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Pentazocine-Induced Antinociception Is Mediated Mainly by μ-Opioid Receptors and Compromised by κ-Opioid Receptors in Mice

Haihua Shu, Masakazu Hayashida,[1] Hideko Arita,[2] Wenqi Huang, Hui Zhang, Ke An, Guiyun Wu, and Kazuo Hanaoka[2]

Department of Anesthesiology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China (H.S., W.H., H.Z., K.A., G.W.); and Department of Anesthesiology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan (H.S., M.H., H.A., K.H.)

ABSTRACT

Pentazocine is a widely used mixed agonist-antagonist opioid. Previous animal studies have demonstrated that pentazocine-induced antinociception displayed a ceiling effect characterized by biphasic dose response with a increasing and then descending analgesia like a bell-shaped curve. This study attempted to clarify the mechanisms underlying such dose-response relationships. ddY and C57BL/6J mice received subcutaneous injection of saline or pentazocine (3, 10, 30, 56, or 100 mg · kg^{-1}), at 120 min after subcutaneous injection of saline, a μ-opioid receptor antagonist clocinnamox mesylate (C-CAM) (5 mg · kg^{-1}), a κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) (10 mg · kg^{-1}), or the combination of C-CAM and nor-BNI. The antinociceptive effects of pentazocine were evaluated using tail pressure, hot plate, tail flick, and acetic acid writhing tests. Without pretreatment with an opioid receptor antagonist, the antinociceptive effects of pentazocine exhibited biphasic bell-shaped dose-response curves peaking at 30 mg · kg^{-1}. C-CAM completely and partly antagonized the antinociception induced by pentazocine at low (3–30 mg · kg^{-1}) and high (56–100 mg · kg^{-1}) doses, respectively. nor-BNI enhanced the antinociception by pentazocine at high doses and turned the later descending portion of the biphasic dose-response curves into a sigmoid curve. The combination of C-CAM and nor-BNI completely abolished the antinociception by pentazocine at all doses. Our results suggest pentazocine produces antinociception primarily via activation of μ-opioid receptors, but at high doses, this μ-opioid receptor-mediated antinociception is antagonized by concomitant activation of κ-opioid receptors. This provides the first reasonable hypothesis to explain the ceiling effects of pentazocine analgesia characterized by a biphasic dose response.

Introduction

The mixed agonist/antagonist opioid pentazocine (PZ) has been widely used for pain management (Hoskin and Hanks, 1991; Fudala and Johnson, 2006). Pentazocine displays μ-, κ-, and δ-opioid receptors selectivity in the ratio 5.4:4.3:1 in radioligand binding experiments in vitro (Raynor et al., 1994) and acts as a partial agonist on the κ-opioid receptor (KOR) and an antagonist or a partial agonist on the μ-opioid receptor (MOR) in vivo (Gutstein and Akil, 2006). Animal studies show that both MORs and KORs can contribute to antinociceptive effects to somatic as well as visceral pain induced by pentazocine (Suzuki et al., 1991; Bidlack et al., 2000). A human study showed that pentazocine produces μ-like subjective effects that can be antagonized by naltrexone, an antagonist more potent in blocking MOR than KOR activity (Preston and Bigelow, 1993). Thus, MORs and/or KORs can be implicated in pentazocine antinociception, but it is still controversial as to which of the MORs and KORs primarily contribute to pentazocine antinociception.

Previous dose-response studies demonstrated that pentazocine exhibits a biphasic dose-response curve and at low to moderate doses produces dose-dependent antinociception, whereas at high doses it produces diminished antinociception

ABBREVIATIONS: PZ, pentazocine; C-CAM, clocinnamox mesylate; nor-BNI, nor-binaltorphimine; KOR, κ-opioid receptor; MOR, μ-opioid receptor; AUC, area under the response-versus-time curve; PLSD, protected least significance difference test; Sal, saline.

