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Paradoxical hyperalgesia induced by μ -opioid agonist endomorphin-2, but not endomorphin-1, microinjected into the centromedial amygdala of the rat

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Abstract

The effects of endomorphin-2 or endomorphin-1 microinjected into the centromedial amygdala on the thermally induced tail-flick response were studied in male CD rats. Microinjection of endomorphin-2 (8.7–35.0 nmol) given into the centromedial amygdala time- and dose-dependently decreased the tail-flick latencies. On the other hand, endomorphin-1 (8–32.6 nmol) given into the same site did not cause any change of the tail-flick latency. However, endomorphin-1 (32.6 nmol) or endomorphin-2 (35.0 nmol) given into the basolateral site of amygdala did not affect the tail-flick latency. Pretreatment with the antiserum against dynorphin A(1–17) (200 μ g) significantly reversed the decrease of the tail-flick latency induced by endomorphin-2. The decrease of the tail-flick latency induced by endomorphin-2 was also blocked by the endomorphin-2 selective μ -opioid receptor antagonist 3-methoxynaltrexone (6.4 pmol) and by the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (30 nmol), but not by the κ -opioid receptor antagonist nor-binaltorphimine (6.6 nmol). It is concluded that endomorphin-2, but not endomorphin-1, given into the centromedial amygdala stimulates a 3-methoxynaltrexone-sensitive μ -opioid receptor subtype to induce the release of dynorphin A(1–17), which then acts on the NMDA receptor, but not κ -opioid receptor for producing hyperalgesia. This conclusion is further supported by the additional findings that dynorphin A(1–17) (2.3 nmol) given into the centromedial amygdala also caused the decrease of the tail-flick latency, which was similarly blocked by the NMDA receptor antagonist MK-801 (30 nmol), but not κ -opioid receptor antagonist nor-binaltorphimine (6.6 nmol).

Keywords

opioid; hyperalgesia; amygdala; brain; rat

1. Introduction

The amygdala plays a central role in the interaction of sensory information, especially pain-related behavior (Pitkanen et al., 1997). The endogenous μ opioids involved in the stress-induced analgesia are probably produced within the amygdala complex, especially the

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central amygdaloid nucleus and stria terminalis (Roberts et al., 1982; da Costa Gomex and Behbehani, 1995; Wiedenmayer et al., 2002). The central amygdaloid nucleus is an important site for pain perception and analgesia produced by opioids through the projection to the periaqueductal gray (Finnergan et al., 2004). The central amygdaloid nucleus receives afferent inputs from the spinal cord dorsal horn and parabrachial nucleus (Bernard et al., 1993; Burstein and Potrebic, 1993). The spinopontoamygdaloid pathway has been shown to specifically transmit nociceptive information (Bernard and Besson, 1990; Bester et al., 2000). Also, this amygdaloid nucleus contains endogenous opioid peptides and all their opioid receptors, including the endomorphin-1 and endomorphin-2 and μ -opioid receptors (Martin-Schild et al., 1999; Paden et al., 1987). The nociceptive threshold is increased following the microinjection of morphine and other μ -opioid agonists into the central and basolateral nuclei (Helmstetter et al., 1993; Rodgers, 1977; 1978). Furthermore, lesions placed in the amygdala reduce the magnitude of systemic morphine analgesia (Manning and Mayer, 1995). Morphine analgesia elicited from the basolateral amygdala appears to be mediated by μ -, but not δ - or κ -opioid receptors (Helmstetter et al., 1995). Thus, the central amygdala may play an important role in both descending pain facilitating and pain inhibitory pathways (Basbaum and Fields, 1984).

We have previously demonstrated that the endogenous μ -opioid peptides endomorphin-1 or endomorphin-2 given into the spinal cord or supraspinally produces antinociception (Tseng et al., 2000; Ohsawa et al., 2000, 2001). However, the antinociceptive effects produced by endomorphin-1 and endomorphin-2 are mediated by the stimulation of different subtypes of μ -opioid receptors. This view is evidenced by the finding that μ -opioid receptor antagonists, 3-methoxynaltrexone blocks the antinociception produced by endomorphin-2, but not endomorphin-1 (Sakurada et al., 2000). Intrathecal treatment with antisense oligodeoxynucleotides against specific exons of the μ -opioid receptor clone leads to a differential loss of antinociception produced by endomorphin-1 and endomorphin-2 (Wu et al., 2002). The analgesic effects produced by endomorphin-2, but not endomorphin-1, is mediated in part by the release of dynorphin acting on the κ -opioid receptors (Tseng et al., 2000; Mizoguchi et al., 2000; Sakurada et al., 2001).

