Jpn. J. Pharmacol. **89**, 229 – 234 (2002)

FORUM MINIREVIEW

Recent Advances in the Search for the μ-Opioidergic System

Differential Mechanism of G-Protein Activation Induced by Endogenous μ-Opioid Peptides, Endomorphin and β-Endorphin

Hirokazu Mizoguchi[1,*], Leon F. Tseng[1], Tsutomu Suzuki[2], Ichiro Sora[3] and Minoru Narita[2]

[1]*Department of Anesthesiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA*
[2]*Department of Toxicology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*
[3]*Department of Psychopharmacology, Tokyo Institute of Psychiatry, Tokyo 156-8585, Japan*

Received December 4, 2001 Accepted March 22, 2002

ABSTRACT—It is well documented that the μ-opioid receptor (MOP-R) is expressed by neurons in several central nervous system regions. Its occupancy with agonist drugs modulate a variety of physiological processes including pain, reward, stress, immune responses, neuroendocrine functions, and cardiovascular control. Based on the receptor binding assay, endomorphin-1 and endomorphin-2 have the highest specificity and affinity for the MOP-R of any endogenous substance so far described in the mammalian nervous system. In contrast, β-endorphin exhibits the strongest actions among endogenous opioid peptides mainly through the MOP-R; however, it also shows the distinct pharmacological actions. Recent cloning and expression studies have indicated that MOP-Rs are seven-transmembrane domain receptors whose actions are mediated through activation of heterotrimeric guanine nucleotide binding proteins (G-proteins). The activation of G-proteins by MOP-Rs can be measured by assessing agonist-induced stimulation of membrane binding of guanosine-5'-*o*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS). The subject of the present review is to focus on the differential mechanism underlying G-protein activation induced by these μ-opioid peptides using the [^{35}S]GTPγS binding assay.

Keywords: Endomorphin, β-Endorphin, G-Protein, [^{35}S]GTPγS binding, μ-Opioid receptor

Introduction

Among the opoid receptors, the μ-opioid receptor (MOP-R) makes a major contribution to opioids-induced antinociception. Since the initial demonstration of MOP-Rs over 25 years ago, investigators have searched for their endogenous ligands. The search led to the discovery of enkephalins (1), endorphins (2) and dynorphins (3) in the 1970's, but they have either low selectivity or efficacy at the MOP-Rs (4). The enkephalins ([Met^5]enkephalin and [Leu^5]enkephalin) are the endogenous ligands for δ-opioid receptors and dynorphin A(1 – 17) is the endogenous ligand for κ-opioid receptors. β-Endorphin had been proposed to be an endogenous ligand for the MOP-Rs (5), since β-endorphin has a moderate affinity for MOP-Rs (4). However, at the present, the pharmacological effects of β-endorphin have been recognized to be mediated by MOP-R and putative ε-opioid receptor (6, 7). Recently, two new endogenous opioid peptides, endomorphin-1 (EM-1) and -2 (EM-2), were isolated from mammalian brain with high selectivity to MOP-Rs (8). Behavioral studies reveal that EM-1 and EM-2 produce profound, naloxone-reversible antinociception in mice after either intracerebroventricular or intrathecal administration (9, 10), suggesting that EM-1 and EM-2 are endogenous selective MOP-R.

The MOP-Rs belong to the superfamily of seven-transmembrane domain receptors that are coupled to G-proteins (11). The occupation of MOP-Rs by agonists leads to the activation of the Gi/Go class of G-proteins, which can be measured by assessing agonist stimulation of membrane binding of the non-hydrolyzable analog of guanosine-5'-triphosphate (GTP), guanosine-5'-*o*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) (12). [^{35}S]GTPγS binding enhanced by the synthetic MOP-R agonist [D-Ala^2,NHPhe^4,Gly-ol^5]enkephalin (DAMGO) has been reported in membranes from

*Corresponding author. FAX: +1-414-456-6507
E-mail: toyama@mcw.edu

human neuroblastoma SH-SY5Y cells (12), SK-N-SH cells (13), C6 glioma cells (14), CHO cells (15), NG108-15 cells (13), rat thalamus (15), rat striatum (16), rat locus coeruleus (17), mouse spinal cord (18) and mouse pons/medulla (19). Such MOP-R agonist-stimulated [^{35}S]GTPγS binding in membrane preparations has provided dynamic measurements of agonist occupation of MOP-Rs and its efficacy for activation of G-proteins.