(Hoskin and Hanks, 1991; Fürst and Hosztafi, 2008). Although buprenorphine exhibits a similar biphasic dose-response curve probably via concomitant activation of opioid receptor-like receptors (Lutfy et al., 2003; Lutfy and Cowan, 2004), mechanisms underlying the biphasic dose-response curve for pentazocine antinociception remain unknown. While a partial opioid receptor agonist commonly exhibits a ceiling effect, maximal analgesia was observed at a certain dose and neither increased nor decreased with higher doses; the partial agonistic property of pentazocine cannot fully explain the later descending portion of its biphasic dose-response curve. Because KOR agonists antagonize MOR-mediated pharmacological effects of morphine, including antinociception, dependence, and tolerance (Pan, 1998; Meng et al., 2005; Shu et al., 2006a, 2008; Chiba et al., 2009), one may hypothesize that coactivation of KORs by pentazocine self-antagonizes MOR-mediated pentazocine-induced antinociception, thereby resulting in the biphasic dose-response curve.

We conducted the present study using two opioid receptor antagonists: clocinnamox mesylate (C-CAM), a selective MOR antagonist that acts as an insurmountable, long-acting MOR antagonist in behavioral experiments (Zernig et al., 1996; Paronis and Woods, 1997), and nor-binaltorphimine (nor-BNI), a selective KOR antagonist that exerts long-lasting activity in vivo (Endoh et al., 1992; Raynor et al., 1994), in an attempt to clarify a receptor subtype primarily responsible for pentazocine antinociception and mechanisms underlying the biphasic dose-response curve of pentazocine-induced antinociception in mice.

Materials and Methods

Animals

This study was approved by the Institutional Laboratory Animal Care and Use Committee of the University of Tokyo and Sun Yat-sen University. Male ddY mice (SLC Japan, Hamamatsu, Japan) weighing 23 to 28 g and male C57BL/6J mice (Animal Center at Sun Yat-sen University, Guangzhou, China) weighing 18 to 22 g were used. Mice were housed in a temperature-controlled (23 ± 2°C) and humidity-controlled (55 ± 10%) environment with a 12-h light/dark cycle and free access to food and water. All experiments were performed in accordance with the guidelines of the Physiological Society of Japan and China regarding the care of experimental animals. All experiments were performed during the light period (7:00 AM-7:00 PM). All behavior tests were performed by a single investigator blinded to the treatment groups. Each of the tail pressure, hot plate, and tail flick tests was repeated three times at intervals of 30 min before the first analgesic drug application to each mouse, the first measurement was omitted, and the mean of the next two was taken as a baseline value.

Behavior Tests

Tail Pressure Test. The tail pressure test was performed using a Randall-Sellito Analgesy Meter (Ugo Basile, Comerio, Italy) as described previously (Shu et al., 2006b). In brief, the distal part of the tail was supported by a plinth while pressure, linearly increasing at a rate of $16 \text{ g} \cdot \text{s}^{-1}$, was applied to the proximal 2 cm of the tail with a cone-shaped pusher. The threshold pressure inducing the first motor response of struggle was measured. The baseline threshold pressure was between 50 and 80 g. A cutoff pressure of 250 g was used to avoid tissue damage.

Hot Plate Test. The hot plate test was performed using an apparatus consisting of an acrylic resin cage and a thermo-controllable aluminum plate (model MK-350B; Muromachi Kikai Co., To-

kyo, Japan) as described previously (Shu et al., 2007). In brief, mice were placed on an aluminum hot plate thermo-controlled at 52 ± 0.5°C. The latency to a positive response, defined as licking of a forepaw or hindpaw, flicking of the hindpaws, or jumping, was measured. A cutoff latency of 30 s was used to avoid tissue damage.

Tail Flick Test. The tail flick test was performed using an automated tail flick analgesimeter (model MK-330A; Muromachi Kikai) as described previously (Shu et al., 2008). In brief, a light beam was focused on the dorsal surface of the tail 2.5 cm from its tip. The latency to an abrupt flick of the tail was measured. The intensity of the light was adjusted so that the baseline latency averaged between 3 and 4 s. A cutoff latency of 10 s was used to avoid tissue damage.

