed in nontreated animals. Deltorphin II administered i.c.v. (5, 15, 50 and 100 mg/toto) induced dose-related EEG alterations. All animals treated with the highest dose (100 mg/toto) showed ictal EEG episodes limited to the hippocampus and consisting of continuous high-voltage spikes. During ictal episodes, rabbit maintained frozen posture and neither wet-dog shakes nor other behavioral alterations or clonic or tonic convulsions were observed. After these ictal episodes, hippocampal EEG voltage showed a progressive slowing in frequency and displayed typical deltorphin II EEG synchronized activity, consisting of high-voltage slow waves with superimposed spikes. This EEG pattern appears 5 to 10 min after deltorphin II and lasts from 30 min to 3hr after deltorphin II administration, depending on the dose. During these tardive EEG alterations, animals remained motionless and exhibited after respiratory depression with loss of corneal reflex and exophthalmos. L-NAME (3-300 mg/i.c.v./mouse) did not induce significant EEG or behavioral changes whereas when injected 15 min before i.c.v. deltorphin II (100 mg/toto) dose dependently prevented the EEG ictal episodes, the spiking activity and the synchronized EEG pattern induced by deltorphin II. Behavioral alterations as well as the frozen posture, the appearance of exophthalmos, loss of the corneal reflex and the respiratory depression induced by deltorphin II were also prevented. The inhibitory effect of L-NAME on deltorphin II seizures was dose-dependently reversed by L-arginine (3-300 mg/i.c.v./mouse) but not by D-arginine. Finally, glyceryl trinitrate on its own (3-300 mg/i.c.v./mouse) significantly increased deltorphin II seizures in the rabbit and it was also able to reverse the inhibition on deltorphin II seizures operated by L-NAME. The results of the present study provide a strong evidence that NO may be involved in the control of brain excitability induced by opioids: further studies are in progress in order to better understand the above involvement.

MOTOR STIMULATION AFTER SINGLE AND REPEATED COCAINE AMINISTRATION IN ORL-1 KNOCKOUT MICE (120)

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We have shown that orphanin FQ/nociceptin blocks cocaine-induced behavioral sensitization or induces a sensitized response to cocaine, depending on the dosing regimen. The present study was designed to evaluate if cocaine-induced motor stimulation and behavioral sensitization are affected in opioid receptor-like (ORL-1) receptor knockout (KO) mice. On day 1, mice were injected with cocaine (15 mg/kg) and total distance traveled was recorded for 1 hr. The same treatment was given for 4 more days. Following a period of withdrawal, mice were tested after a challenge dose of cocaine (7.5 mg/kg) on day 11. The motor stimulatory action of cocaine was significantly attenuated in the ORL-1 KO mice on day 1. However, the ORL-1 KO mice sensitized to cocaine to a greater degree such that there was no significant difference in locomotor response to cocaine challenge on day 11. (Supported by DA 05010; KL was supported by DA00411 from NIDA and a NARSAD Young Investigator Award)

ORPHANIN FQ/NOCICEPIN BLOCKS DEVELOPMENT OF COCAINE-INDUCED BEHVIORAL SENSITIZATION IN RATS (121)

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Previous data from our laboratory indicated that intra-VTA OFQ administration failed to block the development of cocaine sensitization. Given previous reports of rapid tolerance to OFQ, we sought to investigate the effect of escalating doses of OFQ on cocaine-induced behavioral sensitization. Rats were injected either i.c.v. or directly into the VTA with aCSF or OFQ. Rats were then immediately injected with saline or cocaine (20 mg/kg) and total distance traveled was recorded for 1 hr. Similar treatment was given for the next 2 days except the dose of OFQ was doubled each day. Rats were then left untreated for 4 days. On day 8, rats were challenged with cocaine (7.5 mg/kg). The sensitized response, observed in the control group, was completely blocked in rats pretreated with OFQ. The results demonstrate that OFQ is capable of blocking cocaine-induced behavioral sensitization, an effect mediated, at least in part, in the VTA. (Supported by a KO1 award DA00411 from NIDA and a NARSAD Young Investigator Award to KL)

A COMPARATIVE STUDY OF N-METHYL-D-ASPARTATE AND DOPAMINE D1 RECEPTOR LEVELS IN LEWIS AND FISCHER 344 RATS (122)

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Lewis (LEW) and Fischer 344 (F344) rats differ in there behavioral response to the novelty, acute amphetamine and cocaine challenge and susceptibility to drug addiction. To investigate if differences in N-methyl-D-aspartate (NMDA) and dopamine (DA) D1 receptors could be correlated with behavioral differences between these strains, a comparative autoradiographic study of NMDA- and DA D1- receptors within distinct brain regions was undertaken. We found that LEW rats have a significantly greater density of NMDA- receptors in frontal and cingulate cortex, caudate putamen, central amygdaloid nuclei and deep layers of superior colliculus compared to F344 rats. DA D1 densities in some layers of CA1, CA2 and CA3 areas of hippocampus, dentate gyrus, posterolateral thalamic nuclei and substantia nigra reticulata of LEW rats were significantly higher than the levels found in the F344 rats. DA D1 density in cingulate cortex of LEW rats was significantly lower than in F344 rats. These data suggest that differences in NMDA and DA D1 receptors may contribute to diffrences in NMDA and DA related behavior seen between these rat strains.

DESENSITIZATION OF HUMAN DELTA OPIOID RECEPTOR (HDOR) BY ALKALOID (ETORPHINE) AND PEPTIDE (DPDPE AND DELTORPHINE I) AGONISTS : IMPLICATION OF DIFFERENT KINASES (33)

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In the human neuroblastoma SK-N-BE cells, we previously demonstrated that etorphine induced hDOR desensitization, observed on adenylate cyclase (AC). Moreover, this desensitization is closely correlated to receptor phosphorylation (Hasbi et al., 1998) and involves probably the GRK2, the only member of GRK (G-protein coupled receptor kinase) family expressed in the cell line. In order to demonstrate the role of this kinase in hDOR desensitization, we transfected the cDNA of bovine GRK2 or itf the peptides-induced hDOR desensitization. In conclusion, hDOR desensitization is achieved by different kinases dependending on the chemical nature of the agonists. Hasbi et al. (1998) J. Neurochem., 70, 2129-2138.

DIFFERENTIAL ORL1 AND MU OPIOID RECEPTOR MEDIATED CROSS-TOLERANCE IN HUMAN NEURONAL CELLS (83)

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OFQ/N appears to contribute to the development of morphine tolerance as morphine tolerance is greatly reduced following blockade of the ORL1/OFQ/N system. We recently reported that ORL1 and mu opioid receptor (OR) activation produced cross-tolerance in BE(2)-C cells (*Mandyam et al., 2000*). However, in SH-SY5Y cells only OFQ/N pretreatment produced mu-OR cross-tolerance. The mechanisms regulating the cross-tolerance in both cell lines was examined using PKA (H-89) and PKC (Chelerythrine chloride) inhibitors. PKC inhibition blocked OFQ/N mediated desensitization of mu-ORs in BE(2)-C, but not SH-SY5Y cells. DAMGO induced desensitization of ORL1-OR in BE(2)-C cells was not blocked by either inhibitors. When ORL1 receptor cDNA from SH-SY5Y cells was stably transfected in BE(2)-C cells, the receptor was desensitized by DAMGO pretreatment indicating that the differential cross-tolerance was a cell-specific effect. Thus BE(2)-C cells represent an important model system for understanding ORL1 and mu-OR interactions resulting in acute opioid tolerance, and allow us to investigate how this tolerance may be prevented. These studies were supported by funds from NIDA to KMS and CDM.

DOES THE HUMAN MU OPIOID RECEPTOR COUPLE DIFFERENTLY TO Gi1a OR Gi2a ? (22)

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Preferential coupling of mu receptor to various Galpha subunits is still under debate. Here we investigated mu opioid receptor selectivity towards Gi1 and Gi2 subunits using a fusion strategy. Fusions between the human mu opioid receptor and a pertussis-insensitive Gi1ile or Gi2ile subunit were stably expressed in HEK293 cells. Cells were treated with pertusssis toxin and the affinities of the 2 fusions for different agonists were determined and compared to the values obtained with the wild type receptor. [^{35}S]GTPγS binding was used to monitor the coupling efficiency of the receptor to Gi1ile or Gi2ile and to detect a possible modulation by the agonist of this first step of the signalling cascade. The influence of the ligand or the Gialpha subunit on the signal transduction mediated by βγ subunits was also examined using MAP kinase phosphorylation as a test.

IMMUNOMODULATORY PROPERTIES OF BETA-ENDORPHIN IN MICE WITH DIFFERENT IMMUNOREACTIVITY (139)

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In the last few years, the data on the participation of beta-endorphin in the regulation of the immune system functions has been obtained, however, its role has been little studied. The present paper is aimed to determine the beta-endorphin (BER) effect on the humoral immune response in mice with different immunoreactivity and to elucidate the mechanisms of this effect. BER (100 mg/kg, s.c.) enhanced synthesis of hemolysins regardless of the administration time (24 h before, simultaneously or 72 h after mice immunization). Macrophage phagocytic activity and glucose level were increased when the peptide was administered during the productive phase of the immune response. No modulatory effect of the BER on synthesis of corticosteroid hormones was revealed. In stressed mice (6-h immobilization 24 h before immunization) administration of BER in combination with immunizationThus, the beta-endorphin enhanced a specific immune response in intact animals and decreased it in stressed animals. The inhibitory effect of peptide is likely to be mediated through its effect on the adrenal cortex function and/or the hormonal system of the carbohydrate homeostasis regulation.

ATYPICAL PROCESSING OF HUMAN DELTA OPIOID RECEPTOR mRNA IN MALIGNANT CELLS (85)

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A truncated variant of the human delta opioid receptor mRNA was identified in melanoma and neuroblastoma cells. The corresponding receptor protein is predicted to lack 48 amino acids which represent the third cytoplasmic loop. Expression studies in NIH-3T3 fibroblasts and Xenopus oocytes revealed that this receptor variant bound delta ligands (DPDPE and naltrindole) but did not couple to adenylate cyclase nor to inwardly rectifying potassium channels. The mechanism by which 144 nucleotides are removed from the delta receptor mRNA is probably different from alternative splicing since consensus splice recognition sites were absent. A transposon-like process seemed more likely because of a repeated 13 nucleotide motif flanking the excised fragment. Not only human tumor cells expressing the delta receptor naturally did truncate it. Truncation was also observed in NIH3T3 permanent mouse fibroblasts and NG 108-15 rat x mouse neuroblastoma x glioma cells upon transfection. Notably, the rat delta opioid receptor sequence was not truncated in spite of >90% sequence homology in the region under question. This argues for a highly specific process underlying the atypical processing of the human delta receptor mRNA which was performed by all (semi-)malignant cells tested. Further studies may reveal whether this transposon-like processing is involved in tumor genesis or in the generation of novel receptor isoforms.

CONTENTS OF SUBSTANCE P IN BRAIN OF INBRED RATS WITH DIFFERENT LEVEL OF SELF-ADMINISTRATION OF MORPHINE (128)

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The aim of this study was evaluation of contents of Substance P (SP) in hypothalamus, hippocampus and midbrain of inbred WAG/G and Fischer-344 (F-344) rats during development of craving to morphine, using experimental model of intravenous self-administration. Results was shown, that first trying of morphine was more intensive in F-344, then in WAG/G rats. During long-lasting morphine intake F-344 rats decrease their sensitivity to positive reinforcing property of morphine, and intravenous sso. The results of our experiments and data of other investigators led us to decision that any kind of acute or chronic morphine administration induce level of SP in hypothalamus and, possible, in other brain areas. But, only satisfaction of pathological craving to morphine by predisposed animals, decrease level of SP in midbrain.

