Negative modulation of spinal κ-opioid receptor-mediated antinociception by the μ-opioid receptor at selective doses of (−)-pentazocine

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The mixed-action κ-opioid receptor (KOR) agonist, pentazocine, binds to both KOR and the μ-opioid receptor (MOR). Racemic (±)-pentazocine and (−)-pentazocine, each administered systemically, have been shown to produce antinociception in various animal models. In contrast, racemic (±)-pentazocine failed to produce antinociception when administered intrathecally (i.t.). However, whether spinal activation of KOR and MOR by (−)-pentazocine produces antinociception and the relative contribution of KOR and MOR in mediating antinociception remain unknown. Hence, we investigated whether i.t. (−)-pentazocine produces dose-dependent modulation of acute thermal nociception. Drugs were administered intrathecally in Sprague-Dawley rats and tail flick latency was recorded. Pentazocine produced a significant antinociceptive effect that was mediated by KOR and/or MOR at differential doses. MOR blockade restored the antinociceptive effect of an ineffective dose and prolonged the duration of an effective dose of pentazocine. Hence, spinal KOR and MOR mediated the effect of pentazocine. This study provides evidence that spinal MOR negatively modulates the KOR-mediated antinociceptive effect of i.t. pentazocine. *NeuroReport* **29:852–855**

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Introduction

Mixed-action κ-opioid receptor (KOR) agonists, namely, pentazocine, nalbuphine, and butorphanol produce limited to no analgesia or biphasic effects in men following molar extraction [1–3]. Pentazocine produces analgesia in an experimental model of pain [4,5], whereas findings in animal studies are ambiguous [6]. Systemic pentazocine produces antinociception in rats [7,8], and MOR may mediate pentazocine antinociception [9,10].

Each optical isomer of pentazocine affects analgesia differently. (−)-Pentazocine produces opioid receptor-mediated antinociception [7–9,11], whereas, (+)-pentazocine binds to the sigma 1 receptor and inhibits antinociception [11]. Systemic racemic (±)-pentazocine (Talwin) binds to KOR and MOR, and produces analgesia in rodents [11,12]. However, in spite of KOR and MOR being present in the spinal cord, intrathecal (i.t.) racemic (±)-pentazocine did not produce antinociception [13]. Therefore, we investigated (a) whether i.t. (−)-pentazocine produces antinociception in rats, (b) the relative contribution of spinal KOR and MOR in mediating pentazocine antinociception, and (c) whether there is a negative interaction between spinal KOR and MOR.

Materilas and methods

Male Sprague-Dawley rats (250–274 g; Envigo, Indianapolis, Indiana, USA) were housed in an animal care facility approved by the American Association for the Accreditation of Laboratory Animal Care under a 12-h light/12-h dark cycle with free access to water and food. Experimental protocols were approved by the Institutional Animal Care and Use Committee and conformed to the International Association for the Study of Pain guidelines.

A stretched PE-10 cannula (Intramedics, Sparks, Maryland, USA) was implanted in the lumbosacral spinal cord as described [14] in anesthetized (80 mg/kg ketamine and 4 mg/kg xylazine) rats. Briefly, their heads were mounted on a stereotaxic frame, skin above the head incised, atlanto-occipital membrane cleared, a narrow slit made in the dura, and a stretched PE-10 cannula was inserted caudally ~8.8 cm to reach lumbosacral segment. Dental cement was used to secure the cannula and wound clips applied to the skin. Recovery time was 7–14 days.

The tail flick test was carried out as described by Nag and Mokha [14]. Briefly, a noxious heat stimulus was applied on the tail using an analgesia meter (Model 33 T, IITC, Woodland Hills, California, USA). Tail flick latency (TFL) was recorded automatically every 5 min.