G-Protein activation by endogenous μ-opioid peptides

The G-protein activation induced by EM-1 and EM-2 has been reported in the membrane preparation obtained from the mouse spinal cord (18), mouse pons/medulla (20), and mouse periaqueductal gray matter (21). Like a selective MOP-R full agonist DAMGO, EM-1 and EM-2 produce concentration-dependent increases of [^{35}S]GTPγS binding in these membrane preparations, saturating at 10 μM (Fig. 1). The increase of [^{35}S]GTPγS induced by EM-1 and EM-2 are completely attenuated by selective MOP-R antagonist β-funaltrexamine (β-FNA). However, the maximal stimulation of [^{35}S]GTPγS binding induced by EM-1 and EM-2 are approximately 0.6-fold lower than that induced by DAMGO. We could exclude the possibility that the comparable lower levels of endomorphins (EMs)-stimulated [^{35}S]GTPγS binding may result from the quick degradation of EMs by intrinsic enzymes, because no differences in the [^{35}S]GTPγS binding stimulated by EMs were noted in the presence and absence of peptidase/protease inhibitors (Fig. 2). The evidence clearly suggests that endogenous μ-opioid peptides EM-1 and EM-2 may be the partial agonists for MOP-Rs.

Unlike EM-1 and EM-2, β-endorphin (1 nM – 10 μM)

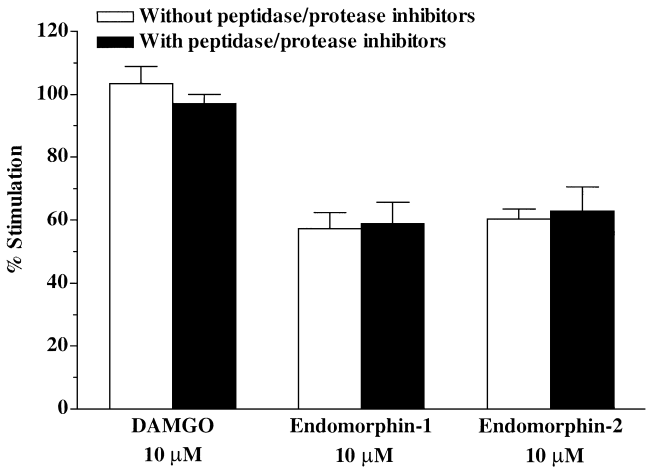

Fig. 2. Effect of the peptidase/protease inhibitors on the increase of [^{35}S]GTPγS binding induced by EM-1, EM-2 and DAMGO in the spinal cord membrane obtained from ICR mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP in the absence /presence of opioid peptides and/or peptidase/protease inhibitors (10 μl/ml of a solution containing 0.2 mg/ml each of bestatin, leupeptin, pepstatin A and aprotinin) for 2 h at 25°C. Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of agonist and represent the mean ± S.E.M. from at least three independent experiments.

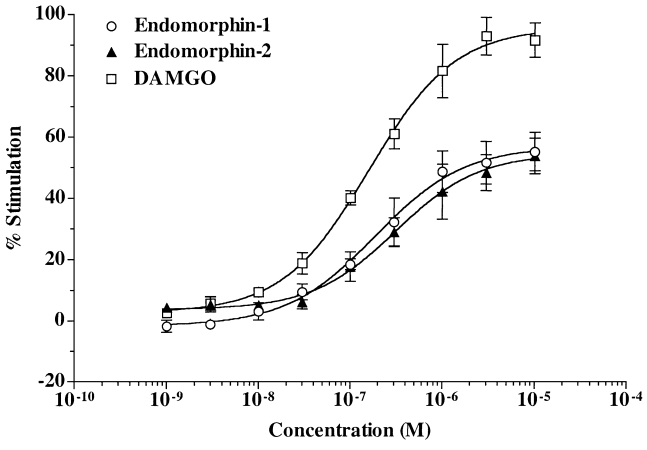

Fig. 1. The increase of [^{35}S]GTPγS binding induced by EM-1, EM-2 and DAMGO in the pons/medulla membrane obtained from ICR mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP in the absence or presence of various concentrations (1 nM – 10 μM) of opioid peptides for 2 h at 25°C. Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of agonist and represent the mean ± S.E.M. from at least three independent experiments.