INTRATHECALLY ADMINISTERED SUBSTANCE P ENHANCES MORPHINE-INDUCED ANTINOCICEPTION THROUGH THE PRODUCTION OF SUBSTANCE P *N*-TERMINUS (2)

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The present study was designed to determine whether the SP *N*-terminal fragment SP(1-7) produced by peptidases in the spinal cord is involved enhancement of morphine-induced antinociception. Morphine(93.8-1000pmol), injected i.t., produced antinociception in the mouse assessed by the capsaicin-induced paw-licking model. The co-administration of SP (50pmol) produced markedly an enhanced antinociceptive effect of morphine (62.5 and 93.8pmol) at sub-threshold doses. This pharmacological effect was reduced significantly by simultaneous injection of phosphoramidon, an inhibitor of endpeptidase-24.11. Pretreatment with [D-Pro^2, D-Phe^7]SP(1-7), a SP(1-7) antagonist, inhibited the enhanced effect of morphine in combination with SP was also inhibited by pretreatment with antisera against SP(1-7). Similarly, antinociception induced by i.t. morphine was enhanced by co-administration of SP(1-7), which was inhibited by [D-Pro^2, D-Phe^7]SP(1-7) and SP(1-7) antisera. SP(5-11), a SP *C*-terminal fragment, was without effect on morphine-induced antinociception. These results suggest that morphine-induced antinociception may be enhanced through SP(1-7) produced by the degradation of i.t. injected SP.

INVOLVEMENT OF GLIAL GLUTAMATE TRANSPORTERS IN MORPHINE DEPENDENCE AND NALOXONE-PRECIPITATED WITHDRAWAL (123)

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We examined the involvement of glial glutamate transporters, GLT-1 and GLAST, in morphine dependence and naloxone-precipitated withdrawal. Rats were rendered morphine-dependent by s.c. implantation of two 75 mg morphine pellets for 5 days. I.c.v. administration of TBOA, a glutamate transporter inhibitor significantly facilitated various naloxone-precipitated withdrawal signs. By northern blot analysis, the expression of GLT-1 mRNA was significantly decreased in the striatum and thalamus of morphine-dependent rats, and significantly increased in the striatum 2 hr after the naloxone-precipitated withdrawal. On the other hand, there were no significant changes in GLAST mRNA level in any brain regions. In vivo microdialysis experiments revealed that extracellular glutamate level was elevated in the striatum and nucleus accumbens, in which the changes of GLT-1 mRNA level were observed, during naloxone-precipitated morphine withdrawal. These results suggest that the changes of GLT-1 expression, which alter the glutamate uptake and affect the glutamatergic transmission efficiency, play a role in the development of morphine dependence and the expression of morphine withdrawal.

[^{3}H]SUPER DALDA BINDING TO BRAIN MEMBRANES (40)

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The dermorphin-derived peptide [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2), also known as Super DALDA, labels μ-opioid receptors with high affinity and selectivity. The receptor mechanism of action of [Dmt^1]DALDA appears distinct from that of morphine, as evidenced by antisense mapping studies where [Dmt^1]DALDA is insensitive to MOR1 exon probes that reduce morphine analgesia. In addition [Dmt^1]DALDA displays a lack of cross-tolerance to morphine. In an attempt to further characterize and elucidate the mechanism of action of [Dmt^1]DALDA, radiolabeled [Dmt^1]DALDA has been synthesized. Binding studies using calf brain membranes reveal highest levels of [^{3}H][Dmt^1]DALDA binding in the striatum. [^{3}H][Dmt^1]DALDA labels μ-receptors with high affinity (K_D = 50pM). K_i values for unlabeled [Dmt^1]DALDA, DAMGO and morphine obtained from competition binding studies were 0.1, 1.8 and 4.6 nM respectively. [^{3}H][Dmt^1]DALDA will clearly prove to be a useful tool with which to study the pharmacology of this unusual peptide and may provide some important answers regarding the mode of action of μ-ligands.
Supported in part by grants DA07242, DA00220, and CA08748.

THE EFFECT OF ADRENALECTOMY AND CORTICOSTERONE REPLACEMENT ON ORPHANIN FQ - INDUCED FOOD INTAKE IN RATS (132)

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Acute central administration of Orphanin FQ (OFQ) has been shown to dose-dependently stimulate food intake and corticosterone (B) release in rats. The aim of this study was to determine whether a relationship exists between OFQ–induced B release and OFQ-induced feeding. In adrenalectomised male rats, the stimulatory effect of OFQ (5nmoles) on food intake was abolished. With a low level of B replacement, (1.975±0.302μg/dl, n=15) OFQ's stimulatory effect on food intake was still negated. However, with higher B replacement (14.10±1.42μg/dl, n=15) the stimulatory effect of OFQ on food intake was fully restored, indicating that the stimulatory effect of OFQ on food intake is dependent on relatively high levels of circulating B. We furthered this study by co-administering the glucocorticoid receptor antagonist, RU486, with OFQ and found that this agent (80μg/2μl) blocked OFQ-induced food intake. Overall, these data demonstrate that B has a permissive effect on OFQ-induced food intake which may be mediated through central GR receptors.

COMPARISION OF THE INHIBITORY EFFECTS OF MU-OPIOID RECEPTOR AGONISTS ON MOUSE GASTROINTESTINAL TRANSIT (10)

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The inhibitory effect of intracerebroventricularly (i.c.v.) administered H-Tyr-D-Arg-Phe-beta-Ala-OH (TAPA), a dermorphin tetrapeptide analogue and highly selective mu1-opioid receptor agonist, on mouse gastrointestinal transit was compared with that of morphine and DAMGO. When administered i.c.v. 5 min before the oral injection of charcoal meal, TAPA (10-100 pmol), morphine (0.25-4 nmol) and DAMGO (20-80 pmol) dose-dependently inhibited gastrointestinal transit of charcoal. The inhibitory effect of each mu-opioid receptor agonist was completely antagonized by naloxone. The inhibitory effect of morphine and DAMGO was significantly antagonized by both beta-funaltrexamine (beta-FNA), and naloxonazine. On the other hand, the inhibitory effect of TAPA was not affected at all by beta-FNA, naloxonazine, nor-binaltorphimine and naltrindole. These results suggest that the inhibitory effect of TAPA on gastrointestinal transit may be mediated through the different opioid receptor mechanism from morphine and DAMGO.

MORPHINE EXPOSURE ALTERS ORL1 RECEPTOR mRNA EXPRESSION AND OFQ-STIMULATED [35S]GTPγS LEVELS (105)

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The orphanin system may interfere with the behavioral and neurochemical effects of morphine. To further address this issue we asked if acute or chronic morphine administration alters expression of orphanin FQ (OFQ) and OFQ receptor (ORL1) mRNA with *in situ* hybridization. Additionally, ORL1 receptor function was examined using OFQ-stimulated [^{35}S]guanylyl-5'-O-(γ-thio)-triphosphate ([^{35}S]GTPγS) autoradiography. In the chronic treatment paradigm, rats were given morphine (10mg/kg, i.p.) or saline for 14 days followed by two weeks of no drug. On day 29, rats received a single injection and were decapitated after 60 min. The acute group received a single injection of morphine or saline on day 29. Chronic morphine increased ORL1, but not OFQ, mRNA levels in the ventral tegmental area (VTA) and substantia nigra compacta (SNc). No effects were observed in these brain regions following acute morphine. At the level of the receptor, OFQ-stimulated [^{35}S]GTPγS levels increased in the VTA and SNc following chronic but not acute morphine. In contrast, levels decreased in the prefrontal cortex following acute but not chronic morphine. Taken together, these studies suggest that morphine influences the expression and function of the ORL1 receptor in a tissue- and time-specific manner.

A TETRAPEPTIDE OF DERMORPHIN ANALOGUE PRODUCES AN EXTREMELY POTENT ANTINOCICEPTIVE EFFECT IN MICE (41)

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FYK-1258 is a newly synthetized analogue of dermorphin *N*-terminal tetrapeptide. We investigated the antinociceptive effect of FYK-1258 in the paw-withdrawal test in mice. Peripheral administration (s.c. or p.o.) of FYK-1258 produced potent antinociception with extraordinary durability. Antinociception induced by intracerebroventricularly (i.c.v.) administered FYK-1258 was about 1000-fold more potent than that of morphine. The antinociceptive effect of i.c.v. FYK-1258 was blocked by pretreatment with b-funaltrexamine (40mg/kg,s.c.) or naloxonazine (35mg/kg,s.c.). Pretreatment with nor-binaltorphimine (4nmol,i.c.v.) inhibited FYK-1258-induced antinociception, whereas morphine-induced antinociception was unaffected by the k-opioid receptor antagonist. In addition, pretreatment with antisera against a-neoendorphin markedly attenuated FYK-1258-induced antinociception. These results suggest that FYK-1258 may stimulate the distinct subtypes of m-opioid receptors through the release of dynorphins. Especially, a-neoendorphin may be involved in the antinociceptive mechanism of FYK-1258.

ANTINOCICEPTIVE AND ANTI-OPIOID EFFECTS OF SPINALLY ADMINISTERED NOCICEPTIN RELATED PEPTIDES IN THE CAPSAICIN TEST (42)

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The present study was designed to compare the effects of intrathecally (i.t.) administered nociceptin (NC), NC(1-13) and NC(1-13)NH_2 on capsaicin-induced nociceptive behavioural response consisting of paw-licking or biting in mice. The i.t. administration of NC produced a dose-dependent antinociceptive effect with an ED_{50} of 830 pmol. The antinociceptive effect of NC(1-13) was weaker than that of NC, whereas NC(1-13)NH_2 was approximately 2.0-fold more potent than NC. NC(1-13)NH_2 exhibited a long-lasting antinociceptive effect as compared with NC or NC(1-13). Antinociception induced by these three peptides were not antagonized by pretreatment with naloxone (1.0 mg/kg, i.p.). When morphine was co-administered i.t. with NC or NC(1-13), the combination potentiated the antinociceptive effect of morphine. On the contrary, NC(1-13)NH_2 co-administered i.t. with morphine, resulted in a significant antagonistic effect on morphine-induced antinociception. These results indicate that i.t. NC(1-13)NH_2 produces an opioid-independent antinociception with opioid antagonistic activity, which is distinctly different from spinal action NC(1-13).

EFFECTS OF ACUTE AND CHRONIC CLONIDINE AND PRAZOSIN ON MORPHINE TOLERANCE AND WITHDRAWAL IN MICE (31)

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Morphine tolerance was induced using a 3-day graded b.i.d.dosage regimen with s.c. doses up to 120 mg/kg. Tolerance was assessed on day 4 as loss of the antinociceptive effect of a test dose of morphine (5 mg/kg), using the tail flick test. Ten h later, withdrawal was induced with naloxone 1 mg/kg. Single doses of clonidine (0.5, 1, 2 and 5 mg/kg) and prazosin (0.5 mg/kg) increased the analgesic potency of the morphine test dose (to 25, 40, 93 and 95%, and 71% of MPE, respectively) (controls receiving only morphine, 14% of MPE). Naloxone-induced vertical jumping and weight loss were reduced by high-dose clonidine (38/15 min and 0.8%/3 h) and by prazosin (26 and 2.1%) (controls, 63 and 6.9%). Repeated administration was less efficacious than acute dosage. Clonidine given alone was analgesic and sedative but prazosin was not.