Acetic Acid Writhing Test. A response to a visceral chemical stimulus was evaluated with the acetic acid writhing test as described previously (Kurihara et al., 2003). In brief, mice received intraperitoneal injection of 0.6% acetic acid solution ($10 \text{ ml} \cdot \text{kg}^{-1}$ body weight). Each mouse was placed in a transparent chamber, and the total number of writhes, i.e., abdominal constriction and whole body stretch, was counted over a 10-min period from 5 to 15 min after the intraperitoneal injection of acetic acid.

Drugs and Chemicals

Pentazocine hydrochloride ($C_{19}H_{27}NO \cdot HCl$) (Pentagin, DL-pentazocine; Sankyo Pharmaceutical, Tokyo, Japan) and nor-BNI [17,17′-(dicyclopropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetrol dihydrochloride] (Sigma, Tokyo, Japan) were dissolved in normal saline ($10 \text{ ml} \cdot \text{kg}^{-1}$ body weight) for subcutaneous injection. C-CAM [14β-(p-chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphin one mesylate] (Tocris Bioscience, Ellisville, MO) was dissolved in dimethyl sulfoxide and diluted with normal saline ($10 \text{ ml} \cdot \text{kg}^{-1}$ body weight) for subcutaneous injection. Acetic acid (Kyowa Hakko Chemical, Tokyo, Japan) was dissolved in normal saline (0.6%; $10 \text{ ml} \cdot \text{kg}^{-1}$ body weight) for intraperitoneal injection. Normal saline was used as placebo for pentazocine, C-CAM, and nor-BNI.

Medication and Measurement Protocols

Antinociceptive Effects to Somatic Pain of Pentazocine in ddY Mice. To evaluate the dose-response relationship of the antinociception to somatic pain by pentazocine and whether pentazocine produces these effects primarily via MORs or KORs, 24 groups of ddY mice received subcutaneous saline or pentazocine (3, 10, 30, 56, or $100 \text{ mg} \cdot \text{kg}^{-1}$) 120 min after pretreatment with subcutaneous placebo saline, subcutaneous C-CAM ($5 \text{ mg} \cdot \text{kg}^{-1}$), subcutaneous nor-BNI ($10 \text{ mg} \cdot \text{kg}^{-1}$), or the combination of subcutaneous C-CAM and nor-BNI ($n = 8$ for each group). The tail pressure, hot plate, and tail flick tests were performed before and at 30, 60, 90, and 120 min after the subcutaneous pentazocine injection.

Antinociceptive Effects to Visceral Pain of Pentazocine in ddY Mice. To evaluate the dose-response relationship of the antinociception to visceral pain by pentazocine and whether pentazocine produces these effects primarily via MORs or KORs, 24 groups of ddY mice received subcutaneous saline or pentazocine (3, 10, 30, 56, or $100 \text{ mg} \cdot \text{kg}^{-1}$) 120 min after pretreatment with subcutaneous placebo saline, subcutaneous C-CAM ($5 \text{ mg} \cdot \text{kg}^{-1}$), subcutaneous nor-BNI ($10 \text{ mg} \cdot \text{kg}^{-1}$), or the combination of subcutaneous C-CAM and nor-BNI ($n = 8$ for each group). Fifty minutes after the subcutaneous pentazocine injection mice received intraperitoneal injection of acetic acid. The amount of writhing in each mouse was counted over the 10-min period from 5 to 15 min after the intraperitoneal acetic acid injection.