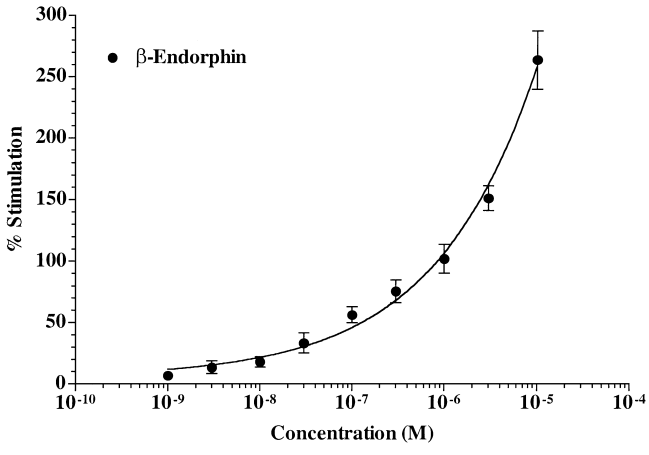

Fig. 3. The increase of [^{35}S]GTPγS binding induced by β-endorphin in the pons/medulla membrane obtained from ICR mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP in the absence/presence of various concentrations (1 nM – 10 μM) of β-endorphin for 2 h at 25°C. Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of agonist and represent the mean ± S.E.M. from at least three independent experiments. Modified from Mizoguchi et al. (19).

produces an unsaturable increase of [^{35}S]GTPγS binding to mouse pons/medulla membrane, affording more than twofold higher binding than that induced by DAMGO at 10 μM (Fig. 3). The increase of [^{35}S]GTPγS induced by β-endorphin is only partially attenuated to 40% of the original stimulation by β-FNA (19). This phenomenon may be caused by the agonist property of β-endorphin for a different type of receptor, the putative ε-opioid receptor, in addition to its agonist property for the MOP-R. In C_6 rat glioma cells expressing a cloned rat MOP-R, β-endorphin shows the same magnitude of increase of [^{35}S]GTPγS binding with DAMGO, suggesting that β-endorphin may have a full agonist property for MOP-Rs (14).

It is somewhat interesting that endogenous μ-opioid peptides EM-1 and EM-2 are partial agonists for MOP-Rs, since most of the endogenous opioid peptides, including [Met^5]enkephalin, [Leu^5]enkephalin, dynorphin A(1 – 17) and β-endorphin show the full agonistic properties for their respective opioid receptors (14, 22). At the present, it is not clear why both endogenous MOP-R full agonist (β-endorphin) and endogenous MOP-R partial agonists (EM-1 and EM-2) exist. Theoretically, a partial agonist, but not a full agonist, can inhibit the pharmacological effect of full agonists acting on same receptor, at the concentration inducing the maximal response. It is of interest to note that EM-1 and EM-2 partially but significantly attenuate the increase of [^{35}S]GTPγS binding induced by 1 μM β-endorphin in the mouse spinal cord (Fig. 4). Considering the endogenous μ-opioidergic system, we propose that EM-1 and EM-2 can negatively modulate the physiological overshooting of μ-opioidergic system induced by β-endorphin (23).

G-Protein activation by endogenous μ-opioid peptides in MOP-R knockout mice

Following the initial cloning of MOP1 (24, 25), a number of splicing variants of MOP-R differing at the intracellular carboxy terminus have been reported (26, 27). At the present, based on the splicing of identified at least 10 exons of the MOP-R gene, 7 subtypes of the MOP-R (MOP1, 1A, 1B, 1C, 1D, 1E and 1F) have been identified. Since all subtypes of the MOP-R contain the sequences encoding the same first 3 exons (exon 1 to exon 3), the presence of these 3 exons seems to be critical for expression of MOP-Rs.