The effect of endomorphin-1 and endomorphin-2 from the central amygdala on the thermally induced nociceptive response has not been studied. The present study was then taken to determine the effects of endomorphin-1 and endomorphin-2 given into the centromedial amygdala on the thermal tail-flick response. We unexpectedly found that the endomorphin-2, but not endomorphin-1, given into the centromedial amygdala produced a decrease of the tail-flick latency. The hyperalgesia induced by endomorphin-2 from the centromedial amygdala was found to be mediated by the release of dynorphin A(1–17) acting on the NMDA receptors, but not κ -opioid receptors.

2. Materials and Methods

2.1. Animals

Male CD rats weighing 300–350 g (Charles River Breeding Laboratories, Wilmington, MA) were used. Animals were housed two per cage in a room maintained at 22 ± 0.5 °C with an alternating 12-h light/dark cycle. Food and water were available *ad libitum*. Animals were used only once. All experiments were approved by and conformed to the guidelines of the Medical College of Wisconsin Animal Care Committee.

2.2. Surgical procedures

Rats were pretreated with methylatropine bromide (5 mg/kg intraperitoneally), anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally) and mounted in a stereotaxic

apparatus (David Kopf instruments, Tujunga, CA). A 23-gauge stainless steel guide cannula 12 mm in length was then implanted unilaterally 3 mm down from the surface of the skull and anchored to the skull with three stainless screws and dental cement. The coordinates for placement of the cannula aiming for centromedial amygdala microinjection were AP -1.88 to -2.00 mm posterior to the bregma and 4.0 mm lateral to the midline; for basolateral amygdala microinjection sites were AP -2.30 to -2.80 mm posterior to the bregma and 4.8 mm lateral to the midline (Paxinos and Watson, 1997). After a recovery period of at least 5 days, animals were used for the experiments.

2.3. Assessment of analgesia

Analgesic responses were measured with the tail-flick test (D'Amour and Smith, 1941). To measure the latency of the tail-flick response, rats were gently held by hand and their tail positioned on the apparatus (Model TF6; EMDIE Instrument Co., Maidens, VA). The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of the heat stimulus was set to provide a pre-drug tail-flick response time of 3 to 4 s. The cutoff time was set at 10 s to minimize tissue damage.

2.4. Drugs and drug administration

Endomorphin-2 (Tyr-Pro- Phe-Phe-NH₂) and endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) were purchased from Calbiochem (La Jolla, CA). Other drugs used were 3-Methoxynaltrexone hydrochloride (3-MNX), (5R,10S)-(+)-5-Methyl-10,11-dihydro 5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), dynorphin A(1–17) (Sigma, St. Louis, MO) and nor-binaltorphimine (nor-BNI) (National Institute of Drug Abuse, Baltimore, MD). The endomorphin-2, endomorphin-1, 3-MNX, MK-801, nor-BNI and dynorphin A(1–17) were dissolved in sterile saline solution (0.9% NaCl solution) (Terashvili et al., 2005). The antiserum against dynorphin A(1–17) was produced by immunization of male New Zealand white rabbits according to the method described in previous publications. The potencies and cross-immunoreactivities of these antisera have been characterized (Tseng and Huang, 1992; Tseng and Collins, 1993; Wu et al., 2002, 2004). The injections into the centromedial and basolateral amygdala were made by hand with a 30-gauge injection needle attached to a microsyringe via polyethylene tubing. The injection needle was inserted directly into the guide cannula and the injection volume was 0.5 µl, which was administered over a 30 s period. The injection needle was left in place for an additional 60 s to ensure complete distribution. The stereotaxic coordinates of the centromedial amygdala injection site were aimed at AP-1.88 to -2.00 mm posterior to the bregma and 4.0 mm lateral to the midline and 8.0 mm down from the surface of the skull; for the basolateral amygdala microinjection were AP-2.30 to -2.80 mm posterior to the bregma, 4.8 mm lateral to the midline and 8.7 mm down from the surface of the skull (Paxinos and Watson, 1997).

2.5. Histological identification of the injection site

At the end of the experiments, rats were injected with 0.5 µl of methylene blue solution (2%) to mark the injection site and were then sacrificed with CO₂. The brains were removed, frozen and sectioned sagittally for microscopic identification of the injection sites. The stereotaxic atlas of rats by Paxinos and Watson (1997) was used as a guide for the identification of anatomical injection sites. Only the data obtained from rats, in which the injection sites were accurately identified to be in the intended centromedial or basolateral amygdala were used for further statistical analysis. Histological examination verified that all the injection sites intended for the centromedial and basolateral amygdala were within the intended region of the brain sites (Fig 1).

