

行政院國家科學委員會專題研究計畫 成果報告

類鴉片 mu 和 kappa 受體在調節痛覺與癢覺功能的行為神經 機制 研究成果報告(精簡版)

計畫類別：個別型
計畫編號：NSC 96-2413-H-004-019-
執行期間：96年11月01日至97年10月31日
執行單位：國立政治大學心理學系

計畫主持人：柯美全

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處理方式：本計畫可公開查詢

中華民國 98 年 03 月 14 日

(計畫名稱)

類鴉片 mu 和 kappa 受體在調節痛覺與癢覺功能的行為神經機制

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共同主持人：NA

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成果報告類型(依經費核定清單規定繳交)： 精簡報告 完整報告

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執行單位：國立政治大學心理系

中 華 民 國 98 年 2 月 28 日

報告內容：

Itch sensation (Pruritus) is a symptom of many clinical disorders that afflicts a large population of humans and is treated by a variety of pharmacological agents with variable success. Little effort has been made to understand the basic mechanisms by which itch sensation is provoked. Recent studies illustrate distinct sensory neurons responding to two different pruritogenic agents and several receptor mechanisms regulating the itch or/and pain sensation. Therefore, it is important to conduct studies using behaving animals to investigate the receptor mechanisms modulating itch and pain sensation. In our previous studies, we have demonstrated that central mu opioid receptors mediate both antinociception and itch/scratching responses and that kappa opioid receptor agonists can inhibit mu opioid receptor agonist-elicited scratching responses without decreasing antinociception. Using pharmacological approaches, we intend to establish other experimental itch models with different pruritogenic agents and to determine the effectiveness of kappa opioid receptor agonists as antipruritics in a broader context in behaving animals. The proposed studies in this project have tested the central hypothesis that activation of kappa opioid receptors attenuates itch evoked by a variety of pruritogenic agents in rodents. In the proposed studies, scratching activity were monitored by video recorders and quantified by observers blind to experimental conditions. The potential attenuation of scratching activity by rationally selected pharmacological agents was studied in different experimental itch models including i.c.v. DAMGO- and bombesin-induced scratching responses. In addition, the warm water tail-withdrawal nociceptive assay was used to measure whether the rodent's nociceptive threshold was changed after receiving different pharmacological agents alone or in combination. This proposal further established experimental models of centrally elicited itch and compare the effectiveness of kappa opioid receptors agonists, opioid receptor antagonists, and histamine receptor antagonists as antipruritics under these conditions. Theses studies provide a substantial contribution to the *in vivo* pharmacology of itch and pain, and establish a translational basis for kappa opioid receptor agonists as potential antipruritics in humans.

During the study period, we have published two peer-reviewed SCI articles and one conference abstract. Below is the list of abstracts derived from this NSC research project.


1. Lai CP. Chen CM. Lin AP. **Ko MC** . (2009)

The roles of mu opioid receptors and bombesin receptors in the modulation of pain and itch sensation.

The 24th Joint Annual Conference of Biomedical Science, 第二十四屆生物醫學聯合學術年會, Taipei, Taiwan.

Itch sensation (pruritus) is a significant clinical problem, but little effort has been made to understand the basic mechanisms of provoked itch sensation. The aim of this study was to establish experimental models of itch in rats by using drugs acting on different receptor types. The intracerebroventricular (i.c.v.) route was chosen for drug delivery because the study focuses on the supraspinal site of action and it is highly relevant to the scratching site when rats use their hind paws for scraping contact on the skin surface of the body. Peptidic agonists, DAMGO and bombesin,

were chosen to investigate how mu opioid receptors and bombesin receptors modulate pain and itch sensation. After i.c.v. administration, DAMGO (0.03-0.3 µg) dose-dependently produced antinociceptive effects in both warm water 52°C tail-withdrawal assay and formalin-induced nociceptive assay. However, the same doses of DAMGO did not significantly elicit itch/scratching responses. In contrast, i.c.v. bombesin (0.03-0.3 µg) dose-dependently elicited profound scratching responses. The highest dose of i.c.v. bombesin 0.3 µg did not change rats' nociceptive threshold manifested as unchanged tail-withdrawal latency in 52C water. It is known that activation of central mu opioid receptors produces both analgesia and itch simultaneously in primates. Scratching responses are absent in rats receiving antinociceptive doses of i.c.v. DAMGO, indicating that rats may not be the species to study opioid receptor-mediated itch. Nevertheless, i.c.v. bombesin-induced scratching responses occurred in both rodents and non-human primates. It stands as a valuable model to further study effects of bombesin-like receptor antagonists and other experimental antipruritics under this context.