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SPLICE VARIANT, MOR-1R, OF THE HUMAN MU OPIOID RECEPTOR GENE(OPRM) (7)

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Alternative splicing is a common mechanism used in eukaryotic gene regulation. Recently, we showed that the mouse mu opioid receptor gene undergoes extensive splicing. Fifteen MOR-1 variants were generated through differential splicing among the fourteen exons of the MOR-1 gene which span over 250kb. Here we report the identification of a novel splice variant, MOR-1R, of the mu opioid receptor gene from human brain. Human MOR-1R was isolated by using a 3'RACE and RT-PCR strategy. Sequece sequences. Expression of the MOR-1R mRNA was examined by Northern blot and RT-PCR analysis. Binding studies in CHO cells stably transfected with the MOR-1R/pcDNA3 construct indicated that MOR-1R encodes a mu opioid receptor. Supported by: NIDA grants (DA00296 to Y.X.P. and (DA07242,DA02615 and DA00220) to G.W.P

MU AND DELTA OPIOID RECEPTORS FORM COMPLEXES IN SH-SY5Y CELLS (84)

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Recent evidence demonstrates that m and d opioid receptors form hetero-oligomers and –dimers in heterologous cells co-transfected with both receptors. In addition, low concentrations of a ligand selective for one receptor modulates the binding of a ligand selective for the other receptor. This occurs both in transfected cells and in SK-N-SH neuroblastoma cells. SH-SY5Y cells, a subclone of SK-N-SH cells, express m and d receptors at a respective ratio of 2:1. The aim of this study was to identify m-d heterodimers and –oligomers in these cells using immunoprecipitation and to characterize the ligand binding profile of the hetero-dimer and –oligomer. Retinoic acid-differentiated cells were solubilized and immunoprecipitated with anti-m opioid receptor antibody conjugated to Protein-A Sepharose beads. After electrophoresis and transfer, the resulting blots were probed with antibodies against m and d receptors. The results demonstrated that in SH-SY5Y cells, m and d receptors exist in hetero-oligomeric and –dimeric forms as well as in monomers. Saturation binding experiments showed that 10 nM naltrindole increased the K_d value of [^{3}H]DAMGO binding 3-fold without changing the B_{max} value. These findings provide a possible explanation for the m-d interactions that are observed *in vivo*. (Supported by grants K05-DA00360, DA03742 and DA07232).

SPINAL ANALGESIC SYNERGY BETWEEN THE ENDOMORPHINS AND CLONIDINE, BUT NOT THE ENDOMORPHINS AND 2-METHYL-5-HT (23)

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Endomorphin-1 (Endo-1) and Endomorphin-2 (Endo-2) are endogenous ligands for mu opioid receptors. Spinal mu opioid analgesia synergizes with alpha2 adrenoceptor agonist analgesia and is blocked by alpha2 antagonists. However, spinal mu opioid analgesia is only additive or less-than-additive with 5-HT agonist analgesia. Accordingly, we used isobolographic analysis to study the interaction of the endomorphins with the alpha2 agonist, clonidine (Clon), and the 5-HT3 agonist, 2-methyl-5-H Thus, the interaction of exogenously administered endomorphins with monoamines in the spinal cord is similar to other mu agonists. Supported by LA BoG Grant HEF-01-22 to DP and a VA Merit Review Grant to JEZ.

EFFECTS OF PROLIFERATION AND DIFFERENTIATION ON ADULT HIPPOCAMPAL PROGENITORS IN RAT (52-1)

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Opioid agonists and antagonists have been shown to have profound effects on brain development. Few reports have investigated the role of G-protein coupled receptors on progenitors/stem cells. This work focus on opioids effects *in vitro* on adult hippocampal progenitors from rat. We first identified opioid receptors on adult hippocampal progenitors and thereafter studied the expression of different cell type-specific markers after 10 days of exposure to different agonists and antagonists against the opioid receptors. No effect of any substances used affected the number of cells entering apoptosis. Opioids against mu- and delta-opioid receptors were able to increase $[Ca^{2+}]_i$. The effects on proliferation and activation through Ras-Raf-MAPK-signaling pathway were studied. Our results show a complex interaction of opioid receptors in these cultures which suggest that these cells might specifically be activated by different combinations of opioid agonists and antagonists.

SHARED PROCESSING IN THE ROSTRAL ACC DURING OPIOID AND PLACEBO ANALGESIA (17)

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Placebo analgesia may be attenuated by the opioid antagonist naloxone which indicates involvement of the endogenous opioid systems. In order to investigate whether similar neural systems are involved in opioid treatment and placebo analgesia, we compared a placebo with an rapidly acting opioid agonist (remifentanil) during a standard pain stimulus, i.e. tonic heat stimulation of 48° C for 70 seconds in a PET study while the stimulation temperature was 38° C in the control conditions. The following conditions were included in the study: 1) painful heat and opioid (POP), 2) painful heat and placebo (PPL), 3) painful heat only (P), 4) non-painful heat and opioid (WOP), 5) non-painful heat and placebo (WPL) and 6) non-painful heat only (W). The subject responses were studied both behaviourally and in terms of differential rCBF responses using PET. Behaviourally, analgesia was induced by both the opioid and placebo treatment. Both the general opioid effect [(POP+WOP)-(P+W)] and the effect of placebo analgesia (PPL-P) induced similar increases of activity in the rostral ACC [x y z; 8 44 12]; Z = 6.8 for the opioid effect and [18 32 14]; Z= 3.3 for the placebo analgesia). The rCBF activity of the rostral ACC correlated with similar regions in the brainstem during placebo treated pain (PPL) and opioid treated pain (POP), but not during untreated pain (P). This study indicates that similar regions are involved in both the general opioid effect and placebo analgesia and support previous hypotheses based on behavioural observations that opioid systems are involved in placebo analgesia.

FORMATION OF HETERODIMERS BETWEEN THE MU-OPIOID RECEPTOR AND THE sst_{2A} SOMATOSTATIN RECEPTOR PROMOTES HOMOLOGOUS CROSS-DESENSITIZATION (86)

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It has been shown that G protein-coupled receptors can interact functionally with each other to form homo- and heterodimers. In the present study, we have examined dimerization of the rat µ-opioid receptor (MOR1) and the sst_{2A} somatostatin receptor in stably transfected HEK 293 cells. We show that MOR1 and sst_{2A} exist as constitutive homodimers at the plasma membrane. Moreover, in co-immunoprecipitation studies using differentially epitope-tagged receptors we provide direct evidence for heterodimerization of MOR1 and sst_{2A}. The heterodimers were stable under reducing conditions and the degree of dimerization was agonist independent. The MOR1-sst_{2A} heterodimer exhibited high affinity binding to both the µ-selective ligand DAMGO and the sst_{2A}-selective ligand L-779,976. Interestingly, exposure of the MOR1-sst_{2A} heterodimer to DAMGO induced desensitization of MOR1 and sst_{2A}. Conversely, exposure of the MOR1-sst_{2A} heterodimer to L-779,976 induced desensitization of sst_{2A} and MOR1.

VENLAFAXINE AND MIRTAZAPINE: COMMON OPIOID-MEDIATED ANTINOCICEPTIVE EFFECTS (24)

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Venlafaxine and mirtazapine are two antidepressant drugs of the new generation. Venlafaxine is a presynaptic drug which blocks the synaptosomal uptake of noradrenaline and serotonin and, to a lesser degree, of dopamine, while mitrazapine is a postsynaptic drug which enhances noradrenergic and 5-HT1A-mediated serotonergic neurotransmission via antagonism of central a2-auto- and hetero-adrenoreceptors. Though exerting their antidepressant action through totally different mechanisms of action of venlafaxine is mainly influenced by all opioid receptor subtypes combined with the a2-adrenergic receptor, while the antinociceptive effect of mirtazapine mainly involves m- and k3-opioid mechanisms. This opioid profile of the two drugs may be one of the explanations to their efficacy in severe depression, and may suggest a possible use in the treatment of chronic pain syndromes.

EFFECTS OF NEONATAL SEPARATION ON ETHANOL INTAKE AND OPIOID RECEPTOR DENSITY IN THE RAT BRAIN (133)

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Accumulating evidence indicates that an animal's response to a drug can be profoundly affected by early environmental influences. Since both ethanol and stress alter endogenous opioid peptide levels the interaction of ethanol and stress could be mediated via the endogenous opioid system. The aim of the present study was to examine if 15 min or 360 min of daily neonatal separation in male Wistar rats until weaning could affect ethanol drinking behaviour at adulthood. We were also interested in the effects of neonatal separation on opioid receptor density in the rat brain. The results from the present study show that 360 min daily neonatal separation until weaning increases voluntary ethanol intake in adult rats compared with rats exposed to 15 min of neonatal separation or control rats. The results also show that exposure to restraint stress at adulthood affects the ethanol intake behaviour. These findings suggest that early experiences, such as neonatal separation, are useful psychobiological predictors of future high ethanol consumption. The effects of neonatal separation on brain opioid receptor density using quantitative autoradiography are under investigation and will also be discussed.

CLONING AND CHARACTERIZATION OF A NEW OPIOID RECEPTOR LIKE FROM ZEBRAFISH (100)

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A new opioid receptor like (ZFOR3) has been cloned and characterizated in the teleost Zebrafish (*Danio rerio*). A complete cDNA sequence of 2.7 kb long that codifies a 377 aminoacid protein was obtained. From hydrophobicity data of the protein sequence of ZFOR3, it can be deducted that it codes seven potential transmembrane domains. Also, genomic analysis of our clone shows that it is formed of four exons distributed in approximate 10 kb. When we compared the aminoacid sequence correspondig to ZFOR3 with sequences deposited at EMBL and GenBank data bases, we find that this protein presents high identity to other opioid receptors: 70%-72% to human, rat and mouse kappa opioid receptor; 62%-64% to human, rat and mouse delta opioid receptor; and 63%-65% to human, rat and mouse mu opioid receptor. Analyzing the degree of identity among ZFOR3 and mu, delta and kappa opioid receptors, we found that the highest degree of identity appears at the transmembrane domains and at intracellular loops. Divergences in sequence are greater in the regions corresponding to extracellular loops 2 and 3. Our findind suggests that ZFOR3 may present differences in ligand selectivity as compared to other opioid receptors. Preliminary binding studies indicate that ZFOR3 might work as a delta opioid receptor.

PRONOCICEPTIN AND PRODYNORPHIN SYSTEMS IN NEUROPATHIC PAIN (21)

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We studied an antiallodynic and antinociceptive effects of nociceptin after i.th. administration as well as the level of pronociceptin and its receptor mRNAs in rats after the ligation of sciatic nerve. Also the level of prodynorphin and k-opioid receptor mRNAs was measured. Behavioural experiments show significant antiallodynic and antinociceptive effects of nociceptin(5-10 ug i.th.). Pronociceptin and nociceptin receptor mRNAs remained unchanged in laminas I-VI of the dorsal horn. However, the higher level of nociceptin receptor in the ventral horn of the spinal cord was observed. In contrast to pronociceptin, prodynorphin mRNA was increased in laminae I-VI of the lumbar spinal cord. Ligation of the sciatic nerve had no influence on the level of the k-opioid receptor mRNA. Our study shows that nerve injury differentially influence pronociceptin and prodynorphin system in the rat spinal cord. Further, the significant changes in nociceptin receptor mRNA level in the ventral horn may indicate that antiallodynic effects of nociceptin may be partly related to the changes in the postsynaptic nociceptin receptors. Supported by a grant KBN no. 4 P05A 093 15.

THE EFFECTS OF NEONATAL SEPARATION ON TISSUE LEVELS OF DYNORPHIN B AND NOCICEPTIN IN WISTAR RATS (134)

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Preweaning rearing conditions can have long-term consequences for neuroendocrine parameters and behavioural performance. The aim of this study was to investigate if neonatal separation can induce long-term neurochemical changes in brain dynorphin B (DYNB) and nociceptin/orphanin FQ (N/OFQ) immunoreactive (ir) levels in male Wistar rats. Neonatal separation for 15 min and 360 min respectively, significantly increased ir-levels of DYNB in the neurointermediate lobe of the pituitary gland as compared to control rats. In the hypothalamus 15 min of neonatal separation significantly increased ir-levels of DYNB. 15 min and 360 min of neonatal separation significantly increased N/OFQ ir-levels in the hypothalamus compared to control rats. In the medial prefrontal cortex rats separated for 15 min had significantly higher ir N/OFQ levels compared to controls. The results indicate that manipulations early in life can induce persistent neurochemical changes.