2.7. Statistical Analysis

The analgesic or hyperalgesic responses (tail-flick latencies) were presented as the mean $\pm$ S.E.M. Two-way analysis of variance (ANOVA) followed by Bonferroni's post-tests, one-way ANOVA followed by Dunnett's post test or unpaired Student's *t* test was used to test the differences between groups. The GraphPad Prism software was used to perform the statistics (version 4.1; GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Effects of endomorphin-2 or endomorphin-1 microinjected into the centromedial or basolateral amygdala on the thermally-induced tail-flick response

Groups of rats were microinjected with different doses of endomorphin-2 (8.7–35 nmol), endomorphin-1 (8.1–32.6 nmol), or vehicle into the centromedial or basolateral amygdala and the tail-flick response was then measured at different times thereafter. Endomorphin-2 (8.7–35.0 nmol) microinjected into the centromedial amygdala time- and dose-dependently inhibited the tail-flick latencies; the decrease of the tail-flick latency developed in 5 to 10 min, reached a maximal effect in 30 to 40 min and returned to the control level 90 to 120 min after endomorphin-2 injection (Fig 2A). On the other hand, endomorphin-1 microinjected into the same centromedial amygdala did not cause any significant change of the tail-flick latency (Fig 2B). Thus, endomorphin-2, but not endomorphin-1, given into the centromedial amygdala selectively produces hyperalgesia. The dose of 35.0 nmol of endomorphin-2 was then chosen for the following experiments.

To determine if another opioid sensitive site in amygdala, basolateral amygdala, is also sensitive to endomorphin-2 or endomorphin-1 for producing hyperalgesia or antinociception, the effects of endomorphin-2 and endomorphin-1 microinjected into basolateral amygdala on the tail-flick response were also studied. Microinjection of either endomorphin-1 (32.6 nmol) or endomorphin-2 (35.0 nmol) microinjected into the basolateral amygdala did not produce any effect (Fig 3).

3.2. Effects of the pretreatment with antiserum against dynorphin A(1–17) on the decrease of the tail-flick latency induced by endomorphin-2

We have previously demonstrated that endomorphin-2 treatment releases dynorphin A(1–17) and the antinociception induced by endomorphin-2 is mediated by the release of dynorphin A(1–17) in the spinal cord (Leitermann et al., 2004; Tseng et al., 2000; Mizoguchi et al., 2006). Antiserum against dynorphin A(1–17) was used to determine if the endomorphin-2-induced hyperalgesia is mediated by the release of dynorphin A(1–17). Groups of rats were pretreated with different doses of antiserum against dynorphin A(1–17) (20–200 μ g) given into the centromedial amygdala 1 h before administration of endomorphin-2 (35 nmol). The tail-flick response was measured 30 min after endomorphin-2 injection. Pretreatment with antiserum against dynorphin A(1–17) at a dose of 200 μ g, but not 20 or 60 μ g, significantly reversed the decrease of the tail-flick latency induced by endomorphin-2 (Fig 4). On the other hand, pretreatment with the normal rabbit serum at the same dose (20–200 μ g) did not affect the decreased tail-flick latency induced by endomorphin-2 (Fig 4).

3.3. Effects of the pretreatment with 3-methoxynaltrexone, nor-BNI or MK-801 on the decreased tail-flick response to endomorphin-2

We have previously demonstrated that endomorphin-2 stimulates the 3-methoxynaltrexone-sensitive μ -opioid receptor subtype to cause the release of dynorphin A(1–17), which then acts on opioid κ -opioid receptors for producing antinociception from the mouse spinal cord (Tseng et al., 2000, Ohsawa et al., 2000, 2001, Sakurada et al., 2001). However, the

hyperalgesia of dynorphin A (1–17) may require activation of the NMDA receptor complex (Shukla and Lemaire, 1994). The experiments were undertaken to determine if the endomorphin-2 acts on the same subtype of μ -opioid receptors for the release of dynorphin A(1–17) and the released dynorphin A(1–17) acts on opioid κ -opioid or NMDA receptors for producing hyperalgesia from centromedial amygdala. Pretreatment with endomorphin-2-sensitive μ -opioid receptor antagonist 3-methoxynaltrexone (6.4 pmol) effectively reversed the decrease of the tail-flick latency induced by endomorphin-2 (35 nmol). Similarly, pretreatment with NMDA receptor antagonist MK-801 (30 nmol) also reversed the decrease of the tail-flick latency induced by endomorphin-2. However, pretreatment with the selective κ -opioid receptor antagonist nor-BNI (6.6 nmol) did not affect the decreased tail-flick latency induced by endomorphin-2. Pretreatment with MK801 or nor-BNI alone did not affect the baseline tail-flick latency (Fig 5).