2. **Ko MC** . Husbands SM. (2009)

Effects of atypical kappa opioid receptor agonists on intrathecal morphine-induced itch and analgesia in primates.
Journal of Pharmacology and Experimental Therapeutics 328(1):193-200.

Itch/Pruritus is the most common side effect associated with spinal administration of morphine given to humans for analgesia. The aim of this study was to investigate the effectiveness of κ opioid receptor (KOR) agonists with diverse chemical structures as antipruritics and to elucidate the receptor mechanism underlying the antipruritic effect in monkeys. In particular, previously proposed non-KOR-1 agonists including nalfurafine (TRK-820), bremazocine, and GR 89696 were studied in various behavioral assays for measuring itch/scratching, analgesia, and respiratory depression. Systemic administration of nalfurafine (0.1-1 µg/kg), bremazocine (0.1-1 µg/kg), or GR 89696 (0.01-0.1 µg/kg) dose-dependently attenuated intrathecal morphine (0.03 mg)-induced scratching responses without affecting morphine antinociception. The combination of intrathecal morphine with these KOR agonists did not cause sedation. In addition, pretreatment with effective anti-scratching doses of nalfurafine, bremazocine, or GR 89696 did not antagonize systemic morphine-induced antinociception and respiratory depression. The dose-addition analysis revealed that there is no subadditivity for nalfurafine in combination with morphine in the antinociceptive effect. Furthermore, the KOR antagonist study revealed that anti-scratching effects of both nalfurafine and a prototypical KOR-1 agonist, U-50488H, could be blocked completely by a selective KOR antagonist, nor-binaltorphimine (3 mg/kg). These findings suggest that the agonist action on KOR mainly contributes to the effectiveness of these atypical KOR agonists as antipruritics and there is no evidence for KOR subtypes or µ opioid antagonist action underlying the effects of these KOR agonists. This mechanism-based study further supports the clinical potential of KOR agonists as antipruritics under the context of spinal opioid analgesia.

3. Xie H. Woods JH. Traynor JR. **Ko MC** . (2008)

The spinal antinociceptive effects of endomorphins in rats: Behavioral and G protein functional studies.
Anesthesia and Analgesia 106(6):1873-1881.

BACKGROUND: Endomorphin-1 and endomorphin-2 are endogenous peptides that are highly selective for mu-opioid receptors. However, studies of their functional efficacy and selectivity are controversial. The aim of this study was to systematically compare the effects of intrathecal (i.t.) administration of endomorphin-1 and -2 on nociception assays and G protein activation with those of DAMGO, a highly efficacious peptidic mu-opioid receptor agonist.

METHODS: Male Sprague-Dawley rats were used. Acute and inflammatory pain models were used to compare the duration and magnitude of antinociception. Agonist-stimulated [³⁵S]GTPγS binding was used to observe the functional activity at the level of the receptor-G protein in both spinal cord and thalamic membranes. In addition, antagonists selective for each receptor type were used to verify the functional selectivity of endomorphins in the rat spinal cord.

RESULTS: Following i.t. administration, endomorphin-1 and -2 produced less antinociceptive effects than DAMGO in the model of acute pain. Concentration-response curves for DAMGO-, endomorphin-1-, and endomorphin-2-stimulated [³⁵S]GTPγS binding revealed that both endomorphin-1 and -2 produced less G protein activation (i.e., ~50-60%) than DAMGO did in the membranes of spinal cord and thalamus. In addition, i.t. endomorphin-induced antinociception was blocked by μ-opioid receptor selective dose of naltrexone (p<0.05), but not by delta- and kappa-opioid receptor antagonists, naltrindole and nor-binaltorphimine (p>0.05).

CONCLUSIONS: Endomorphins are partial agonists for G protein activation at spinal and thalamic mu-opioid receptors. Both *in vivo* and *in vitro* measurements together suggest that DAMGO is more efficacious than endomorphins. Spinal endomorphins' antinociceptive efficacy may range between 53 and 84% depending on the intensity and modality of the nociceptive stimulus.