(−)-Pentazocine-succinate (125–500 nmol/10 μl sterile water), nor-binaltorphimine (nor-BNI) (26 nmol/10 μl sterile saline), a selective KOR antagonist [15], and CTAP (34 nmol/10 μl sterile saline), a selective MOR antagonist [16], were administered i.t. CTAP [17] and

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nor-BNI were administered 5 min and 18 h, respectively, before pentazocine. Using 18-h time interval, 26 nmol nor-BNI selectively antagonizes KOR [18]. Each rat was tested once and euthanized [150 mg/kg; intraperitoneal; Beuthanasia (Schering-Plough Animal Health Corporation, Union, New Jersey, USA)]. (−)-Pentazocine was provided by the National Institute on Drug Abuse (NIH, Bethesda, Maryland, USA); nor-BNI and CTAP were purchased from Sigma Aldrich (St. Louis, Missouri, USA).

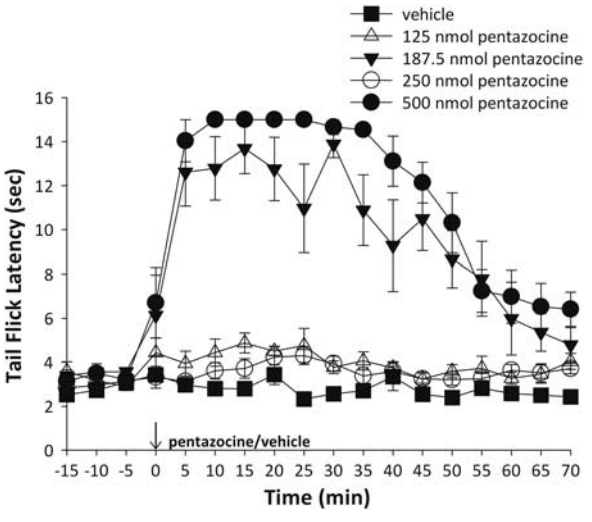
Data were analyzed by repeated measures analysis of variance followed by Bonferroni post-hoc or independent *t*-test ($P < 0.05$; SPSS Inc., Chicago, Illinois, USA). Data are plotted from 63 rats in 13 groups as mean ± SEM.

Results

Pentazocine significantly increased the TFL in a dose-dependent manner [Fig. 1; time: $F_{(17,510)} = 32.41$, $P < 0.01$; dose: $F_{(4,30)} = 61.02$, $P < 0.01$; time × dose: $F_{(68,510)} = 12.5$, $P < 0.01$]. High and low doses of pentazocine (187.5 and 500 nmol), but not the middle dose (250 nmol), increased TFL for up to 40 min (post-hoc $P < 0.05$) with peak effect at 10–15 min postinjection ($P < 0.05$). Baseline TFL values were comparable between various groups throughout this study. The highest dose of pentazocine (500 nmol) did not induce sedation or motor impairment as measured by rota rod test (data not shown).

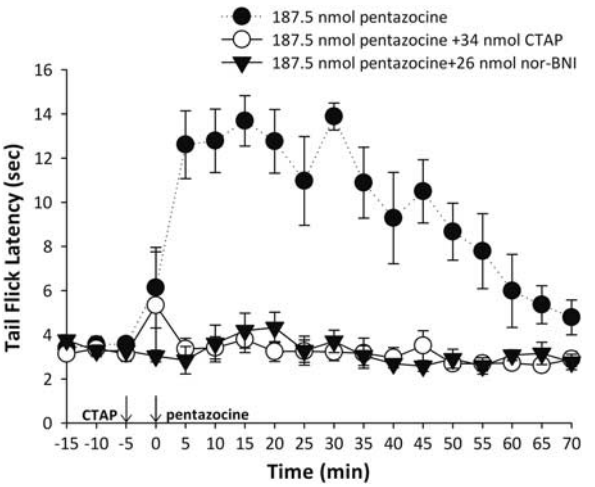
Both nor-BNI and CTAP blocked the antinociceptive effect of pentazocine [Fig. 2; time: $F_{(17,187)} = 5.74$, $P < 0.01$; group: $F_{(2,11)} = 22.59$, $P < 0.01$; and time × group: $F_{(34,187)} = 5.73$, $P < 0.01$] throughout the time course (all $P < 0.05$). Hence, the effect of the lowest effective dose of pentazocine is likely mediated by KOR or MOR.