3.4. Effects of dynorphin A(1–17) microinjected into the centromedial amygdala on the thermally-induced tail-flick response

The results of the experiments described above indicate that endomorphin-2 given into centromedial amygdala releases dynorphin A(1–17). Thus, microinjection of dynorphin A(1–17) itself given into the same centromedial amygdala would mimic the effect of endomorphin-2 to produce hyperalgesia. The effect of dynorphin A (1–17) given into the centromedial amygdala on the tail-flick response was then studied. Groups of rats were microinjected with different doses of dynorphin A (1–17) (0.23–2.3 nmol) or vehicle into the centromedial amygdala and the tail-flick response was then measured 10 min thereafter. Dynorphin A(1–17) (0.23–2.3 nmol) microinjected into the centromedial amygdala dose-dependently caused the decrease of the tail-flick latency (Fig 6). Thus, like endomorphin-2, dynorphin A(1–17) given into the same centromedial amygdala produces hyperalgesia.

3.5. Effects of nor-BNI or MK-801 on the decrease of the tail-flick response to dynorphin A(1–17)

The experiments were then performed to determine if the hyperalgesia induced by dynorphin A(1–17) is mediated by the stimulation of NMDA receptor or κ -opioid receptors. Groups of rats were pretreated with the NMDA receptor antagonist MK-801 (30 nmol) or the selective κ -opioid receptor antagonist nor-BNI (6.6 nmol). Pretreatment with MK-801 significantly reversed the decrease of the tail-flick latency induced by dynorphin A(1–17). However, pretreatment with the selective κ -opioid receptor antagonist nor-BNI (6.6 nmol) did not affect the decreased tail-flick latency induced by dynorphin A(1–17) (Fig 7).

4. Discussions

4.1 Endomorphin-1 or endomorphin-2 given into the centromedial and basolateral amygdala does not produce antinociception

Microinjection of μ -opioid receptor agonists such as [D-Ala²-N-methyl-Phe⁴, Gly-OI⁵]-enkephalin or morphine given into the amygdala has been reported to produce antinociception (analgesia) (Helmstetter et al., 1993, 1995; Rodgers, 1977, 1978). In as much as the antinociception induced by either endomorphin-1 or endomorphin-2 given supraspinally is mediated by the stimulation of μ -opioid receptors, it was speculated that both endomorphin-1 or endomorphin-2 would also utilize the same descending pain control pathways as that of other μ -opioid agonists such as morphine and [D-Ala²-N-methyl-Phe⁴, Gly-OI⁵]-enkephalin to produce antinociception. Paradoxically, we found in the present study that the selective μ -opioid ligand endomorphin-1 given into the centromedial amygdala did not produce any antinociception and endomorphin-2 given into the same site produces hyperalgesia. However, both endomorphin-1 and endomorphin-2 microinjected into the basolateral amygdala did not produce any antinociception or hyperalgesia. The reason for

the failure of endomorphin-1 to produce any antinociception from the centromedial or basolateral amygdala is not clear. Our discussion will focus on the neural mechanism of hyperalgesia induced by endomorphin-2 from centromedial amygdala.

4.2. The hyperalgesia induced by endomorphin-2 is mediated by the stimulation of a 3-methoxynaltrexone-sensitive μ -opioid receptor subtype

Endomorphin-1 or endomorphin-2 given intrathecally or intracerebroventricularly in mice produces antinociception. However, the antinociceptive effects produced by endomorphin-1 and endomorphin-2 are mediated by the stimulation of different subtypes of μ -opioid receptors (Tseng, 2002). This is evidenced by the finding that pretreatment with the μ -opioid receptor antagonist 3-methoxynaltrexone selectively inhibits the antinociception induced by endomorphin-2, but not endomorphin-1, whereas β -funaltrexamine inhibits both (Sakurada et al, 2000). Pretreatment with endomorphin-1 or endomorphin-2 given intrathecally or intracerebral periaqueductal gray attenuates the antinociception produced by morphine. The phenomenon has been defined as antianalgesia. The antianalgesia induced by endomorphin-2, but not endomorphin-1, is blocked by the pretreatment with 3-methoxynaltrexone (Terashvili et al., 2005; Wu et al., 2003). We found in the present study that the hyperalgesia induced by endomorphin-2 given into the centromedial amygdala was blocked by the pretreatment with 3-methoxynaltrexone. The findings indicate that these pharmacological effects induced by endomorphin-2 are mediated by the stimulation of the same 3-methoxynaltrexone-sensitive μ -opioid receptor subtype.