REPEATED CENTRAL INJECTIONS OF D-TYR[11]NEUROTENSIN ALTER MORPHINE-INDUCED LOCOMOTOR ACTIVITY (125)

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We have previously shown that repeated central injections of D-Tyr[11]neurotensin (D-NT), sensitize to amphetamine-induced locomotion in rodents. This experiment was aimed at determining whether repeated exposure to D-NT also sensitizes to the locomotor activating effects of morphine, and involves 5-HT3 receptors. Experiments were performed on male Long-Evans rats. During the induction phase, locomotor activity was measured for two hours, every second day for four days (Day 1,3,5 and 7) this effect was not prevented by tropisetron. These results suggest that increased central neurotensin transmission may alter behavioral responses to morphine, an effect that may not require activation of 5-HT3 receptors.

DIFFERENTIAL EFFECTS OF ENDOMORPHIN-1 AND ENDOMORPHIN-2 ON BEHAVIORAL ACTIVITY: MOR-1 ANTISENSE PROFILE (30)

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Two tetrapeptides, endomorphin-1 and endomorphin-2 were identified in 1997 and thought to be the endogenous ligands for the mu opioid receptor (Zadina et al., 1997). Several studies have confirmed the mu selectivity of these peptides through antagonist studies (Tseng et al., 2000; Goldberg et al.,1998 etc), G-protein downregulation (Sanchez-Blazquez et al., 1999), and antisense mapping studies (Rossi et al., in review). Our lab has done further pharmacological evaluation of these two peptideprofiles as shown by the tailflick test, however the activity assay provided several indices of differential responses between endomorphin-1 and endomorphin-2. Endomorphin-2(4ug) significantly blocked ambulatory activity,while endomorphin-1(10ug) increased stereotypy.These results and others further indicate that endomorphin-1 and endomorphin-2 may have different roles in the modulation of pain.

INVOLVEMENT OF SPINAL k-OPIOID RECEPTORS IN TYR-D-ARG-PHE-SARCOSINE(TAPS)-INDUCED ANTINOCICEPTION (43)

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Tyr-D-Arg-Phe-sarcosine (TAPS), an N-terminal tetrapeptide analogue of dermorphin, has been found to possess potent antinociceptive effects. The present study was conducted to characterize the antinociceptive effect of TAPS after intrathecal (i.t.) injection. Antinociceptive activity was measured by the tail flick test in mice. The i.t. injection of TAPS dose-dependently suppressed the tail-flick response with an ED50 value of 0.94 pmol. The antinociceptive effect of TAPS was significantly attenuated by pretreatment with b-funaltrexamine, naloxonazine (NLZ), nor-binartorphimine or antisera against dynorphin B(1-13), but not antisera against dynorphin A(1-17). Especially, antinociception induced by TAPS was more sensitive to NLZ than that induced by Tyr-D-Arg-Phe-b-Ala (TAPA) or DAMGO. ID50 values for s.c. NLZ on tail-flick antinociception produced by i.t. TAPS and TAPA were 2.9 and 12.0 mg/kg, respectively, whereas DAMGO-induced antinociception was insenstive to NLZ. The results indicate that i.t. TAPS may stimulate m-opioid receptors sensitive to NLZ in the spinal cord, which subsequently induce the release of dynorphins that act on k-opioid receptors to produce antinociception.

METABOLISM OF ENDOMORPHIN-2 BY SYNAPTIC MEMBRANES OF MOUSE BRAIN (49)

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Endomorphin-2 (Tyr-Pro-Phe-PheNH_2; EM2) is known to possess high affinity for the mu-opioid receptor. Although the function of EM2 as a transmitter or modulator may cease through the rapid enzymatic process in the synapse of brain, its inactivation in the synaptic region has still remained obscure. The present study was conducted to examine metabolism of EM2 by synaptic membranes of mouse brain. Major metabolites of EM2 were Tyr, Tyr-Pro, Phe, PheNH_2 and Phe-PheNH_2. Both the degradation of EM2 and the accumulation of major metabolites were inhibited by specific inhibitors of dipeptidyl peptidase IV. On the other hand, the accumulation of Phe-PheNH_2 was increased in the presence of bestatin, an aminopeptidase inhibitor, whereas that of Phe and PheNH_2 was decreased. Furthermore, purified dipeptidyl peptidase IV hydrolyzed EM2 at the cleavage site, Pro^2-Phe^3 bond. Thus, degradation of EM2 by mouse brain synaptic membranes seems to take place mainly through the cleavage of Pro^2-Phe^3 bond by dipeptidyl peptidase IV, followed by release of Phe and PheNH_2 from the liberated fragment, Phe-PheNH_2 by aminopeptidase.

REGULATION OF OPIOID RECEPTORS AFTER CHRONIC MORPHINE ADMINISTRATION IN AMPHIBIANS (87)

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Opioid agents have been shown to elicit antinociception through three distinct types of receptors (mu, kappa, and delta) in mammals. Previous behavioral and binding data from this laboratory has suggested the presence of a single opioid binding site in the amphibian, *Rana pipiens*. The present study was performed to investigate the regulation of this unique opioid receptor by chronic administration of morphine. Antinociception was assessed using a behavioral assay and opioid receptors were characterized by radioligand binding techniques. Daily systemic injections of morphine (100 nmol/g body weight) produced a tolerance time-course curve which was significantly different than the saline control group. Saturation analysis with [3H]-naloxone of brain homogenates was used to obtain affinity (Kd) and receptor density (Bmax) values. The saline control group yielded similar values as in untreated animals. The morphine-treated group showed no changes in affinity but a significant decrease in receptor density in brain tissue. These studies suggest that the regulation of amphibian opioid receptors is consistent with that observed in mammalian studies.

FILAMIN-1 BINDS TO THE CARBOXY TAIL OF THE HUMAN MU OPIOID RECEPTOR (104)

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To detect proteins capable of interacting with the mu opioid receptor, we used a yeast two-hybrid screen. The human mu opioid receptor carboxy tail (C-tail, aa 333-400) was used as bait to screen a human brain cDNA library. Sequencing of several positive cDNA inserts resulted in the identification of several encoded proteins, which may interact specifically with the mu opioid receptor C-tail. Because of our interest in the interaction of opioid receptors with the cytoskeleton, we investigated a DNA insert, which encoded the C-terminal portion of human filamin-1 (ABP 280). Direct binding of filamin-1 to the C-tail of the human m opioid receptor was demonstrated by column overlay of the GST-fusion protein of the receptor C-tail and the His-fusion protein of the C-terminal portion of filamin-1. Mapping of the interacting regions between filamin and the human mu opioid receptor C-tail is in progress. We believe that the association of filamin with the receptor protein could be of importance in the interaction of the receptor with the actin-cytoskeleton. The implications of such an interaction for receptor anchoring, regulation and signaling is under investigation (supported by grants R01-DA00017 and KO5-DA00364 to EJS).

IDENTIFICATION OF A NEURORESTRICTIVE SUPPRESSOR ELEMENT (NRSE) IN THE HUMAN MU-OPIOID RECEPTOR GENE (89)

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Analysis of the DNA sequence of the human MOR gene revealed that a region overlapping the start codon was substantially homologous to a DNA element named the neurorestrictive suppressor element (NRSE). Transient transfection experiments in the L929 and HEK non-neural cell lines showed that expression of a MOR promoter/reporter gene construct was suppressed by inclusion of this MOR NRSE. Expression from a thymidine kinase promoter was also suppressed by inserting the MOR NRSE. The MOR NRSE did not suppress expression of the reporter gene constructs in neural derived cell lines, IMR-32 and neuro2a. The transcription factor REST binds NRSE enacting the suppression of transcription. Co-transfection of REST made IMR-32 cells sensitive to the MOR NRSE mediated suppression of reporter gene expression. Electrophoretic mobility shift experiments revealed that oligonucleotides containing the MOR NRSE were bound by a factor from nuclear extracts of non-neural cell lines, HeLa and Jurkat. This binding was specifically competed by oligonucleotides containing NRSE sequences previously shown to suppress transcription through REST. (NIDA K21-DA 002700 to MLA and DA-00017 and K05-00364 to EJS)

L-NAME PREVENTS EEG AND BEHAVIORAL ALTERATIONS INDUCED BY DELTORPHIN II IN THE RABBIT (113)

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Central administration of deltorphin II (selective d opioid receptor agonist) produces EEG seizure activity in rats and in rabbits, associated with wet-dog shakes, myoclonic twitches and convulsive activity. These deltorphin II-induced effects were antagonized in rats by pretreatment with naltrexone. Opioid peptide naloxone-reversible EEG seizures are reported after i.c.v. peptide administration in rats and rabbits. In rabbits, EEG seizures induced by opioid peptide were unaccompained by convulsive motor activity and wet-dog shakes were also present. Recently, we have demonstrated that L-N^{G}-nitro arginine methyl ester (L-NAME) was able to reduce EEG and behavioural alterations induced by morphine (less selective opioid receptor agonist) in the rabbit indicating an important interaction between nitric oxide (NO) and opioid system. The present study was undertaken to verify whether L-NAME was able to influence deltorphin II-induced EEG seizure in rabbits. I.c.v. administered (20 ml/toto) did not induce EEG or behavioral alterations. Also, power spectral EEG recorded from cortex and hippocampus were similar to those observed in nontreated animals. Deltorphin II administered i.c.v. (5, 15, 50 and 100 mg/toto) induced dose-related EEG alterations. All animals treated with the highest dose (100 mg/toto) showed ictal EEG episodes limited to the hippocampus and consisting of continuous high-voltage spikes. During ictal episodes, rabbit maintained frozen posture and neither wet-dog shakes nor other behavioral alterations or clonic or tonic convulsions were observed. After these ictal episodes, hippocampal EEG voltage showed a progressive slowing in frequency and displayed typical deltorphin II EEG synchronized activity, consisting of high-voltage slow waves with superimposed spikes. This EEG pattern appears 5 to 10 min after deltorphin II and lasts from 30 min to 3hr after deltorphin II administration, depending on the dose. During these tardive EEG alterations, animals remained motionless and exhibited after respiratory depression with loss of corneal reflex and exophthalmos. L-NAME (3-300 mg/i.c.v./mouse) did not induce significant EEG or behavioral changes whereas when injected 15 min before i.c.v. deltorphin II (100 mg/toto) dose dependently prevented the EEG ictal episodes, the spiking activity and the synchronized EEG pattern induced by deltorphin II. Behavioral alterations as well as the frozen posture, the appearance of exophthalmos, loss of the corneal reflex and the respiratory depression induced by deltorphin II were also prevented. The inhibitory effect of L-NAME on deltorphin II seizures was dose-dependently reversed by L-arginine (3-300 mg/i.c.v./mouse) but not by D-arginine. Finally, glyceryl trinitrate on its own (3-300 mg/i.c.v./mouse) significantly increased deltorphin II seizures in the rabbit and it was also able to reverse the inhibition on deltorphin II seizures operated by L-NAME. The results of the present study provide a strong evidence that NO may be involved in the control of brain excitability induced by opioids: further studies are in progress in order to better understand the above involvement.