In addition, there are data have been collected and have not been published yet.

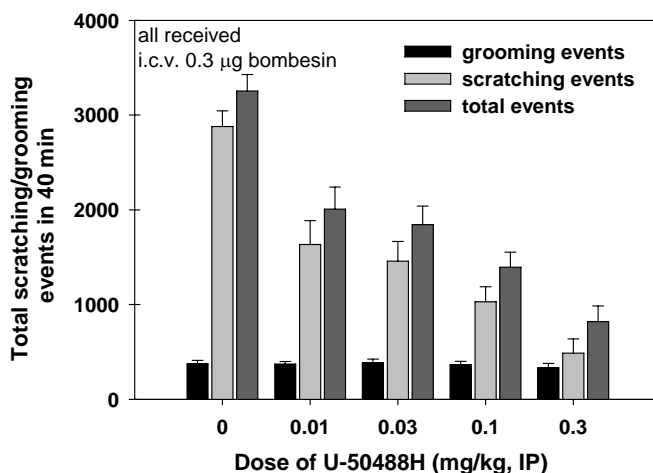


Figure 1. Effects of U-50488H on i.c.v. bombesin-induced scratching responses in rats. Bombesin was administered 5 min before observation. U-50488H was administered 15 min before i.c.v. bombesin. Each value represents mean ± S.E.M. (n=6).

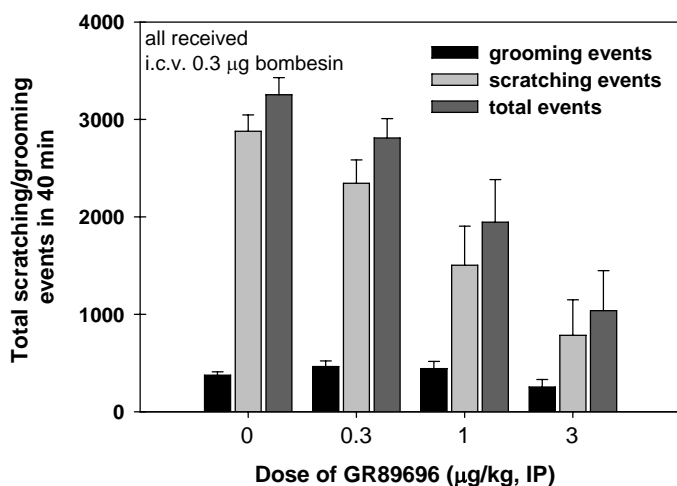


Figure 2. Effects of GR89696 on i.c.v. bombesin-induced scratching responses in rats. Bombesin was administered 5 min before observation. GR89696 was administered 15 min before i.c.v. bombesin. Each value represents mean \pm S.E.M. (n=6).

Figure 1 illustrates the effects of intraperitoneal administration of U-50488H on intracerebroventricular bombesin-induced scratching responses in rats. Pretreatment with U-50488H dose-dependently attenuated intracerebroventricular bombesin-induced scratching [$F(4,25) = 21.8$; $p < 0.05$]. Post hoc comparisons indicated that U-50488H from 0.03 to 0.3 mg/kg significantly attenuated scratching. In addition, figure 2 illustrates the effects of intraperitoneal administration of GR89696 on intracerebroventricular bombesin-induced scratching responses in rats. Pretreatment with GR89696 dose-dependently attenuated intracerebroventricular bombesin-induced scratching [$F(3,20) = 9.0$; $p < 0.05$]. Post hoc comparisons indicated that GR89696 from 1 to 3 $\mu\text{g}/\text{kg}$ significantly attenuated scratching. It is worth noting that anti-scratching doses of both U-50488H and GR89696 do not produce antinociceptive effects in rats.