4.3. Dynorphinegic mechanism for endomorphin-2-induced hyperalgesia from the centromedial amygdala

We found in the present study that endomorphin-2 given into the centromedial amygdala produced hyperalgesia, but not antinociception (analgesia). The hyperalgesia produced by endomorphin-2 is blocked by the pretreatment with antiserum against dynorphin A(1–17), indicating that the effect is mediated by the release of dynorphin A(1–17). The finding is consistent with the previous findings that the analgesia and antianalgesia induced by endomorphin-2 from spinal cord or periaqueductal gray, respectively, are also mediated by the release of dynorphin A(1–17) (Tseng et al., 2000; Ohsawa et al., 2000, 2001; Sakurada et al., 2001; Wu et al., 2003). In a biochemical study, the release of dynorphin A(1–17) by endomorphin-2 from the rat spinal cord has been demonstrated (Leitermann et al., 2004). Thus, if the hyperalgesia induced by endomorphin-2 is mediated by the release of dynorphin A(1–17), the administration of dynorphin A(1–17) itself into the same centromedial amygdala would mimic the effect of endomorphin-2 to produce hyperalgesia as well. We found in the present study that dynorphin A(1–17) given into same site as endomorphin-2 also produced hyperalgesia. Thus, dynorphin A(1–17) either endogenously released by endomorphin-2 or exogenously given into centromedial amygdala produces hyperalgesia.

4.4. Both hyperalgesia induced by endomorphin-2 and dynorphin A(1–17) are mediated by NMDA receptors, but not κ opioid receptors

We found in the present study that the hyperalgesia induced by endomorphin-2 given into the centromedial amygdala is blocked by NMDA receptor antagonist MK-801, but not κ -opioid receptor antagonist nor-BNI, indicating that the NMDA but not κ -opioid receptors are involved in hyperalgesia induced by endomorphin-2 from the centromedial amygdala. Similarly, the hyperalgesia induced by dynorphin A(1–17) given into the same centromedial amygdala was also blocked by MK801 but not nor-BNI. Thus, both hyperalgesic effects induced by endomorphin-2 and dynorphin A(1–17) are similarly mediated by the stimulation of NMDA receptors, but not κ -opioid receptors.

Although dynorphin A(1–17) is an endogenous opioid peptide, the nociceptive actions of dynorphin A(1–17) requires activation of the NMDA receptors (Shukla and Lemaire, 1994). Allodynia is a painful sensation elicited by a stimulation that does not normally provoke pain. When induced by exogenous dynorphin, allodynia, like hyperalgesia, is inhibited by intrathecal pretreatment with NMDA receptor antagonists MK801 and LY235959, a competitive NMDA receptor antagonist, but is unaffected by the opioid receptor antagonist naloxone (Vanderah et al., 1996; Laughlin et al., 1997). Dynorphin interact directly with the NMDA receptor complex in the in vitro receptor binding studies (Massardier and Hunt, 1989). Here, dynorphin has two opposing actions on NMDA receptor ligands: potentiating binding of competitive antagonists at the glutamate recognition site but inhibiting binding of non-competitive antagonists, such as MK801, within the ion channel (Shukla et al., 1997). Our present findings are consistent with previous studies that dynorphin acts directly on NMDA receptors and the inhibitory action of dynorphin cannot be blocked by the non-selective opioid receptor antagonist naloxone, or by the specific κ -opioid receptor antagonist nor-BNI (Chen et al., 1995; Caudle and Dubner, 1998).

Thus, endomorphin-2 can induce either analgesia or hyperalgesia depending on the brain sites injected. Endomorphin-2 microinjected into the centromedial but not from basolateral amygdala induces hyperalgesia. The hyperalgesia induced by endomorphin-2 is mediated by the stimulation of a selective μ -opioid receptor subtype, which subsequently induces the release of dynorphin A(1–17) acting on NMDA receptor, but not κ -opioid receptor.

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References

- Basbaum AI, Fields HL. Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. *Annu. Rev. Neurosci.* 1984; 7:309–338. [PubMed: 6143527]
- Bernard JF, Besson JM. The spino(trigemino)pontoamygdaloid pathway : electrophysiological evidence for an involvement in pain processes. *J. Neurophysiol.* 1990; 63:473–490. [PubMed: 2329357]
- Bernard JF, Alden M, Besson JM. The organization of the efferent projection from the pontine parabrachial area to the amygdala complex: a *phaseolus vulgaris* leucoagglutinin (PHA-L) study in the rat. *J. Comp. Neurol.* 1993; 329:201–229. [PubMed: 8454730]
- Bester H, Chapman V, Besson JM, Bernard JF. Physiological properties of the lamina I spinoparabrachial neurons in the rat. *J. Physiology.* 2000; 83:2239–2259.
- Burstein R, Potrebic S. Retrograde labeling of neurons in the spinal cord that project directly to the amygdala or the orbital cortex in the rat. *J. Comp. Neurol.* 1993; 335:469–485. [PubMed: 8227531]
- Caudle RM, Dubner R. Ifenprodil blocks the excitatory effects of the opioid peptide dynorphin 1–17 on NMDA receptor-mediated currents in the CA3 region of the guinea pig hippocampus. *Neuropeptides.* 1998; 32:87–95. [PubMed: 9571650]
- Chen L, Gu Y, Huang LYM. The opioid peptide dynorphin directly blocks NMDA receptor channels in the rat. *Journal of Physiology.* 1995; 482.3:575–581. [PubMed: 7537820]
- Da Costa Gomez TM, Behbehani MM. An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. *Brain Res.* 1995; 689:21–31. [PubMed: 8528703]
- D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* 1941; 72:74–79.
- Finnegan TF, Chen SR, Pan HL. Effects of the μ opioid on excitatory and inhibitory synaptic inputs to periaqueductal gray-projecting neurons in the amygdala. *J. Pharmacol. Exp. Ther.* 2004; 312:441–448. [PubMed: 15388784]