NALTREXONE FOR IMPULSIVITY, OBSESSIONALITY AND RISK TAKING BEHAVIORS (135)

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Naltrexone an opiod antagonist, is used in opiod dependence to block the effect of opiates and in Alcohol Dependence Syndrome as an anti craving agent. The effect of naltrexone on impulsivity, risk taking behavior and obsessive symptoms are not known. We report psychiatric cases who had substance use disorder, obsessionality, impulsivity and risk taking behavior. A 20 year old unmarried male, with 10 a year illness characterized initially by inability to control impulses, kleptomaniac and homosexual activities, followed by obsessive sexual fantasies with high-risk sexual behavior, poor control of anger and solvent abuse from the age of 15 year. Naltrexone 50mg/day significantly reduced the craving for the solvent as well as obsessive sexual fantasies and anger outburst in 6 weeks. A 41 year old married male had obsessive symptoms since childhood and developed alcohol harmful use during adulthood. He had poor impulse control and severe craving for alcohol which resulted in poor adherence to treatments. Obsessive symptoms and craving responded significantly to Naltrexone 50 mg/day. These patients had poor insight into severity of symptoms and used denial and rationalization to their behavior. The above cases indicate the role of endogenous opiods in the genesis of impulsivity, obsessionality and substance abuse as well as potential therapeutic benefits of opiod antagonists in the treatment.

PHOSPHORYLATION SITES ON δ-OPIOID RECEPTOR AND AGONIST-INDUCED RECEPTOR DESENSITIZATION (90)

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The consequence of the agonist-induced phosphorylation of the d-opioid receptor has not been elucidated completely. The dogma for the G protein-coupled receptor such as the opioid receptor predicts that rapid receptor desensitization would follow receptor phosphorylation. Previous studies from our laboratory have demonstrated that rapid desensitization of the d-opioid receptor requires both the phosphorylation and the internalization of the receptor. Since then, we have also identified the DPDPE-induced phosphorylation of the mouse d-opioid receptor occurred hierarchically at the Thr^{358} and Ser^{363} residues of the carboxyl tail, with Ser^{363} being the first one to be phosphorylated. Thus, in the current studies, the consequence of the agonist-induced phosphorylation of these 2 Ser/Thr residues on the desensitization and internalization of the receptor was examined. Several d-opioid receptor mutants, T358A, T358D, S363A, S363D, and T358DS363D were stably expressed in the EcR293 cells in which the level of receptor expression was controlled by culturing the cells in ponasterone A. The receptor mutant in which all the Ser and Thr residues were converted to Ala with the exception of Ser^{363} was also stably expressed in the same cells. The ability of 1 μM DPDPE to desensitize these receptor mutants were compared to the cells expressing the wild type ?-opioid receptor. Both the rapid desensitization (minutes) and the slow desensitization (hours), represented by the ability of 1 μM DPDPE to inhibit the forskolin-stimulated intracellular cAMP production after agonist pretreatment, were determined at both high and low receptor concentrations of these mutants and wild type. The rates of the agonist-induced receptor internalization, as determined by FACS analysis, in these cell lines expressing the mutant and wild type receptors were also examined. The role of agonist-induced phosphorylation of the Thr^{358} and Ser^{363} in these cellular processes will be discussed. (Supported in part by NIDA grants: DA07339, DA11806 and DA0564)

NOCICEPTIN-INDUCED INTERNALIZATION AND RECYCLING OF THE HUMAN OPIOID-RECEPTOR-LIKE 1 (hORL1) INVOLVES BETA-ARRESTIN 2 (44)

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Nociceptin/orphanin FQ (NC) promotes the internalization of the human opioid receptor-like 1 (hORL1) via clathrin-coated pits in a time- and concentration-manner. This process is more rapid and effective in CHO cells stably transfected with the cloned hORL1 (79% of cell surface receptors are lost after 10 min exposure to 1 mM NC) than in SK-N-BE cells expressing the native receptor, where 57% of cell surface receptors are lost after 30 min. hORL1 expressed in CHO-K1 cells may recycle partially in the continued presence of NC. After short (30 min) exposure to NC, hORL1 internalization is partially reversible and recycling is dependent on acid phosphatases. NC-induced internalization is significantly reduced by the peptide [Nphe1]nociceptin(1-13)NH2, a new selective ORL1 antagonist, which by itself does not affect hORL1 internalization. Over-expression of rat b-arrestin increases NC-promoted internalization of the hORL1 whereas the dominant negative mutant rat b-arrestin 2 (319-410) inhibits this event. These data show a more pronounced internalization pattern of the cloned receptor expressed in heterologous cells in comparison to the endogenous hORL1 occurring in neuronal cells.

MORPHINE-INDUCED IN VIVO RELEASE OF SPINAL CHOLECYSTOKININ IS MEDIATED BY DELTA-OPIOID RECEPTORS - EFFECT OF PERIPHERAL AXOTOMY (45)

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Morphine and other opioid agonists induce spinal in vivo release of the antiopioid peptide cholecystokinin (CCK). In the present in vivo microdialysis study, the morphine-induced release of CCK-like immunoreactivity (CCK-LI) in the dorsal horn was completely blocked by the delta-opioid antagonist naltrindole, but not the mu-opioid receptor antagonist CTOP. In addition, systemic administration of the delta-opioid receptor agonist BW373U86 and spinal administration of the delta2-opioid recept administration of morphine and DAMGO altered the spinal CCK-LI.release in axotomized animals. The present data indicate that the delta-opioid receptor mediates morphine-induced CCK release in the spinal cord.

TEMPORAL DYNAMICS OF CELLULAR OPIOID AND OPIOID RECEPTOR GENE-EXPRESSION DURING HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) (91)

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Activity-dependent alterations in neuronal gene-expression are pivotal for long-term but not short-term adjustment of synaptic strength. As a model of synaptic plasticity hippocampal LTP was induced by perforant path stimulation in freely moving rats. LTP-induction could be inhibited by naloxone. Changes in the mRNA-expression of the endogenous opioid system members were analysed by in situ hybridization with ^{35}S-labelled riboprobes at 0.5, 2, 6, 12 and 24 h after stimulation. LTP transsynaptically induced an early and sustained up-regulation pf preprodynorphin (PDYN) mRNA levels in denate gyrus (DG) granule cells. Constitutive prepronociceptin mRNA expression was detected in the DG and the ammons horn (CA 1,3). m-opioid receptor (MOR) mRNA levels were high in (most likely GABAergic) interneurons of the DG and CA1,3 but virtually absent from granule cells. Major LTP-induced changes in MOR mRNA levels were, however, not observed. Thus, increased PDYN but not MOR gene-expression may be involved in the maintenance of hippocampal LTP.

TOLERANCE AND CROSS-TOLERANCE STUDIES WITH [DMT[1]]DALDA (5)

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We previously reported that [Dmt[1]]DALDA (SD; Dmt-D-Arg-Phe-Lys-NH_2) is a highly selective mu-opioid agonist that is significantly more potent and longer-acting than morphine after i.t. and s.c. administration. In addition, we reported minimal cross-tolerance to SD in morphine-tolerant mice, suggesting that tolerance may be less of a problem with highly selective mu-opioid agonists. However, more recent data have shown significant tolerance and cross-tolerance after repeated s.c. or i.t. SD. Subcutaneous SD in mice (5xED50; *qd* x 7d) resulted in a rightward shift in ED50 for both SD (3.3x) and M (3.2x). Similarly, sc M (5xED50; *bid* x 7d) produced a similar shift in ED50 for M (3.4x) and SD (2.1x). Interestingly, tolerance and cross-tolerance between SD and M were much more profound with i.t. administration. Intrathecal SD in rats (10xED50; *qd* x 3d) resulted in a 4-fold shift in ED50; while *bid* injections produced a 40-fold shift in ED50. An escalating dose paradigm for 7d resulted in profound tolerance and the inability to determine ED50 for either SD or M even at doses 1000xED50. Reasons that may account for the profound tolerance to SD in the spinal cord will be discussed.

PEPTIDE SECRETION FROM THE BRAIN TO THE BLOOD VIA MULTIDRUG RESISTANCE ASSOCIATED PROTEIN (6)

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The blood-brain barrier protects the brain from circulating compounds. Recently, we demonstrated the ability of the brain to secrete a wide range of peptides directly into the circulation via p-glycoprotein (Pgp). Multidrug resistance associated protein (MRP), a 190 kDa protein, has long been known to cause intrinsic multidrug resistance. This protein, similar to Pgp, is categorized in the ATP-binding cassette (ABC) superfamily of transporter proteins. Several reports indicate that MRP confers a pattern of drug resistance similar to Pgp. *In vitro* studies have shown that MRP and Pgp efflux the same anti-tumor agents with different affinities. An antisense oligodeoxynucleotide targeting MRP was utilized to modulate the expression of MRP in the brain. Immunohistochemical and RT-PCR studies demonstrated a dramatic decrease in expression of MRP protein and mRNA, respectively, in the choroid plexus of animals treated with antisense targeting MRP compared to saline and mismatch controls. Following i.c.v. administration of ^{125}I-$-endorphin and ^{125}I-DPDPE, blood levels of these compounds were decreased by 60% and 80%, respectively, in antisense treated animals compared to control animals. The decrease in MRP expression in the brain also significantly enhanced the analgesic potency of systemic morphine but conversely, diminished the activity of centrally administered morphine. MRP1 knockout mice displayed similar results. Thus, this study identifies another transporter protein, MRP, able to transport endogenous substances from the brain into the blood, thus suggesting another important pathway for the brain/body communication.

INOTROPIC AND CARDIOPROTECTIVE ACTION OF [DMT[1]]DALDA (13)

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[Dmt[1]]DALDA (SD; Dmt-D-Arg-Phe-Lys-NH_2) is a potent mu-opioid analgesic that transiently increases blood pressure after iv administration. In the isolated perfused guinea pig heart, SD (10^{-7}M) significantly increased contractile force (F) with no effect on heart rate (HR) or coronary flow. The increase in F was blocked by naloxone (N; 10^{-6}M). In contrast, F, HR and flow were all significantly decreased by morphine (M; 10^{-6}M). Global ischemia (30 min) produces severe cardiac dysfunction in the perfused heart. Pretreatment with brief ischemic episodes, M and N all provided limited protection against ischemic cardiac dysfunction. Pretreatment with SD significantly protected against ischemia-reperfusion injury and preserved contractile function. When administered after global ischemia, SD still protected against cardiac dysfunction whereas M had no effect and N only provided short-term protection. These data indicate that SD has inotropic and cardioprotective actions that are mediated only partly by its action at the mu receptor. In addition to being a superior analgesic for cardiothoracic surgery, SD may be useful for treatment of heart failure and coronary artery disease.

EVALUATION OF DAMCK, A MU OPIOID RECEPTOR SPECIFIC AFFINITY LABEL *IN VIVO* AND *IN VITRO* (62)

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The mu opioid receptor selective opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) was equipped with a chloromethyl ketone group at the C-terminal. The resulting compund Tyr-D-Ala-Gly-MePhe-CH_2-Cl (DAMCK) retained the mu receptor specificity of the parent compound. Nanogram doses of DAMCK administered *intracerebroventricularly* produced dose-dependent, opioid receptor mediated, profound antinociception measured with the rat tail-flick assay. The effect persisted for 4 hrs that was only partially reversed by naloxone indicating irreversible labeling of opioid receptors. This finding was confirmed by measuring wash-resistant blockade of [^{3}H]DAMGO binding in crude membranes of certain brain regions, e.g. amygdala, hippocampus, hypothalamus, PAG. Chronic administration of DAMCK for 5-8 days resulted in the development of analgesic tolerance. This was accompanied by a decrease in the density of surface mu opioid sites. Covalent labeling of proteins of about 50 kDa was detected by SDS-PAGE and fluorography in membranes affinity labeled by [3H]DAMCK *in vitro*. It is concluded that DAMCK is able to bind irreversibly to mu opioid receptors both *in vivo* and *in vitro*. Supported by OTKA T-033062 research fund and the Foundation for the Hungarian Peptide and Protein Research.