Antinociceptive Effects of Pentazocine in C57BL/6J Mice. To exclude the possibility that the biphasic dose-response curve of pentazocine analgesia is limited to special mouse strains, 24 groups of C57BL/6J mice received subcutaneous saline or pentazocine (3, 10, 30, 56, or $100 \text{ mg} \cdot \text{kg}^{-1}$) 120 min after pretreatment with subcutaneous placebo saline, subcutaneous C-CAM ($5 \text{ mg} \cdot \text{kg}^{-1}$), subcutaneous nor-BNI ($10 \text{ mg} \cdot \text{kg}^{-1}$), or the combination of subcutaneous

C-CAM and nor-BNI ($n = 8$ for each group). The tail pressure were performed before and at 30, 60, 90, 120, 150, and 180 min after the subcutaneous pentazocine injection.

Statistical Analysis

Antinociception by pentazocine was quantified as either direct measurement data or the area under the response-versus-time curve (AUC). To avoid problems related to multiple comparisons of data repeatedly measured over time, we considered the AUC the best summary of the antinociceptive effect of pentazocine. The AUC was calculated according to the following formula based on the trapezoid rule: AUC = 0.5 h × (the sum of data measured at set time points)/2.

Data are expressed as mean ± S.E.M or median (range) according to data types. Changes in continuous variables over time were analyzed with repeated-measures analysis of variance, followed by Fisher's protected least significance difference test (PLSD). Intergroup comparisons were made with analysis of variance and Fisher's PLSD. Changes in categorical data were analyzed with Mann-Whitney test. $P < 0.05$ was considered statistically significant.

Results

Antinociception to Somatic Pain by Pentazocine in ddY Mice Pretreated with Saline. In the tail pressure test, pentazocine at 3 mg · kg^{-1}, at 10, 30, and 56 mg · kg^{-1}, and at 100 mg · kg^{-1} produced significant antinociception to mechanical pain, compared with saline, at 30 min, at 30, 60, and 90 min, and at 30 and 60 min, respectively ($p < 0.05$ by Fisher's PLSD) (Fig. 1A). The AUCs were more with pentazocine (10, 30, 56, and 100 mg · kg^{-1}) than with saline, with pentazocine at 10 mg · kg^{-1} compared with 3 mg · kg^{-1}, and with pentazocine at 30 mg · kg^{-1} compared with 10 mg · kg^{-1} ($p < 0.05$ by Fisher's PLSD) (Fig. 2A), whereas the AUCs were less with pentazocine at 56 and 100 mg · kg^{-1} compared with 10 and 30 mg · kg^{-1} ($p < 0.05$ by Fisher's PLSD) (Fig. 2A). These results indicated that pentazocine at 3 to 100 mg · kg^{-1} produced significant antinociception to mechanical pain, demonstrating a biphasic dose response like a bell-shaped curve, peaking at 30 mg · kg^{-1}.

In the hot plate test, only pentazocine at 30 mg · kg^{-1} produced significant antinociception to thermal pain, compared with saline, at 30 min ($p < 0.05$ by Fisher's PLSD) (Fig. 1B). The AUC was significantly more only with pentazocine at 30 mg · kg^{-1} than with saline ($p < 0.05$ by Fisher's PLSD) (Fig. 2B). These results indicated that only pentazocine at 30 mg · kg^{-1} produced significant antinociception to thermal pain in the hot plate test, and the dose-response curve was biphasic, peaking at 30 mg · kg^{-1}.

In the tail flick test, pentazocine at 10 and 30 mg · kg^{-1} produced significant antinociception to thermal pain, compared with saline, at 30 min and at 30 and 60 min, respectively ($p < 0.05$ by Fisher's PLSD) (Fig. 1C). The AUCs were more with pentazocine at 10 and 30 mg · kg^{-1} compared with saline, and with pentazocine at 30 mg · kg^{-1} compared with 10 mg · kg^{-1} ($p < 0.05$ by Fisher's PLSD), whereas they were not more with pentazocine at 3, 56, and 100 mg · kg^{-1} compared with saline ($p > 0.05$ by Fisher's PLSD) (Fig. 2C). These results indicated that pentazocine at 10 and 30 mg · kg^{-1}, but not at 3, 56, or 100 mg · kg^{-1}, produced significant antinociception to thermal pain in the tail flick test, and the dose-response curve was biphasic, peaking at 30 mg · kg^{-1}.