Fig. 1



Intrathecal (−)-pentazocine produced dose-dependent antinociception. It significantly increased tail flick latency at 187.5 and 500 nmol (but not 125 and 250 nmol) doses in comparison to the vehicle-treated group between time points 5 and 50 min (all $P < 0.05$).

Fig. 2



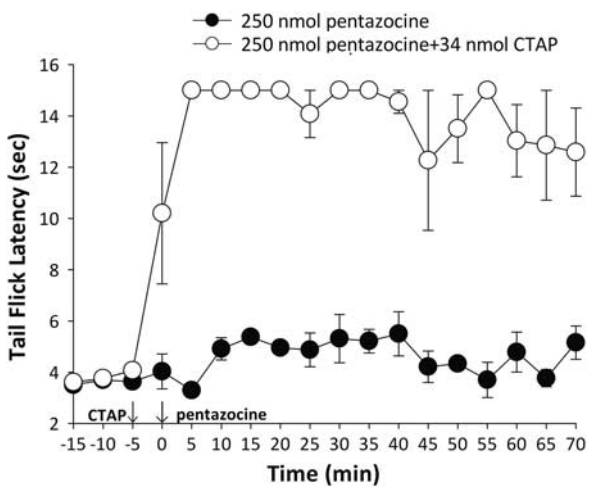
Spinal κ-opioid receptor (KOR) or μ-opioid receptor (MOR) mediated the antinociceptive effect of the low dose of pentazocine (187.5 nmol). The increase in tail flick latency by pentazocine was blocked by either nor-binaltorphimine (nor-BNI) or CTAP pretreatment throughout the duration of the experiment (all $P < 0.05$ as compared with pentazocine only group) (Dotted line represents data replotted from Fig. 1 for instant comparison).

Interestingly, intermediate dose (250 nmol) of pentazocine administered following CTAP pretreatment induced a significant increase in TFL, which was long lasting and persisted throughout the time course [Fig. 3; Time: $F_{(17,68)} = 11.1$, $P < 0.01$; group: $F_{(1,4)} = 1047.97$, $P < 0.01$; time × group: $F_{(17,68)} = 7.34$, $P < 0.01$]. Further, CTAP significantly prolonged the effect duration of the highest dose (500 nmol) of pentazocine [Fig. 4; time: $F_{(17,289)} = 21.2$, $P < 0.01$; group: $F_{(4,17)} = 86.18$, $P < 0.01$; time × group: $F_{(68,289)} = 11.02$, $P < 0.01$]. Nor-BNI blocked pentazocine's effect. Hence, MOR negatively modulates the KOR-mediated effect even at the highest dose of pentazocine.