- Helmstetter FJ, Bellgowan PSF, Poore LH. Microinfusion of mu but not delta or kappa opioid agonists into the basolateral amygdala results in inhibition of the tail-flick reflex in pentobarbital-anesthetized rats. *J. Pharmacol. Exp. Ther.* 1995; 275:381–388. [PubMed: 7562575]
- Helmstetter FJ, Bellgowan PS, Tershner SA. Inhibition of the tail-flick reflex following microinjection of morphine into the amygdala. *NeuroReport.* 1993; 4:471–474. [PubMed: 8513121]
- Laughlin TM, Vanderah T, Lashbrook JM, Nichols ML, Ossipov MH, Porreca F, Wilcox GL. Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors. *Pain.* 1997; 72:253–260. [PubMed: 9272810]
- Leitermann RJ, Terashvili M, Mizoguchi H, Wu HE, Chen F, Clithero A, Tseng LF. Increased release of immunoreactive dynorphin A(1–17) from the spinal cord after intrathecal treatment with endomorphin-2 in anesthetized rats. *Eur. J. Pharmacol.* 2004; 504:177–183. [PubMed: 15541419]
- Manning BH, Mayer DJ. The central nuclei of the amygdala contributes to the production of morphine antinociception in the tail-flick test. *J. Neurosci.* 1995; 15:8199–8213. [PubMed: 8613754]
- Martin-Schild S, Gerall AA, Kastin AJ, Zadina JE. Differential distribution of endomorphin-1 and endomorphin-2 like immunoreactivities in the CNS of the rodent. *J. Comp. Neurol.* 1999; 405:450–471. [PubMed: 10098939]
- Massardier D, Hunt PF. A direct non-opiate interaction of dynorphin-(1–13) with the N-methyl-D-aspartate (NMDA) receptor. *Eur. J. Pharmacol.* 1989; 170:125–126. [PubMed: 2575525]
- Mizoguchi H, Narita M, Wu H, Narita M, Suzuki T, Nagase H, Tseng LF. Differential involvement of μ 1-opioid receptors in endomorphin- and betaendorphin- induced G-protein activation in the mouse pons/medulla. *Neuroscience.* 2000; 100:835–839. [PubMed: 11036217]
- Mizoguchi H, Watanabe H, Hayashi T, Sakurada W, Sawai T, Fujimura T, Sakurada T, Sakurada S. Possible involvement of dynorphin A-(1–17) release via μ 1-opioid receptors in spinal antinociception by endomorphin-2. *J. Pharmacol. Exp. Ther.* 2006; 317:362–368. [PubMed: 16394196]
- Ohsawa M, Mizoguchi H, Narita M, Chu M, Nagase H, Tseng LF. Differential mechanisms mediating descending pain controls for antinociception induced by supraspinally-administered endomorphin-1 and endomorphin-2 in the mouse. *J. Pharmacol. Exp. Ther.* 2000; 294:1106–1111. [PubMed: 10945866]
- Ohsawa M, Mizoguchi H, Narita M, Nagase H, Kampine JP, Tseng LF. Differential antinociception induced by spinally administered endomorphin-1 and endomorphin-2 in the mouse. *J. Pharmacol. Exp. Ther.* 2001; 298:592–597. 2001. [PubMed: 11454920]
- Paden CM, Krall S, Lynch WC. Heterogenous distribution and upregulation of mu, delta and kappa opioid receptors in the amygdala. *Brain Res.* 1987; 418:349–355. [PubMed: 2823964]
- Paxinos, GW.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1997.
- Pitkanen A, Savander V, LeDoux JE. Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci.* 1997; 20:517–523. [PubMed: 9364666]
- Roberts GW, Woodhams PL, Polak JM, Crow TJ. Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. *Neuroscience.* 1982; 7:99–131. [PubMed: 6176906]
- Rodgers RJ. Elevation of aversive threshold in rats by intra-amygdala injection of morphine sulfate. *Pharmacol. Biochem. Behav.* 1977; 6:385–390. [PubMed: 882576]
- Rodgers RJ. Influence of intra-amygdaloid opiate injections on shock thresholds, tail-flick latencies and open field behaviour in rats. *Brain Res.* 1978; 153:211–390. [PubMed: 679048]
- Sakurada S, Hayashi T, Yuhki M, Fujimura T, Murayama K, Yonezawa A, Sakurada C, Takeshita M, Zadina JE, Kastin AJ. Differential antagonism of endomorphin-1 and endomorphin-2 spinal antinociception by naloxonazine and 3-methoxynaltrexone. *Brain Res.* 2000; 88:1–8. [PubMed: 11033087]
- Sakurada S, Hayashi T, Yuhki M, Orito T, Zadina JE, Kastin AJ, Fujimura T, Murayama K, Sakurada C, Sakurada T, Narita M, Suzuki T, Tan-No K, Tseng LF. Differential antinociceptive effects induced by intrathecally administered endomorphin-1 and endomorphin-2 in the mouse. *Eur. J. Pharmacol.* 2001; 427:203–210. [PubMed: 11567650]
- Shukla VK, Lemaire S. Non-opioid effects of dynorphins: possible role of the NMDA receptor. *Trends Pharmacol. Sci.* 1994; 15:420–424. [PubMed: 7855907]