Effects of C-CAM on Antinociception to Somatic Pain in ddY Mice by Pentazocine. After pretreatment with C-CAM (5 mg · kg^{-1}), pentazocine at 3, 10, or 30 mg · kg^{-1} did not produce significant antinociception to mechan-
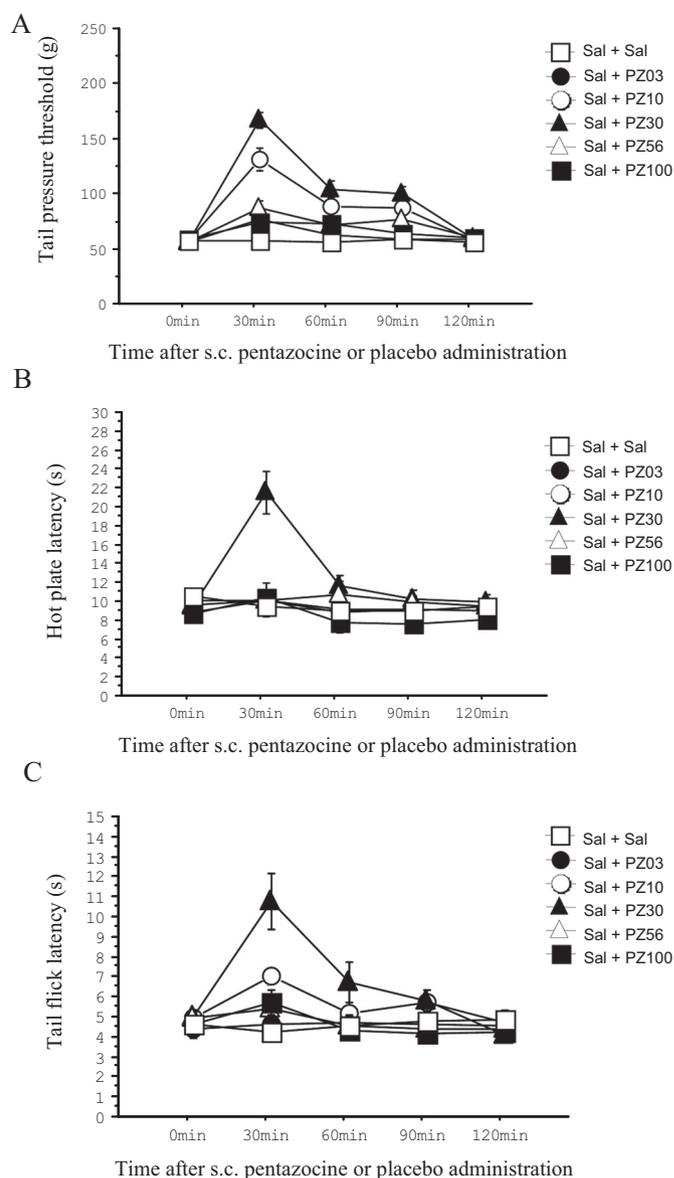

Fig. 1. Dose response of antinociceptive effects to somatic pain of pentazocine. Six groups of mice received subcutaneous normal saline or pentazocine at 3, 10, 30, 56, or 100 mg · kg^{-1} at 120 min after pretreatment with subcutaneous normal saline, (Sal + Sal, Sal + PZ03, Sal + PZ10, Sal + PZ30, Sal + PZ56, and Sal + PZ100 groups; $n = 8$ for each group). The tail pressure threshold (g) (A), hot plate latency (s) (B), and tail flick latency (s) (C) immediately before (0 min) and at 30, 60, 90, and 120 min after the subcutaneous pentazocine injection are shown as mean ± S.E.M.