Collectively, this project has conducted most proposed experiments and allows researchers to learn more about the functions of mu and kappa opioid receptors in the modulation of pain and itch sensation. Both mu and kappa opioid receptor agonists can produce antinociceptive effects. Nevertheless, mu and kappa opioid receptors have opposite effects in eliciting itch sensation. Although central administration of mu opioid receptor agonists can not elicit itch/scratching responses in rats, it is well known in the literature that activation of central mu opioid receptors evokes itch/scratching responses in primates. Nevertheless, the present project provides additional evidence that central administration of bombesin can produce profound scratching responses and such responses can be attenuated by pretreatment with kappa opioid receptor agonists. These results further support the therapeutic potential of kappa opioid receptor agonists, regardless of drugs' selectivity for kappa opioid receptor subtypes, as antipruritics in a broader context. Furthermore, intracerebroventricular bombesin-induced scratching can be used as a rodent model of itch to study the functions of central bombesin receptors and to compare the effectiveness of kappa opioid receptor agonists and bombesin receptor antagonists as antipruritics in the future.

Effects of Atypical κ -Opioid Receptor Agonists on Intrathecal Morphine-Induced Itch and Analgesia in Primates

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ABSTRACT

Itch/pruritus is the most common side effect associated with spinal administration of morphine given to humans for analgesia. The aim of this study was to investigate the effectiveness of κ -opioid receptor (KOR) agonists with diverse chemical structures as antipruritics and to elucidate the receptor mechanism underlying the antipruritic effect in monkeys. In particular, previously proposed non-KOR-1 agonists, including nalfurafine [TRK-820, 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[N-methyl-*trans*-3-(3-furyl)acrylamido]morphinan], bremazocine [(\pm)-6-ethyl-1,2,3,4,5,6-hexahydro-3-[(1-hydroxycyclopropyl)methyl]-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol], and GR 89696 [4-[(3,4-dichlorophenyl)acetyl]-3-(1-pyrrolidinylmethyl)-1-piperazinecarboxylic acid methyl ester] were studied in various behavioral assays for measuring itch/scratching, analgesia, and respiratory depression. Systemic administration of nalfurafine (0.1–1 μ g/kg), bremazocine (0.1–1 μ g/kg), or GR 89696 (0.01–0.1 μ g/kg) dose-dependently attenuated intrathecal morphine (0.03 mg)-induced scratching responses without affecting morphine antinociception. The combination of intrathecal morphine with

these KOR agonists did not cause sedation. In addition, pretreatment with effective antiscratching doses of nalfurafine, bremazocine, or GR 89696 did not antagonize systemic morphine-induced antinociception and respiratory depression. The dose-addition analysis revealed that there is no subadditivity for nalfurafine in combination with morphine in the antinociceptive effect. Furthermore, the KOR antagonist study revealed that antiscratching effects of both nalfurafine and a prototypical KOR-1 agonist, U-50488H [*trans*-(\pm)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide], could be blocked completely by a selective KOR antagonist, nor-binaltorphimine (3 mg/kg). These findings suggest that the agonist action on KOR mainly contributes to the effectiveness of these atypical KOR agonists as antipruritics, and there is no evidence for KOR subtypes or μ -opioid antagonist action underlying the effects of these KOR agonists. This mechanism-based study further supports the clinical potential of KOR agonists as antipruritics under the context of spinal opioid analgesia.

Spinal administration of μ -opioid receptor agonists is an important method for pain management. In particular, it is a widely used therapy for obstetric analgesia (Cousins and Mather, 1984; DeBalli and Breen, 2003). However, itch/pruritus is the most common side effect of spinal opioid administration, and it reduces the value of spinal opioids for pain relief (Cousins and Mather, 1984; Ganesh and Maxwell, 2007). Previous studies have demonstrated that the same μ -opioid receptors mediate both analgesic and itch/scratching responses in primates (Ko and Naughton, 2000; Ko et al.,

2004). Therefore, opioid receptor antagonists such as naloxone are not ideal antipruritics to be used under this context because such compounds can reverse opioid analgesia concurrently (Rawal et al., 1986; Cohen et al., 1992; Wang et al., 1998). It is important to identify specific pharmacological agents that can inhibit spinal opioid-induced itch without attenuating analgesia.

The κ -opioid receptor (KOR) seems to be a promising target because several studies suggest that KOR agonists are potentially useful as antipruritics. For example, scratching was a prominent withdrawal sign in monkeys treated chronically with and withdrawn from a selective KOR agonist, U-50488H (Gmerek et al., 1987). Many withdrawal symptoms from opioids appear to be opposite to the acute effects of agonist administration (Heishman et al., 1989; Kishioka et

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doi:10.1124/jpet.108.143925.