The knockout approaches for the MOP-R gene is a valuable tool in defining the role of the MOP-R in the physiological response induced by μ-opioid peptides. The several laboratories successfully have developed the mice lacking MOP-R with the exon specific deletion of MOP-R gene (28 – 31). At the present, two types of MOP-R knockout mice are available; one is a knockout mouse with a disruption of exon 1 (Fig. 5A and references 29 and 31), and another is a knockout mouse with a disruption of exon 2 and 3 (28, 30). In both MOP-R knockout mice, [^{3}H]DAMGO binding as a ligand for the identification of MOP-R is completely abolished in the both spinal cord and pons/medulla (Fig. 5A, manuscript in preparation, references 20 and 32). We clearly found that the increase of [^{35}S]GTPγS binding induced by EM-1 and EM-2 are also completely abolished in the pons/medulla obtained from both knockout mice (Fig. 5B, manuscript in preparation). In contrast, the increase of [^{35}S]GTPγS binding induced by β-endorphin is only partially attenuated in the pons/medulla obtained from both knockout mice with the same magnitude of attenuation (Fig. 5B, manuscript in preparation, reference 33). These findings provide further evidence for the distinct mechanism of G-protein activation by EMs and β-endorphin.

As expected, there must be no difference between these two types of MOP-R knockout mice on the G-protein activation, since the first 3 exons (exon 1 to exon 3) of MOP-R gene is critical for expression of MOP-Rs. It, therefore, is

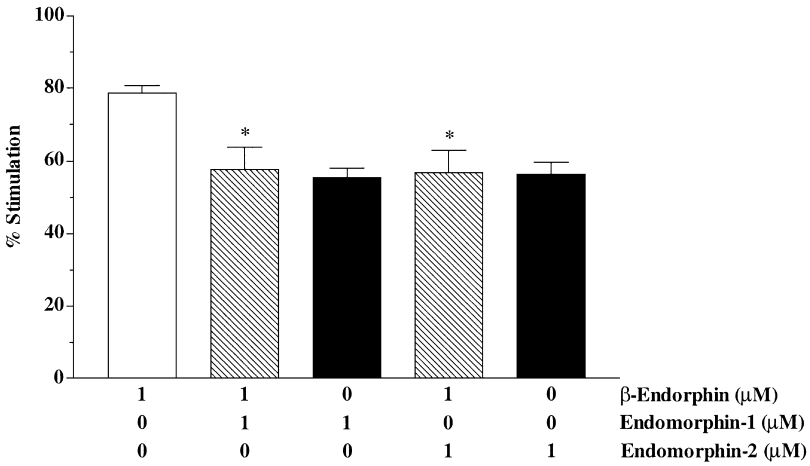

Fig. 4. Effects of EM-1 and EM-2 on the increase of [^{35}S]GTPγS binding induced by β-endorphin in the spinal cord membrane obtained from ICR mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP in the absence/presence of opioid peptides for 2 h at 25°C. Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of agonist and represent the mean ± S.E.M. from at least three independent experiments. *P<0.05 vs β-endorphin alone. Modified from Mizoguchi et al. (23).

(A)

[^{3}H]DAMGO binding	Wild-type mice	KO mice
B_{max} (fmol/mg protein)	72.29 ± 3.42	n.d.
K_d (nM)	0.72 ± 0.08	n.d.

(B)