- Shukla VK, Prasad JA, Lemaire S. Nonopioid motor effects of dynorphin A and related peptides: structure dependence and role of the N-methyl-D-aspartate receptor. *J Pharmacol. Exp. Ther.* 1997; 283:604–610.
- Terashvili M, Wu HE, Leitermann RJ, Sun HS, Clithero AD, Tseng LF. Differential mechanisms of antianalgesia induced by endomorphin-1 and endomorphin-2 in the ventral periaqueductal gray of the rat. *J. Pharmacol. Exp. Ther.* 2005; 312:1257–1265. [PubMed: 15542622]
- Tseng LF. The antinociceptive properties of endomorphin-1 and endomorphin-2 in the mouse. *Jpn. J. Pharmacol.* 2002; 89:216–220. [PubMed: 12184724]
- Tseng LF, Collins KA. Spinal involvement of both dynorphin A and met-enkephalin in the antinociception induced by intracerebroventricularly administered bremazocine but not morphine in the mouse. *J. Pharmacol. Exp. Ther.* 1993; 266:1430–1438. [PubMed: 8103794]
- Tseng LF, Huang FY. Release of immunoreactive [Met5]enkephalin and cholecystokinin from the spinal cord by β -endorphin administered intraventricularly in the pentobarbital-anesthetized rat. *Eur. J. Pharmacol.* 1992; 215:309–312. [PubMed: 1396996]
- Tseng LF, Narita M, Suganuma C, Mizoguchi H, Ohsawa M, Nagase H, Kampine JP. Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse. *J. Pharmacol. Exp. Ther.* 2000; 292:576–583. [PubMed: 10640294]
- Vanderah T, Laughlin T, Lashbrook J, Nichols M, Wilcox G, Ossipov M, Malan T, Porreca F. Single intrathecal injections of dynorphin A or κ -dynorphins produce long-lasting allodynia in rats: blockade by MK801 but not naloxone. *Pain.* 1996; 68:275–281. [PubMed: 9121815]
- Wiedenmayer CP, Noailles PA, Angulo JA, Barr GA. Stress-induced preproenkephalin mRNA expression in the amygdala changes during early ontogeny in the rat. *Neuroscience.* 2002; 114:7–11. [PubMed: 12207949]
- Wu HE, Mizoguchi H, Terashvili M, Leitermann RJ, Hung KC, Fujimoto JM, Tseng LF. Spinal pretreatment with antisense oligodeoxynucleotides against exon-1, -2 or -8 of μ -opioid receptor clone leads to differential loss of spinal endomorphin-1 and endomorphin-2-induced antinociception in the mouse. *J. Pharmacol. Exp. Ther.* 2002; 303:867–873. [PubMed: 12388674]
- Wu HE, Sun HS, Darpolar M, Leitermann RJ, Kampine JP, Tseng LF. Dynorphinergic mechanism mediating endomorphin-2-induced antianalgesia in the mouse spinal cord. *J. Pharmacol. Exp. Ther.* 2003; 307:1135–1141. [PubMed: 14557378]
- Wu HE, Thompson J, Sun H, Leitermann RJ, Fujimoto JM, Tseng LF. Non-opioid mechanism mediating morphine-induced antianalgesia in the mouse spinal cord. 2004; 310:240–246.

Fig. 1.
Coronal sections of the atlas of Paxinos and Watson (1997) showing the injection sites for drugs and saline vehicle at the centromedial and basolateral amygdala.

Fig. 2.