COMPARISON OF NOCISTATIN LEVELS IN CSF AMONG CHRONIC, ACUTE, NON-PAIN PATIENTS AND NORMAL VOLUNTEERS (4)

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We have developed a unique identification method using HPLC combined with RIA specific for nocistatin, and identified two forms of human nocistatin-17 (h-NST-17) and –30 (h-NST-30) together with their precursors in brain and cerebrospinal fluid (CSF) (NeuroReport 10, 1537, 1999; 11, viii, 2000). Our aim is to find possible relationships between pain perception and CSF levels of nocistatin. CSF samples were collected from chronic pain (>6 months), acute pain (<1 week) and non-pain (hospitalized) patients. Further, 6 normal CSF samples donated by male volunteers were analyzed.. The CSF extracts were subjected to HPLC with a ODS column and the eluates were collected, 1 ml/min, and nocistatin-like immunoreactivity determined by RIA. The amounts of three NST-IRs in CSF obtained from volunteers appeared to be lower than those of other patients.

NOCICEPTIVE BEHAVIOR PRODUCED BY INTRATHECAL DYNORPHIN PEPTIDES IN MICE (3)

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Intrathecal (i.t.) administration of big dynorphin (1-10 fmol), a prodynorphin-derived peptide consisting of dynorphin A (Dyn A) and dynorphin B (Dyn B), to mice produced the biting and/or licking of the hindpaw and the tail along with slight hindlimb scratching directed toward the flank, which peaked at 5-15 min after an injection. Dyn A produced a similar response, though the doses required were higher (0.1-30 pmol) whereas Dyn B was practically inactive even at 1000 pmol. The behavior s mediated through the activation of the NMDA receptor ion-channel complex by acting on the polyamine but not the glycine recognition site and does not involve opioid or non-NMDA glutamate receptor mechanisms in the mouse spinal cord.

EFFECTS OF DEXTROMETHORPHAN ON MORPHINE ADDICTION (125)

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Continuous use of morphine may lead to the development of tolerance, physical dependence and psychological craving to morphine. NMDA receptor antagonists have been reported to be able to block the development of morphine tolerance and dependence. Dextromethorphan (DM) is an antitussive drug and also have NMDA receptor antagonist property. In this study, we have investigated the effects of DM on morphine addiction. Male Sprague Dawley rats (250-350g) were separated into four groups: control;AA and HVA in Nucleus Accumbens were determined by microdialysis and HPLC. Acute morphine or M+DM increased their levels similarly. After the treatment of morphine or M+DM for 3 days, the basal level of DOPAC and 5-HIAA significantly increased compared with the level before drug treatment.

MU AND ORL1 RECEPTOR CROSS TOLERANCE IS MEDIATED BY G PROTEIN-COUPLED RECEPTOR KINASE 2 (GRK2) UPREGULATION (25)

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Chronic morphine treatment results in morphine tolerance and increased GRK2 and orphanin FQ/nociceptin (OFQ/N) levels in the brain; morphine tolerance is reduced when the actions of GRK2 and OFQ/N are blocked. The role of GRK2 and OFQ/N in the development of mu receptor tolerance was examined in two human neuroblastoma cell lines natively expressing ORL1 and mu opioid receptors. Chronic (24 h) treatment of BE(2)-C and SH-SY5Y cells with DAMGO desensitized both mu and ORL1 receptor-medies mu receptors via ERK1/2 induction of GRK2, while the homologous desensitization of ORL1 receptors occurs via ERK1/2-dependent and -independent pathways. This work was supported by an NIH grant (DA10738) to KMS.

IDENTIFICATION OF COMPOUNDS THAT MODULATE N/OFQ TRANSCRIPTION (52-2)

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Nociceptin/Orphanin FQ has significant effects on analgesia, anxiety, and a variety of other CNS actions. Small molecule receptor agonists and antagonists have now been shown to act as anxiolytics and analgesics respectively. Another method for alteration of these responses could be the modulation of the endogenous levels of N/OFQ. To explore this possibility, we have cloned the promoter region of the human prepro N/OFQ and inserted it into the pSEAP reporter vector. This allows us to determine transcriptional activity by measuring secreted alkaline phosphatase in transfected cells. By plating cells in a 96-well format, compounds can be rapidly screened for ability to up- or down-regulate the production of alkaline phosphatase, and thus N/OFQ. Initially, we have screened a large number of steroid structures and found two compounds that modulate transcriptional activity. One compounds causes a slight (25%) increase in transcription at low concentrations, and the other compound significantly decreases (60%) transcription at higher concentrations. We now expect to inject these and other compounds into mice to determine whether they can modulate N/OFQ levels in vivo.

IN VITRO STUDY OF ENDOMORPHINS' METABOLISM (48)

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In vitro metabolism of Endomorphins (EM1: H-Tyr-Pro-Trp-Phe-NH2, EM2: H-Tyr-Pro-Phe-Phe-NH2) was examined in rat brain homogenate, in membrane and cytosolic fraction of that. Kinetics of the metabolism of EMs was characterized by analysing the digestion mixtures with HPLC, then velocity constants and half-lives were calculated based on pseudo first-order kinetics (t1/2 (EM1 in brain homogenate) = 4.94 min, t1/2 (EM2 in brain homogenate) = 3.81 min). Some general and specific enzyme inhibitoroducts of the catabolism are amino acids, and the fragments, Tyr-Pro-OH and Pro-Trp-Phe-NH2 were present as intermediates. Biological examination of the fragments of EM1 and EM2 identified and also the synthesized theoretical ones revealed that the fragments had low or zero affinity for m opioid receptors. Therefore the catabolism of EMs results in deactivation of them. Supported by OTKA T030086.

EXCLUSIVE LIGANDS FOR MU OPIOID RECEPTORS (63)

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Endomorphins (EM1: H-Tyr-Pro-Trp-Phe-NH2, EM2: H-Tyr-Pro-Phe-Phe-NH2) are endogenous peptides acting at m opioid receptors. Tritiated endomorphins with high specific radioactivity labelled in different positions were prepared for in vitro binding, signalling and degradation studies and characterisation of different EM analogues. Precursor peptides prepared by solid phase peptide synthesis contain iodinated tyrosine or phenylalanine or dehydroproline. Supported by OTKA T030086.

NEW ENDOMORPHIN ANALOGUES USING Dmt IN POSITION 1 (64)

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Two new endomorphin analogues (Dmt-Pro-Trp-Phe-NH_2 and Dmt-Pro-Phe-Phe-NH_2) were designed and synthesized using Boc-2',6'-dimethyl-tyrosine by solid phase peptide synthesis. The more hydrophobic peptides were characterized by radioreceptor assays in rat brain membrane preparation, and MVD and GPI in vitro assays, and [^{35}S]GTPγS binding. According to the results both peptides were more active (10 and 100 times) and less selective for μ opioid receptors compared to the parent peptides in binding assays. Both analogues are partial agonist in the in vitro assays and [^{35}S]GTPγS assays. In acute pain antinociceptive activity Dmt-EM2 in tail-flick and paw pressure tests was much more effective than EM2. Interesting to note the peptide bond between Dmt and Pro was cis and trans bond and cis peptide bonds are favourable and this is unexpected ration (2:1). The ratio was determined by 2D-NMR and HPLC in low temperature. Supported by OTKA T0030086.

THE INFLUENCE OF NOISE STRESS ON CENTRAL OPIOID SYSTEM (26)

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Noise was thought to be the culprit in inducing stress because it affected our health in many ways. The effects of noise on the central nervous system have shown in many studies. Unfortunately, there are few reports focused on its effects on central opioid system. Therefore, we attempted to understand the effects of noise on those neurons, and tried to find out their mechanisms. In the preliminary experiment, when mice were exposed to different intensities of noise (60~110 dB(A)), the pain threshold was increased in a dose-dependent manner which was determined with the writhing response induced by acetic acid and with the licking time induced by formalin test. In this study, we attempted to investigate the antinociceptive mechanisms induced by noise stress in the formalin test. The results obtained are as follows: naloxone (a μ-opioid receptor antagonist; 1 mg/kg, i.p.), ß-FNA (a d-opioid receptor antagonist; 5, 10 μg, i.c.v.) and naltrindole (ad-opioid receptor antagonist; 1,5 mg/kg, i.p.) reversed the antinociceptive effect of noise, but nor-BNI (a ?-antagonist; 1μg, i.c.v.) did not affect. From the above results, it was suggested that the noise stress induced-pain threshold increase might primarily be related to the μ- and d-opioid receptors in the opioid system.

INVOLVEMENT OF MODALITY-SPECIFIC LOSS OF NOCICEPTOR SIGNALING IN THE MORPHINE-INSENSITIVE NEUROPATHIC PAIN (20)

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It is known that morphine shows no analgesic action on the neuropathic pain due to deafferentation. Here we intended to clarify molecular mechanisms underlying this weakness of morphine by use of the peripheral nociception test evaluating the flexor reflex upon intraplantar (i.pl.) injection of various algogenic substances. Previously we classified such responses into three types of nociceptors-mediated ones, and demonstrated the morphine analgesia specific for type I nociceptors which are by BK. Pharmacological characterization revealed that BK stimulated B1 receptors instead of B2 receptors in such neuropathic pain model mice. These results suggest that the loss of nociception signal due to type I nociceptors may be involved in the resistance of neuropathic pain to morphine. [Supported by HFSP]

PKC-INVOLVEMENT IN THE ACUTE TOLERANCE TO PERIPHERAL MORPHINE ANALGESIA IN THE MOUSE CAPSAICIN TEST (27)

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In our previous reports we demonstrated the PKC involvement in the acute morphine tolerance in our unique peripheral nociception test in mice (JPET 293, 662-669, 2000; J. Neurosci. 21, 2967-2973, 2001). In this experiment we evaluated the opioid-mediated inhibition of nociceptive flexor responses by bradykinin which drives type I nociceptors using substance P as a spinal nociceptive neurotransmitter. Here we attempted to confirm this hypothesis by use of different widely used tests. As morcute tolerance was abolished by the co-administration of calphostin C with the first morphine. On the other hand, the DAMGO-induced analgesia did not show acute tolerance. All these results support our hypothesis of agonists-specific acute tolerance and its PKC involvement in the different nociception test. [Supported by HFSP]

LEPTIN AND THE MELANOCORTIN RECEPTOR AGONIST, MT II, AUGMENT AMPHETAMINE REWARD (129)

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Recent discoveries have been made concerning the regulation of feeding and body weight by leptin, insulin and a variety of orexigenic and anorexigenic neuropeptides. Of particular interest are the POMC-derived, a-MSH, and agouti-related protein which are agonist and antagonist, respectively, at melanocortin receptors (MCR). This study examined effects of i.c.v. injections of insulin, leptin, and the MCR agonist and antagonist, MTII and SHU9119, respectively, on overnight food intake and amphetamine reward. Amphetamine reward was measured in terms of the threshold-lowering effect on lateral hypothalamic self-stimulation (LHSS). Leptin (2 mg) and MTII (1 mg) both decreased food intake but augmented amphetamine (50 mg) reward. SHU9119 (1 mg) increased food intake but had no effect on amphetamine reward. These findings suggest that acute stimulation of the leptin-MCR axis, while suppressing appetite and the incentive effects of food, may facilitate other expressions of incentive motivation mediated by the brain dopamine system.