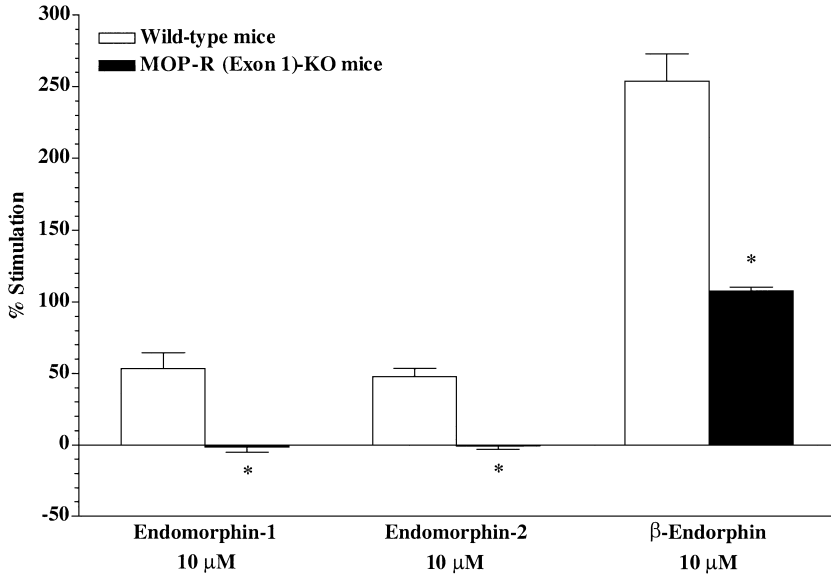

Fig. 5. μ-Opioid receptor (exon 1)-knockout mouse. A: Scatchard plot analysis of [^{3}H]DAMGO binding to membranes from the pons/medulla of wild-type and knockout (MOP-R-KO) mice. Membranes were incubated with [^{3}H]DAMGO at 0.2 – 20 nM for 2 h at 25°C. The specific binding was defined as the difference in binding observed in the absence and presence of 10 μM unlabeled naloxone. A representative experiment that was replicated three times is shown. n.d.: not detected. B: The increase of [^{35}S]GTPγS binding induced by EM-1, EM-2 and β-endorphin in the pons/medulla membrane obtained from wild-type mice and knockout mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP in the absence/presence of opioid peptides for 2 h at 25°C. Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of agonist and represent the mean ± S.E.M. from at least three independent experiments. *P<0.05 vs Wild-type mice.

likely that the development of other MOP-R knockout mice with a disruption of another exon (exon 4 to exon 10) of MOP-R gene may provide new insights into the distinct mechanisms underlying G-protein activation induced by EMs and β-endorphin.

G-Protein activation by endogenous μ-opioid peptides in the MOP-R subtype knockdown mouse

Upon cloning of the MOP-R and identification of its splicing variants, the MOP-R had been further classified into the putative μ_1- and μ_2-opioid receptors (MOP1-R, MOP2-R) (34, 35). The putative MOP1-R shows a high affinity for both opioid peptides and opioid alkaloids. In contrast, the putative MOP2-R has a higher affinity for opioid alkaloids than for opioid peptides. The CXBK mice, recombinant inbred mice of the C57BL/6ByJ and BALB/cByJ strains, have been known as putative MOP1-R knockdown mice and used as an experimental tool for identification of putative MOP1-R- or MOP2-R-mediated physiological responses. In the receptor binding assay, CXBK mice reveal lower density of putative MOP1-Rs without showing a concomitant decrease in putative

MOP2-Rs in several brain regions (36). It is of interest to note that MOP-R agonists show weaker analgesic effects and less locomotor stimulation in the CXBK mice as compared to C57BL/6ByJ mice (37).

We have demonstrated that the differences between EMs and β-endorphin on the G-protein activation in the CXBK mice (38). The increases of [^{35}S]GTPγS binding induced by EM-1 and EM-2 in the pons/medulla membrane obtained from CXBK mice are approximately 0.6-fold lower than those in the pons/medulla membrane obtained from C57BL/6ByJ mice. The increase of [^{35}S]GTPγS binding induced by EMs are completely blocked by β-FNA in both strains. More interestingly, there is no difference in the β-endorphin-induced increase of [^{35}S]GTPγS binding to the pons/medulla membrane between CXBK and C57BL/6ByJ mice. Taken together with the data that the increase of [^{35}S]GTPγS binding induced by β-endorphin is partially attenuated in mice lacking MOP-R gene, it is most likely that G-protein activation by β-endorphin is, at least in part, mediated by the stimulation of the putative MOP2-R, but not by the putative MOP1-R.

Since a new classification of MOP-R has been proposed based on the splicing variance of MOP-R gene (26, 27), the old classification of MOP-R (putative MOP1-R and putative MOP2-R) is likely to be getting inefficient and inaccurate. At the present, it is unclear which new classified opioid receptor subtypes correlates to the putative MOP1-R and putative MOP2-R. Further sutudies using the molecular approach will help to identify the existence of MOP-R subtypes involved in the differential role of EMs and β-endorphin.

Conclusion

A growing body of evidence indicates a certain difference between both endogenous μ-opioid peptides EMs and β-endorphin. Several recent studies have demonstrated that these differences may result from the MOP-R subtype. Further studies on the identification of the MOP-R subtypes for each endogenous μ-opioid peptides may provide valuable in furthering our understanding of the physiological function of the endogenous opioidergic system and pave the way for new therapeutic strategies for the control of pain.