ABBREVIATIONS: KOR, κ -opioid receptor; U-50488H, *trans*-(\pm)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide; nalfurafine (TRK-820), 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 β -[N-methyl-*trans*-3-(3-furyl)acrylamido]morphinan; nor-BNI, nor-binaltorphimine; bremazocine, (\pm)-6-ethyl-1,2,3,4,5,6-hexahydro-3-[(1-hydroxycyclopropyl)methyl]-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol; GR 89696, 4-[(3,4-dichlorophenyl)acetyl]-3-(1-pyrrolidinylmethyl)-1-piperazinecarboxylic acid methyl ester.

The Spinal Antinociceptive Effects of Endomorphins in Rats: Behavioral and G Protein Functional Studies

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BACKGROUND: Endomorphin-1 and endomorphin-2 are endogenous peptides that are highly selective for μ -opioid receptors. However, studies of their functional efficacy and selectivity are controversial. In this study, we systematically compared the effects of intrathecal (i.t.) administration of endomorphin-1 and -2 on nociception assays and G protein activation with those of [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO), a highly effective peptidic μ -opioid receptor agonist.

METHODS: Male Sprague-Dawley rats were used. Acute and inflammatory pain models were used to compare the duration and magnitude of antinociception. Agonist-stimulated [³⁵S]GTP γ S binding was used to observe the functional activity at the level of the receptor-G protein in both spinal cord and thalamic membranes. In addition, antagonists selective for each receptor type were used to verify the functional selectivity of endomorphins in the rat spinal cord.

RESULTS: After i.t. administration, endomorphin-1 and -2 produced less antinociceptive effects than DAMGO in the model of acute pain. Concentration-response curves for DAMGO-, endomorphin-1-, and endomorphin-2-stimulated [³⁵S]GTP γ S binding revealed that both endomorphin-1 and -2 produced less G protein activation (i.e., approximately 50%–60%) than DAMGO did in the membranes of spinal cord and thalamus. In addition, i.t. endomorphin-induced antinociception was blocked by μ -opioid receptor selective dose of naltrexone ($P < 0.05$), but not by δ - and κ -opioid receptor antagonists, naltrindole and nor-binaltorphimine ($P > 0.05$).

CONCLUSIONS: Endomorphins are partial agonists for G protein activation at spinal and thalamic μ -opioid receptors. Both *in vivo* and *in vitro* measurements together suggest that DAMGO is more effective than endomorphins. Spinal endomorphins' antinociceptive efficacy may range between 53% and 84% depending on the intensity and modality of the nociceptive stimulus.

(Anesth Analg 2008;106:1873-81)

The μ -opioid receptors are G protein-coupled receptors that play a pivotal role in the analgesic effects of opioid receptor agonists used clinically.^{1,2} Given that intrathecal (i.t.) administration of opioids is one of the most frequently used methods of analgesia in humans,³⁻⁵ it is important to study the functions of spinal μ -opioid receptors. In particular, the peptidic μ -opioid receptor agonists are of interest due to their enzymatic degradation and low toxicity.⁶

Endomorphin-1 and endomorphin-2 are endogenous opioid peptides isolated from bovine and human

brains, and both peptides have high affinity and selectivity for μ -opioid receptors.^{7,8} Endomorphins have been implicated in a broad range of physiological functions including antinociceptive, cardiovascular, respiratory, digestive, rewarding, and endocrine responses.^{9,10} One important issue is the efficacy of endomorphins in activating μ -opioid receptors and subsequent antinociceptive effects manifested *in vivo*. Previous studies measuring the degree of agonist-stimulated G protein activation have shown that endomorphins act as partial agonists in the mouse spinal cord membranes and cell lines expressing μ -opioid receptors.^{11,12} In contrast, other studies indicate that endomorphins are full agonists in the rat thalamic membranes and cell lines expressing μ -opioid receptors.^{13,14} Although the antinociceptive effects of spinally administered endomorphins have been studied in rodents,¹⁵⁻¹⁸ there are few studies that directly compare the relative degrees, potencies, and durations of antinociceptive effects of i.t. endomorphins with those of i.t. [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO), a highly effective peptidic μ -opioid receptor agonist.¹⁷ In particular, there is no study using both *in vivo* and *in vitro* measurements at the same

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