IDENTIFICATION OF INTRACELLULAR PROTEINS THAT INTERACT WITH MU RECEPTOR USING A YEAST-BASED GENETIC ASSAY (92)

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Over the past few years several groups have provided increasing evidence that opioid receptors are regulated by numerous mechanisms designed to rapidly modify the receptor signaling following ligand activation. In this study, we examined the molecular basis for the cellular regulation of μ receptor and, specifically, to identify novel intracellular proteins that participate in the cellular regulation of μ receptor signaling, using a yeast-based genetic assay to screen proteins that may associate with the μ receptor. The Clontech Matchmaker two-hybrid system was used to screen a rat brain library with baits corresponding to intracellular domains of the μ receptor. Initial experiments were performed using the C-terminus of the μ receptor. DNA fragment corresponding to this domain was generated by PCR and subcloned into the GAL4 DNA binding domain of pAS2-1. The fusion construct was used to screen a rat brain library. Several plasmid DNA from positive clones were isolated. The identity and the function of these positive clones are currently under investigation. Important new information leading to the development of novel therapeutic strategies in the treatment drug abuse may be acquired through the characterization of the molecules that regulate μ receptor signaling.

POSTNATAL MU-OPIOID RECEPTOR SUBCELLULAR TARGETING IN THE RAT CAUDATE-PUTAMEN NUCLEUS (93)

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A specific role for opioids in the regulation of neurogenesis in the developing caudate-putamen nucleus (CPN) is suggested by the early appearance of mu-opioid receptors (MORs) in this region. We used electron microscopic immunocytochemistry to determine whether there are age-dependent changes in subcellular targeting of MOR in the rat CPN. Sections were processed for detection of antisera against MOR at postnatal (P0, P5, P10, P15, P20) and adult age points. During P0–P5, MORs were mainly localized in somatodendritic profiles, some of which were connected by dendrodendritic membrane specializations. At P10, a few spines and axon terminals showed immunoreactivity to MOR. After postnatal third week, the peak of synaptogenesis, MOR distribution patterns in spiny dendrites and terminals were already similar to those of adult. Our results indicate that there are age-dependent changes in MOR distribution from cytoplasmic to plasmalemmal and from postsynaptic to presynaptic sites. These data support the hypothesis that the plasmalemmal availability of MOR is correlated with the period of maximal synaptogenesis. (Supported by grants from NIDA DA14214 for HW and DA04600 for VMP)

THE RAT MOR-1C SPLICE VARIANT IS EXPRESSED BY SEROTONERGIC AND NONSEROTONERGIC BULBOSPINAL NEURONS (88)

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Spinally projecting neurons of the rostral ventromedial medulla (RVM) express the cloned μ-opioid receptor (MOR1). To test whether MOR1 splice variants are also expressed, we have obtained a cDNA fragment from rats that is highly homologous to the carboxyl terminus of mouse MOR-1C. Combining in situ hybridization for MOR-1C with immunocytochemistry for tryptophan hydroxylase (TPH) and retrograde tract-tracing, we found that many spinally projecting RVM cells expressed MOR-1C mRNA and that some also expressed TPH. In addition, MOR-1C-immunoreactivity coexisted with serotonin-immunoreactivity in the neck of the spinal dorsal horn, the intermediate gray, and the ventral horn. We conclude that many bulbospinal serotonergic neurons express the MOR-1C variant and that activation of MOR-1C might modulate spinal serotonin release. Supported by DA09642 and DA 05466.

PI3 KINASE: ROLE IN OPIOID DESENSITIZATION IN DORSAL ROOT GANGLION NEURONS (103)

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We have previously shown that activation of mu receptors inhibits high voltage-gated Ca2+ channel (VGCC) currents in dorsal root ganglion (DRG) neurons, and the inhibitory effect diminishes with prolonged exposure to the agonist. This study further examined the involvement of phosphoinositide 3 (PI3) kinase in the desensitization process. The VGCC currents were recorded with whole-cell voltage-clamp techniques from cultured mouse DRG neurons. Brief applications of DAMGO (1 micro M, 1-2 min) reduced VGCC currents by 33+/-6% (n=13). The inhibitory action decreased in neurons pretreated with DAMGO for 1 hr and nearly completely disappeared after 24 hr pretreatment. Co-administration of a PI3 kinase inhibitor wortmannin attenuated chronic DAMGO-induced desensitization. Following 1 or 24 hr preincubation with both drugs, acute DAMGO still produced a 39+/-11% or 20 +/-7% reduction in Ca2+ currents, significantly greater than the corresponding effect in neurons pretreated with DAMGO alone. These results suggest that PI3 kinase may play a crucial role in mu opioid desensitization in DRG neurons.

ENHANCED μ-OPIOID RESPONSES IN THE SPINAL CORD BY A DELETION OF PROTEIN KINASE Cg GENE (95)

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The present study was investigated whether a deletion of the protein kinase C (PKC) g gene could result in any changes in spinal m-opioid receptor-mediated antinociception and activation of G-proteins. These two spinal m-opioid receptor-mediated functions were enhanced in PKCg knockout mice as compared to wild-type mice. In contrast, there were no changes in d- and k-opioid- or ORL-1 receptor-mediated activation of G-proteins between wild-type and PKCg knockout mice. Deletion of PKCg had no effect on mRNA product of spinal m-opioid receptors, whereas it caused an increase in maximal binding of the m-opioid receptor agonist [^{3}H]DAMGO in spinal cord membranes. These findings suggest that the loss of PKCg gene may protect the functional m-opioid receptors from degradation by phosphorylation in the spinal cord, leading to an enhancement of spinal m-opioidergic systems to activate G-protein and antinociception. The present data provide direct evidence for the critical role of PKCg isoform in the negative modulatory pathway for spinal m-opioidergic systems.

CHRONIC MORPHINE ALTERS G-PROTEIN FUNCTION IN CELLS EXPRESSING THE CLONED MU RECEPTOR (94)

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Chronic morphine (MOR) treatment uncouples the μOR and its G protein in cell culture and animal models. In the present study, hMOR-CHO cells were incubated with 1 μM of morphine (or no drug) for 20 hr. Subsequently, we assessed DAMGO- and MOR-stimulated [^{35}S]-GTP-γ-S binding and MOR-mediated inhibition of forskolin-stimulated cAMP accumulation. Using a single concentration of [^{35}S]-GTP-γ-S (0.05 nM), chronic morphine treatment did not change basal [^{35}S]-GTP-γ-S binding, shifted the MOR ED50 from 59 nM to 146 nM and decreased the maximal stimulation (Emax) from 201 % to 177 %. Similar results were observed with DAMGO. Binding surface analysis resolved two [^{35}S]GTPγS binding sites (high affinity and low affinity sites). In control cells, MOR stimulated [^{35}S]-GTP-γ-S binding by increasing the Bmax of the high affinity site. In MOR-treated cells, MOR stimulated [^{35}S]-GTP-γ-S binding by decreasing the high affinity Kd without changing the Bmax. The overall degree of stimulation was similar. MOR-treatment shifted the ED50 for MOR-inhibition of forskolin-stimulated cAMP accumulation almost 10-fold. These results suggest that MOR tolerance is associated with altered G-protein function.

ENDOMORPHIN-1 AND –2 INDUCE NALOXONE-PRECIPITATED WITHDRAWAL SYNDROMES IN RATS (116)

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In 1997, endomorphin-1 (EM1) and –2 (EM2) were identified as the most specific endogenous m-opioid ligands. These two peptides have shown analgesic effects and many other opioid functions. In the present study, we attempt to investigate the possible ability of endomorphins to induce naloxone-precipitated withdrawal in comparison with that induced by morphine. Using the previously established scoring system in rats, twelve withdrawal signs (chewing, sniffing, grooming, wet-dog shakes, stretching, yawning, rearing, jumping, teeth grinding, ptosis, diarrhea, and piloerection) were observed and scored following naloxone (4 mg/kg, i.p.) challenge. Compared with the sham control, EM1 and EM2 (20mg, i.c.v., b.i.d. for 5 days) both produced significant withdrawal syndromes which was with similar severity to that induced by the same dose of morphine. There was no difference between EM1, EM2 and morphine-treated group for withdrawal signs, except for grooming. EM1 and EM2 induced more grooming than that caused by morphine. Although EM1 and EM2 both led to the withdrawal, they displayed different potency for some syndromes and suggest their distinct regulations. These results indicate a close relationship between m-opioid receptors and morphine withdrawal. It was also implied that EM1 and EM2 could initiate certain mechanism involved opiate dependence.

THE OPIOID-INDUCED REGULATION OF MITOGEN ACTIVATED PROTEIN KINASES CASCADE AT THE CELLULAR LEVEL (126)

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The inhibition of neuronal excitability via the regulation of cAMP levels or Ca^{2+} conductance represents the traditional mechanism of action of opioids. However, opioids can also activate mitogen-activated protein kinases (MAPK). The opioid-induced increase of MAPK activity was shown to initiate m-opioid receptor desensitization. The activated MAPK can also translocate to the nucleus where it can activate or induce transcription factors thereby leading to the expression of several genes. The present study was undertaken to evaluate the direct effect of opioids on MAPK activity in Neuro2a MOR1 cells expressing m-opioid receptors. We report here that opioids, via m-opioid receptors exert a stimulatory effect on MAPK phosphorylation. In contrast, prolonged opioid treatment decreased the levels of phoshorylated MAPK. Moreover, withdrawal from the drug enhanced the opioid-induced inhibition of MAPK phosphorylation. We have also found that the opioid-regulated MAPK activity opposed the adaptation in the well-known cAMP system. The stimulatory effects of opioids on MAPK activity had similar pharmacological profiles and concentration-response relationships to those reported for the inhibitory effects of opioids on cAMP pathway. Our results provide evidence that, at the cellular level, cAMP and MAPK cascade can serve opposing functions and may contribute to the development of tolerance and addiction. This study was supported by KBN grant P05A.107.20

INTRA VTA SP_{1-7} MODULATES NUCLEUS ACCUMBENS DOPAMINE IN THE RAT DURING MORPHINE WITHDRAWAL (127)

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The effect of intraventral tegmental area injection of the substance P fragment SP_{1-7} on nucleus accumbens dopamine release during naloxone-precipitated morphine withdrawal was studied using microdilysis. The result showed that injection of the peptide prior to naloxone challenge up-regulated the dopamine levels in nucleus accumbens and frontal cortex. Moreover, SP_{1-7} was found to enhance level of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) in these brain areas. This result supports our previous observation that SP_{1-7} may affect the mesolimbic dopamine system, e.g. by decreasing the dopamine D2 receptor transcript in nucleus accumbens, as previously shown. It thus evidences an ability of the peptide to inhibit the decrease of dopamine release caused by morphine withdrawal. Therefore, we believe that SP_{1-7} may be involved in a mechanism underlying the regulation of the reaction to opioid withdrawal.