REFERENCES

1 Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA and Morris HR: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature **258**, 577 – 579 (1975)

2 Tseng L-F, Loh HH and Li CH: β-Endorphin as a potent analgesic by intravenous injection. Nature **263**, 239 – 240 (1976)

3 Goldstein A, Tachibana S, Lowney LI, Hunkapiller M and Hood L: Dynorphin (1-13), an extraordinarily potent opioid peptide. Proc Natl Acad Sci USA **76**, 6666 – 6670 (1979)

4 Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI and Reisine T: Pharmacological characterization of the cloned κ-, δ-, and μ-opioid receptors. Mol Pharmacol **45**, 330 – 334 (1994)

5 Reisine T and Pasternak GW: Opioid analgesics and antagonists. *In* Goodman and Gilman's: The Pharmacological Basis of Therapeutics, Edited by Hardman JG and Limbird LE, pp 521 – 556, McGraw-Hill, New York (1996)

6 Nock B: κ and ε opioid receptor binding. *In* The Pharmacology of Opioid Peptides, Edited by Tseng LF, pp 29 – 56, Harwood Academic Publishers, Singapore (1995)

7 Tseng LF: Mechanisms of β-endorphin-induced antinociception. *In* The Pharmacology of Opioid Peptides, Edited by Tseng LF, pp 249 – 269, Harwood Academic Publishers, Singapore (1995)

8 Zadina J, Hackler L, Ge L-J and Kastin AJ: A potent and selective endogenous agonist for the μ-opioid receptor. Nature **386**, 499 – 502 (1997)

9 Stone LS, Fairbanks CA, Laughlin TM, Nguyen HO, Bushy TM, Wessendorf MW and Wilcox GR: Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2. NeuroReport **8**, 3131 – 3135 (1997)

10 Tseng LF: Recent advances in the search for the μ-opioidergic system: the antinociceptive properties of endomorphin-1 and endomorphin-2 in the mouse. Jpn J Pharmacol **89**, 216 – 220 (2002)

11 Chen Y, Mestek A, Liu J, Hurley A and Yu L: Molecular cloning and functional expression of a μ-opioid receptor from rat brain. Mol Pharmacol **44**, 8 – 12 (1993)

12 Traynor JR and Nahorski SR: Modulation by μ-opioid agonist of guanosine-5'-*O*-(3-[^{35}S]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. Mol Pharmacol **47**, 848 – 854 (1995)

13 Breivogel CS, Selley DE and Childers SR: Acute and chronic effects of opioids on δ and μ receptor activation of G proteins in NG108-15 and SK-N-SH cell membranes. J Neurochem **68**, 1462 – 1472 (1997)

14 Alt A, Mansour A, Akil H, Medzihradsky F, Traynor JR and Woods JH: Stimulation of guanosine-5'-*O*-(3-[^{35}S]thio)triphosphate binding by endogenous opioids acting at a cloned mu receptor. J Pharmacol Exp Ther **286**, 282 – 288 (1998)

15 Selley DE, Sim LJ, Xiao R, Liu Q and Childers SR: μ-Opioid receptor-stimulated guanosine-5'-*O*-(γ-thio)-triphosphate binding in rat thalamus and cultured cell lines: signal transduction mechanisms underlying agonist efficacy. Mol Pharmacol **51**, 87 – 96 (1997)

16 Sim LJ, Selley DE, Dworkin SI and Childers SR: Effects of chronic morphine administration on μ opioid receptor-stimulated [^{35}S]GTPγS autoradiography in rat brain. J Neurosci **16**, 2684 – 2692 (1996)

17 Selley DE, Nestler EJ, Breivogel CS and Childers SR: Opioid receptor-coupled G-protein in rat locus coeruleus membranes: decrease in activity after chronic morphine treatment. Brain Res **746**, 10 – 18 (1997)

18 Narita M, Mizoguchi H, Oji GS, Tseng EL, Suganuma C, Nagase H and Tseng LF: Characterization of endomorphin-1 and -2 on [^{35}S]GTPγS binding in the mouse spinal cord. Eur J Pharmacol **351**, 383 – 387 (1998)

19 Mizoguchi H, Narita M, Nagase H and Tseng LF: Activation of G-proteins in the mouse pons/medulla by β-endorphin is mediated by the stimulation of μ- and ε-receptors. Life Sci **67**,

2733 – 2743 (2000)

20 Mizoguchi H, Narita M, Oji DE, Suganuma C, Nagase H, Sora I, Uhl GR, Cheng EY and Tseng LF: The μ-opioid receptor gene-dose dependent reductions in G-protein activation in the pons/medulla and antinociception induced by endomorphins in μ-opioid receptor knockout mice. Neuroscience **94**, 203 – 207 (1999)