Discussion

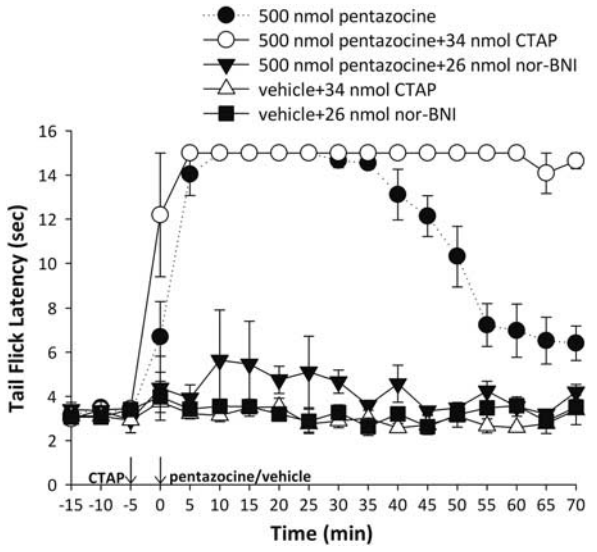
This is the first study to show that (i) i.t. (−)-pentazocine produces antinociception; (ii) KOR or MOR mediates this effect, and (iii) spinal MOR negatively modulates KOR-mediated antinociception. The effect of the low effective dose of pentazocine (187.5 nmol) was mediated by KOR or MOR. MOR-mediated effect of pentazocine shown here is consistent with a previous report in mice [9]. Other studies using systemic pentazocine, nalbuphine, and butorphanol have also reported similar findings as observed here [7,8,19–21]. However, this study provides direct evidence of spinal KOR and MOR mediating i.t. pentazocine's effect. Previous evidence indicates a KOR-induced reduction of MOR-mediated

Fig. 3



Spinal μ-opioid receptor (MOR) negatively modulates the κ-opioid receptor (KOR)-mediated antinociceptive effect of pentazocine (250 nmol). CTAP, injected 5 min before pentazocine produced a significant increase in tail flick latency from 5 to 70 min in comparison to pentazocine only treated group (all $P < 0.05$).

Fig. 4



μ-Opioid receptor (MOR) blockade prolonged the κ-opioid receptor (KOR)-mediated antinociceptive effect of 500 nmol pentazocine. The increase in tail flick latency produced by 500 nmol pentazocine was blocked by nor-binaltorphimine (nor-BNI); however, it was significantly prolonged by CTAP (time points 45 – 70 min; all $P < 0.05$ as compared with pentazocine only group). Hence, the antinociceptive effect of 500 nmol pentazocine was mediated by KOR (Dotted line represents data replotted from Fig. 1 for instant comparison).

effect in the brainstem [22–24]. Here, we show the reverse, that is, MOR negatively modulates the KOR-mediated antinociceptive effect of pentazocine. The effect of high dose of pentazocine (500 nmol) was KOR-mediated, and modulated negatively by the MOR as CTAP prolonged the duration of its effect. An ineffective dose (250 nmol) of pentazocine also produced antinociception with CTAP pretreatment. We speculate that selective doses of pentazocine may enhance coupling of MOR to the stimulatory G-protein (G_s) and/or produce facilitation by disinhibition as is known for opioids. There is some support for such paradoxical finding in a clinical study showing that a hyperalgesic dose of nalbuphine (5 mg) produced analgesia in men when co-administered with naloxone [25].

We contemplate two possible mechanisms for abolishment of antinociceptive effect of low dose of pentazocine by either KOR or MOR blockade: (i) activation of spinal KOR and MOR by pentazocine leads to two distinct signal transduction pathways converging on a common intracellular messenger, or (ii) pentazocine binds to a KOR–MOR heterodimer to induce antinociception. Heterodimerization has been shown between opioid receptors [26]. It will require future studies to reveal the exact mechanism.

Similar to the present finding, a nonlinear dose–response curve was reported in a clinical study where 5 mg nalbuphine produced hyperalgesia, 10 mg was ineffective, and 20 mg produced short-lasting analgesia in men [3]. Further, pentazocine produced antinociception in rats during the early phase of mechanical pain test, followed by hyperalgesia that was mediated by presumably supraspinal opioid-receptor like 1 receptor [12]. As we injected pentazocine i.t., observed effects would be mediated by spinal KOR and MOR.

These results lead us to conclude that i.t. pentazocine produces dose-dependent antinociception. KOR or MOR mediates this effect at the low doses and MOR negatively modulates the KOR-mediated effect at higher doses of pentazocine. Spinal administration of selective doses of (–)-pentazocine, with or without MOR blockade, might help the development of an effective pain treatment strategy.

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Conflicts of interest

There are no conflicts of interest.

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