DIFFERENTIAL EFFECTS OF REGULATORS OF G PROTEIN SIGNALING (RGSS) ON ADENYLATE CYCLASE INHIBITION MEDIATED BY μ AND δ OPIOID RECEPTORS (97)

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We investigated effects of RGS proteins on inhibition of forskolin-stimulated adenylate cyclase (AC) mediated by m and d opioid receptors. One RGS or the vector and the m or d opioid receptor were transiently co-expressed in HEK293 cells. Both morphine and the selective agonist DAMGO or DPDPE dose-dependently inhibited AC in cells transfected with the μ or δ opioid receptor. Co-expression of RGS1, RGS2, RGS4, RGS9, RGS10, GAIP significantly reduced morphine- or DAMGO-induced inhibition of AC mediated by the m receptor, but did not affect the effects of morphine or DPDPE mediated by the d receptor, except RGS9. To delineate molecular basis of the differential effects of RGS, we generated two chimeras m/dC and d/mC receptors, in which the C-terminal domains were exchanged. Expression of RGS4 or GAIP decreased DPDPE-induced inhibition of AC activity in cells transfected with the δ/μC receptor. In contrast, RGS4 and GAIP had no effect on DAMGO-induced AC inhibition mediated by the μ/δC receptor. Thus, RGS proteins had differential effects on the m and d receptors and the C-terminal domain appeared to contribute to the differences. (supported by Natural Science Foundation of China and NIH grants of USA)

ROLE OF CHOLECYSTOKININ IN THE REDUCTION OF ENDOMORPHIN-2-INDUCED ANTINOCICEPTION IN DIABETIC MICE (46)

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The role of cholecystokinin in the reduction of endomorphin-2-induced antinociception in diabetic mice was examined using tail-flick test. There was no significant difference in the antinociceptive effect of endomorphin-1 (EM-1, 1-10 μg, i.c.v.) between non-diabetic and diabetic mice. On the other hand, the antinociceptive effect of endomorphin-2 (EM-2, 3-30 μg, i.c.v.) in diabetic mice was significantly less than that in non-diabetic mice. Cholecystokinin octapeptide (CCK-8, 1-10 ng, i.c.v.) dose dependently reduced the antinociceptive effects of EM-1 and EM-2 in non-diabetic mice. However, in diabetic mice, CCK-8 significantly inhibited the antinociceptive effect of EM-1, but not of EM-2. In non-diabetic mice, CI-988 (0.3 ng, i.c.v.), a selective CCK_B receptor antagonist, had no significant effect of either EM-1- or EM-2-induced antinociception. In diabetic mice, while CI-988 (0.3 ng, i.c.v.) had no significant effect of EM-1-induced antinociception, it dose dependently enhanced the antinociceptive effect of EM-2. The results indicated that the reduction of EM-2-induced antinociception in diabetic mice may be due, at least in part, to the activation of CCK_B receptors.

FUNCTIONAL EXPRESSION OF MOUSE DELTA-OPIOID RECEPTOR AND HUMAN NPFF2-RECEPTOR IN *Xenopus laevis* OOCYTES

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We have expressed the MYC-tagged mouse delta-opioid receptor (DOR) and FLAG-tagged neuropeptide FF - receptor 2 (NPFF2) in *Xenopus laevis* oocytes. Localization of the receptors in unstimulated and NPFF/ Leu-enkephalin stimulated oocytes was studied with fluorescence immunohistochemistry. Staining of oocyte sections with an anti-MYC or anti-FLAG antibody showed that the receptors were located on the cell surface. Double stainings with both anti-MYC and anti-FLAG antibodies did not show significant co-localization of receptors even after stimulation.Cell extracts prepared from the stimulated oocytes were immunoprecipitated with MYC-specific antibody. In the Western blot analysis of the precipitates with FLAG-specific antibody no co-immunoprecipitated receptors were observed. The functionality of the receptors was tested by stimulating the cells with a stable analogue of NPFF or Leu-enkephalin. The currents were measured in physiological solution (10 mM HEPES pH 7,5; 115 mM NaCl; 2,5 mM KCl; 1,8 mM $CaCl_2$) at –80 mV holding potential. The ligands produced dose-dependent outward currents when the receptors were either co-expressed or they were expressed alone. The NPFF-responses were not blocked by 100 nM naltrindole. The results imply that the stimulation of the cells with either DOR agonist or NPFF analogue does not result in significant complex formation between DOR and NPFF2. As observed in other expression systems, the DOR is internalized after agonist stimulation. In contrast, it seems that in this system NPFF2 receptor is not internalized after agonist treatment.

Supplement to the Abstract Book

INHIBITION OF NEUROPATHIC PAIN BY SELECTIVE ABLATION OF BRAIN-STEM MEDULLARY CELLS EXPRESSING THE MU OPIOID RECEPTOR

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The hypothesis that chronic pain from L5/L6 spinal nerve ligation (SNL) is due to tonic activation of descending pain facilitation mechanisms was explored by selective ablation of mu opioid receptor (MOR) expressing cells in the RVM by the mu agonist-toxin conjugate dermorphin-saporin (DERM-SAP). Rats received a single RVM injection of DERM, SAP, or DERM-SAP. After 28 days, these rats received sham- or SNL surgery and their responses to non-noxious or noxious stimuli were tested 7 days later. The RVM pretreatments did not alter responses in sham rats. DERM and SAP pretreated SNL rats showed allodynia and hyperalgesia; DERM-SAP pretreated rats, however, were not different from sham control. The latter showed a selective loss of MOR expressing cells in the RVM. RVM DERM-SAP also fully reversed the established allodynia/hyperalgesia in SNL rats by day 14. RVM pretreatment with $-funaltre-xamine blocked the effects of DERM-SAP. These data, together with findings of blockade of SNL induced pain with RVM lidocaine or lesion of the dorsolateral funiculus, suggest that tonic activation of descending facilitation is essential for the expression of neuropathic pain. Supported by DA 11823.

ANTIDIURETIC EFFECT OF A NOVEL PEPTIDE: BV8

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From skin secretions of Bombina frog, a small protein termed BV8 has been isolated. In male rats BV8 (0.05-5 mg/rat, i.c.v.; 1-5 mg/rat, s.c.) delayed (1-3 hr) renal water excretion following an intra-gastric bolus of water (4% body weight). 90 min after injection, control rats eliminated 9.5 ± 0.5 ml urine while s.c. BV8-injected rats eliminated 7.0 ± 0.6 ml urine (1 mg/rat) or 3.75 ± 1.4 ml urine (5 mg/rat). BV8-induced antidiuresis was completely antagonized by i.p. administration of the V_2 vasopressin receptor antagonists: (1-Adamantaneacetyl[1],D-Tyr(Et)[2], Val[4], Abu[6], Arg[8,9])-Vasopressin (150 mg/kg) and ($d(CH_2)_5$ [1],D-Ile[2], Ile[4],Arg[8], Ala-NH_2 [9])-Vasopressin (30 mg/kg,), by i.p. administration of the a_2-adrenergic agonist clonidine (40 mg/kg), and by i.p. administration of the NMDA antagonist, MK801 (0.3 and 1 mg/kg). BV8 displayed no antidiuretic actions in the Brattleboro rats. Vasopressin and oxytocin plasma levels were evaluated, by radioimmunoassay in rats after s.c. injection of 5 mg of BV8. Vasopressin and oxytocin plasma levels 45 min (11.2 ± 0.9 pg/ml; 60 ± 6 pg/ml) and 90 min (13.5 ± 1.2 pg/ml; 65 ± 8 pg/ml) after BV8 injection were significantly higher respect to control values (4.7 ± 0.5 pg/ml; 14.8 ± 3 pg/ml).

LACK OF OPIOID TOLERANCE IN PRODYNORPHIN "KNOCK-OUT" MICE.

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Peripheral nerve injury and opioid tolerance have common features including tactile allodynia and thermal hyperalgesia, decreased spinal opioid potency and upregulation of spinal dynorphin (DYN). DYN may normally produce antinociception but may be pronociceptive in pathological states. Here, prodynorphin "knock-out" (KO) and wild-type (WT) mice were studied for changes in sensitivity to non-noxious mechanical and noxious radiant heat after morphine or placebo pellet. After 5 days of treatment, WT, but not KO, mice developed antinociceptive tolerance. Morphine, but not placebo pellet produced a time-related increased sensitivity to non-noxious and noxious stimuli in WT, but not in KO, mice. Spinal DYN levels were significantly increased by morphine in WT mice. *I.th.* antiserum to DYN (DYN A/S) blocked morphine-induced abnormal pain in WT mice and reversed tolerance. *I.th.* DYN A/S did not alter sensitivity to non-noxious or noxious stimuli in KO mice. Pre-immune serum had no effect. These data suggest that spinal DYN upregulation after chronic morphine may promote opioid tolerance and abnormal pain. Supported by DA 12656.

MU OPIOID RECEPTOR MEDIATED ANTINOCICEPTIVE EFFECT OF BUPRENORPHINE IS ENHANCED IN ORL-1 KNOCKOUT MICE (121-2)

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Buprenorphine (BUP), a mixed mu, delta and kappa opioid receptors agonist/antagonist, has recently been shown to act as an agonist at the opioid receptor-like (ORL-1) receptor. In the present study we determined the ability of BUP to activate both MAP kinase and AKT activity in CHO cells transfected with either mu opioid or ORL-1 receptors. The antinociceptive effect of BUP was also determined in ORL-1 as well as in the mu opioid receptor Knockout (KO) mice. BUP activated both MAP kinase and AKT via mu opioid and ORL-1 receptors in vitro. The antinociceptive effect of BUP was totally abolished in the mu opioid receptor KO mice, but the response was enhanced in the ORL-1 KO mice. The present data suggest that the mu opioid receptor-mediated antinociceptive effect of BUP is compromised by its ability to activate the ORL-1 receptor. (Supported by NIDA DA 05010; KL was supported by a KO1 award DA00411 from NIDA and a NARSAD Young Investigator Award during these studies)

NEUROPEPTIDE FF – A LINK BETWEEN THE IMMUNE AND NERVOUS SYSTEMS?

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The aim was to study the expression of neuropeptide FF (NPFF) in cells of the immune system and to characterize its function there. Neuropeptide FF, an octapeptide of the RF-amide peptide family involved mainly in pain modulation is expressed in the CNS, the spleen and in the Jurkat cell line. NPFF may thus also play a role as an immunomodulator. Quantitative PCR, immunostaining and FACS –analysis were used to study expression of NPFF in a number of cells of the immune system. Thus far, NPFF mRNA has been detected in U251, A549 MG, Tepa, and several Jurkat cell lines, as well as in several different immune cell types and human tissue cDNA panels. Also, both Th1- and Th2-cells generated *in vitro* expressed NPFF mRNA during both their differentiation and activation and a slight augmentation in expression could be noted during activation in contrast to a slight decrease during differentiation in both the Th1- and Th2-cells. Experiments were normalized using housekeeping genes. However, results also suggest that the levels of NPFF mRNA in U251, A549, or in lymphocytes studied were not sufficiently high or the RNA is not processed to yield sufficient amounts of the active peptide NPFF to be detected using immunoscreening with NPFF antisera. Further examinations will be carried out to determine the levels of NPFF expression in other immune cells and to reveal its role in the immune system/these cells.

EFFECTS OF ACUTE AND CHRONIC OPIOID RECEPTOR ACTIVATION ON REGULATION OF ADENYLYL CYCLASE TYPE VIII SPLICE VARIANTS

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AC-VIII has been shown to be an important target for in vivo modulation by chronic opioid exposure. We previously reported that acute agonist activation of $G_{i/o}$-coupled receptors inhibits AC-VIII activity, while agonist withdrawal following chronic activation of these receptors induces AC-VIII superactivation. Three splice variants of AC-VIII have been identified, and termed AC-VIII-A, B and C (with AC-VIII-B missing the glycosylation domain and AC-VIII-C lacking most of the C_{1b} area). We report here that AC-VIII-A and B, but much less so C, are inhibited by acute m-opioid and D_2-dopaminergic receptor activation, suggesting that the C_{1b} area of AC-VIII has an important role in the AC inhibition via $G_{i/o}$-coupled receptor activation. While the AC isoforms differed in their capacity to be inhibited by acute agonist exposure, agonist withdrawal after prolonged treatment led to a similar superactivation of all three splice variants, with no significant change in AC-VIII expression. We found that G_{bg} dimers have an important role in the superactivation process. Moreover, AC-VIII superactivation was not affected by preincubation with a permeable cAMP analog, indicating that it does not depend on the agonist-induced reduction in cAMP levels. The superactivated AC-VIII-A, B and C were similarly reinhibited by reapplication of agonist (morphine or quinpirole), bringing the activity back to control levels. These results demonstrate differences in the agonist inhibition of the AC-VIII splice variants before, but not after, superactivation. *Supported by NIDA (DA 6265) and the US-Israel Binational Science Foundation.*

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The meeting rooms and lodging are set among acres of forest and sand dunes. Our meeting will be in conjunction with the International Cannabinoid Research Society, and we look forward to a stimulating time in California.

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