21 Narita M, Mizoguchi H, Narita M, Dun NJ, Hwang BH, Endoh T, Suzuki T, Nagase H, Suzuki T and Tseng LF: G-protein activation by endomorphins in the mouse periaqueductal gray matter. J Biomed Sci **7**, 221 – 225 (2000)

22 Zhu J, Luo L-Y, Li J-G, Chen C and Liu-Chen L-Y: Activation of the cloned human kappa opioid receptor by agonists enhances [^{35}S]GTPγS binding to membranes: determination of potencies and efficacies of ligands. J Pharmacol Exp Ther **282**, 676 – 684 (1997)

23 Mizoguchi H, Wu H-E and Narita M: Partial agonistic action of endomorphins in the mouse spinal cord. Neurosci Lett **310**, 66 – 68 (2001)

24 Chen Y, Mestek A, Liu J, Hurley A and Yu L: Molecular cloning and functional expression of a μ-opioid receptor from rat brain. Mol Pharmacol **44**, 8 – 12 (1993)

25 Wang JB, Imai Y, Eppler CM, Gregor P, Spivak CE and Uhl GR: μ opiate receptor: cDNA cloning and expression. Proc Natl Acad Sci USA **90**, 10230 – 10234 (1993)

26 Pasternak GW: Incomplete cross tolerance and multiple mu opioid peptide receptors. Trends Pharmacol Sci **22**, 67 – 70 (2001)

27 Pasternak GW: Insights into mu opioid pharmacology: the role of mu opioid receptor subtypes. Life Sci **68**, 2213 – 2219 (2001)

28 Matthes HWD, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le MM, Dolle P, Tzavara E, Hanoune J, Roques BP and Kieffer BL: Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature **383**, 819 – 823 (1996)

29 Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, Miner LL and Uhl GR: Opiate receptor knockout mice define μ receptor roles in endogenous nociceptive responses and morphine-induced analgesia. Proc Natl Acad Sci USA **94**, 1544 – 1549 (1997)

30 Loh HH, Liu HC, Cavalli A, Yang WL, Chen YF and Wei LN: μ opioid receptor knockout in mice: effects on ligand-induced analgesia and morphine lethality. Mol Brain Res **54**, 321 – 326 (1998)

31 Schuller AG, King MA, Zhang J, Bolan E, Pan YX, Morgan DJ, Chang A, Czick ME, Unterwald EM, Pasternak GW and Pintar JE: Retention of heroin and morphine-6β-glucuronide analgesia in a new line of mice lacking exon 1 of MOR-1. Nat Neurosci **2**, 151 – 156 (1999)

32 Narita M, Mizoguchi H, Narita M, Sora I, Uhl GR and Tseng LF: Absence of G-protein activation by μ-opioid receptor agonists in the spinal cord of μ-opioid receptor knockout mice. Br J Pharmacol **126**, 451 – 456 (1999)

33 Mizoguchi H, Wu H-E, Narita M, Hall FS, Sora I, Uhl GR, Nagase H and Tseng LF: Antagonistic property of buprenorphine for putative ε-opioid receptor-mediated G-protein activation by β-endorphin in pons/medulla of the μ-opioid receptor knockout mouse. Neuroscience (in press)

34 Pasternak GW, Childers SR and Snyder SH: Opiate analgesia: evidence for mediation by a subpopulation of opiate receptors. Science **208**, 514 – 516 (1980)

35 Wolozin BL and Pasternak GW: Classification of multiple morphine and enkephalin binding sites in the central nervous system. Proc Natl Acad Sci USA **78**, 6181 – 6185 (1981)

36 Moskowitz AS and Goodman R: Autoradiographic analysis of MU_1, MU_2, and delta opioid binding in the central nervous system of C57BL/6BY and CXBK (opioid receptor-deficient) mice. Brain Res **360**, 108 – 116 (1985)

37 Moskowitz AS, Terman GW, Carter KR, Morgan MJ and Liebeskind JC: Analgesic, locomotor and lethal effects of morphine in the mouse: strain comparisons. Brain Res **361**, 46 – 51 (1985)

38 Mizoguchi H, Narita M, Wu H-E, Narita M, Suzuki T, Nagase H and Tseng LF: Differential involvement of μ_1-opioid receptors in endomorphin- and β-endorphin-induced G-protein activation in the mouse pons/medulla. Neuroscience **100**, 835 – 839 (2000)