Effect of endomorphin-1 and endomorphin-2 microinjected into the centromedial amygdala on the thermal tail-flick response. Groups of rats were microinjected with different doses of endomorphin-1 (8.0–32.6 nmol; A), endomorphin-2 (8.7–35.0 nmol; B) or vehicle (0.5 μ l) into the centromedial amygdala and the tail-flick responses were measured at different times thereafter. Two-way ANOVA followed by Bonferroni's post-test was used to test the difference between groups. For the group of rats injected with endomorphin-1 versus vehicle (A): interaction $F_{(27, 240)} = 0.77$, treatment $F_{(3, 240)} = 14.89$, time $F_{(9, 240)} = 7.56$. For the group of rats injected with endomorphin-2 versus vehicle (B): interaction $F_{(27, 270)} = 2.43$,

treatment $F_{(3, 270)} = 70.30$, time $F_{(9, 270)} = 9.39$; $N = 6$ to 8 rats in each group; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig. 3.

Effect of endomorphin-1 and endomorphin-2 microinjected into the basolateral amygdala on the thermal tail-flick response. Groups of rats were microinjected with endomorphin-1 (32.6 nmol), endomorphin-2 (35.0 nmol) or vehicle (0.5 μ l) into the basolateral amygdala and the tail-flick responses were measured at different times thereafter. Two-way ANOVA followed by Bonferroni's post-test was used to test the difference between groups. For the group of rats injected with endomorphin-1 versus vehicle into the basolateral amygdala: interaction $F_{(5, 66)} = 0.82$, treatment $F_{(1, 66)} = 2.05$, time $F_{(5, 66)} = 2.23$; For the group of rats injected with endomorphin-2 versus vehicle: interaction $F_{(5, 78)} = 0.48$, treatment $F_{(1, 78)} = 0.19$, time $F_{(5, 78)} = 2.58$; $N = 6$ to 8 rats in each group.

Fig. 4.

Pretreatment with antiserum against dynorphin A(1–17) (A/S Dyn) blocks the hyperalgesia induced by endomorphin-2 from the centromedial amygdala. Groups of rats were pretreated with different doses (20 to 200 µg) of A/S Dyn or normal rabbit serum given into the centromedial amygdala 1 h before microinjection of endomorphin-2 into the centromedial amygdala and the tail-flick responses were measured 30 min after endomorphin-2 injections. Each column represents the mean and the vertical bar represents the S.E.M. with 6 to 8 rats in each group. Two-way ANOVA followed by Bonferroni's post-test was used to test the difference between groups. For rats pretreated with A/S Dyn versus vehicle followed by endomorphin-2 challenge, interaction $F_{(3, 53)} = 15.72$, treatment $F_{(1, 53)} = 0.004$, time $F_{(3, 53)} = 7.86$; * $p < 0.01$.

Fig. 5.

Effects of the pretreatment with 3-methoxynaltrexone (3-MNX), norbinaltorphimine (nor-BNI) or MK-801 on the hyperalgesia induced by endomorphin-2 from the centromedial amygdala. Groups of rats were pretreated with 3-MNX (6.4 pmol) 25 min, nor-BNI (6.6 nmol) 24 h, MK-801 (30 nmol) 20 min or saline vehicle given into the centromedial amygdala before microinjection of endomorphin-2 and the tail-flick responses were measured 30 min after endomorphin-2 injection. Each column represents the mean and the vertical bar represents the S.E.M. with 6 to 8 rats in each group. Students's *t* test was used to test the difference between groups; For rats pretreated with nor-BNI versus vehicle followed by endomorphin-2 challenge $t=1.65$; For rats pretreated with MK-801 versus vehicle followed by endomorphin-2 challenge $t=8.77$; * $p < 0.01$, ** $p < 0.001$.

Fig. 6. Effect of dynorphin A(1–17) microinjected into the centromedial amygdala on the thermal tail-flick response. Groups of rats were microinjected with different doses of dynorphin A(1–17) (0.23–2.3 nmol) or vehicle (0.5 μ l) into the centromedial amygdala and the tail-flick responses were measured 10 min thereafter. One-way ANOVA followed by Dunnett's post-test was used to test the difference between groups, $N = 6$ to 8 rats in each group; * $p < 0.001$.

Fig. 7.

Effects of the pretreatment with nor-binaltorphimine (nor-BNI) or MK-801 on the hyperalgesia induced by dynorphin A(1–17) from the centromedial amygdala. Groups of rats were pretreated with nor-BNI (6.6 nmol) 24 h, MK-801 (30 nmol) 20 min or saline vehicle given into the centromedial amygdala before microinjection of dynorphin A(1–17) and the tail-flick responses were measured 10 min after dynorphin A(1–17) injection. Each column represents the mean and the vertical bar represents the S.E.M. with 6 to 8 rats in each group. Students's *t* test was used to test the difference between groups; For rats pretreated with nor-BNI versus vehicle followed by dynorphin A(1–17) challenge $t=1.77$; For rats pretreated with MK-801 versus vehicle followed by dynorphin A(1–17) challenge $t=5.26$; * $p < 0.001$.