ical pain, compared with saline, in the tail pressure test ($p > 0.05$ by Fisher's PLSD), whereas pentazocine at 56 and 100 mg · kg^{-1} produced significant antinociception to mechanical pain at 30 and 60 min ($p < 0.05$ by Fisher's PLSD) (Fig. 3A). The AUCs with pentazocine (3, 10, and 30 mg · kg^{-1}) were not more than the AUC with saline in the C-CAM-treated mice, and the AUCs with pentazocine (3, 10, and 30 mg · kg^{-1}) were significantly less in the C-CAM-treated than the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 2A). On the other hand, the AUCs with pentazocine (56 and 100 mg · kg^{-1}) were significantly more than the AUC with saline in the C-CAM-treated mice ($p < 0.05$ by Fisher's PLSD), and the AUCs with pentazocine (56 and 100 mg · kg^{-1}) were not differ-
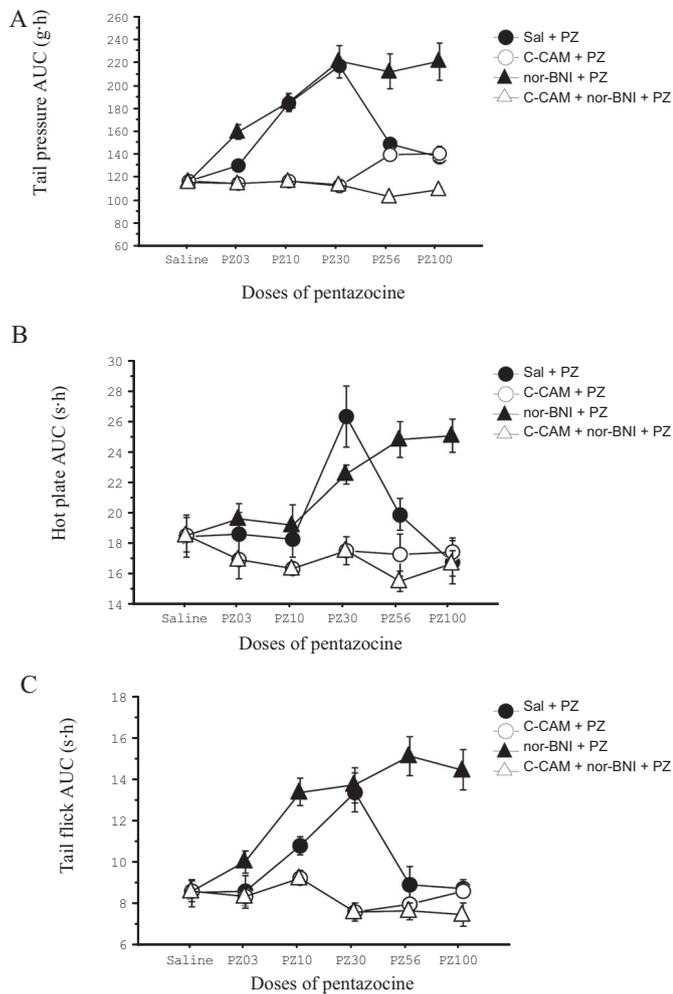
Fig. 2. The calculated AUC representing the antinociceptive effect to somatic pain of pentazocine. Abscissa: doses of pentazocine (Sal, PZ03, PZ10, PZ30, PZ56, PZ100). Ordinate: tail pressure AUC (g·h) represent the antinociception assessed by the tail pressure test (A), hot plate AUC (s·h) represent the antinociception assessed by the hot plate test (B), and tail flick AUC (s·h) represent the antinociception assessed by the tail flick test (C). ● (Sal + PZ) indicate pretreatment with saline (as a placebo for C-CAM and/or nor-BNI). ○ (C-CAM + PZ) indicate pretreatment with C-CAM (5 mg·kg^{-1}). ▲ (nor-BNI + PZ) indicate pretreatment with nor-BNI (10 mg·kg^{-1}). △ (C-CAM + nor-BNI + PZ) indicate pretreatment with both C-CAM and nor-BNI. Data are expressed as mean ± S.E.M.
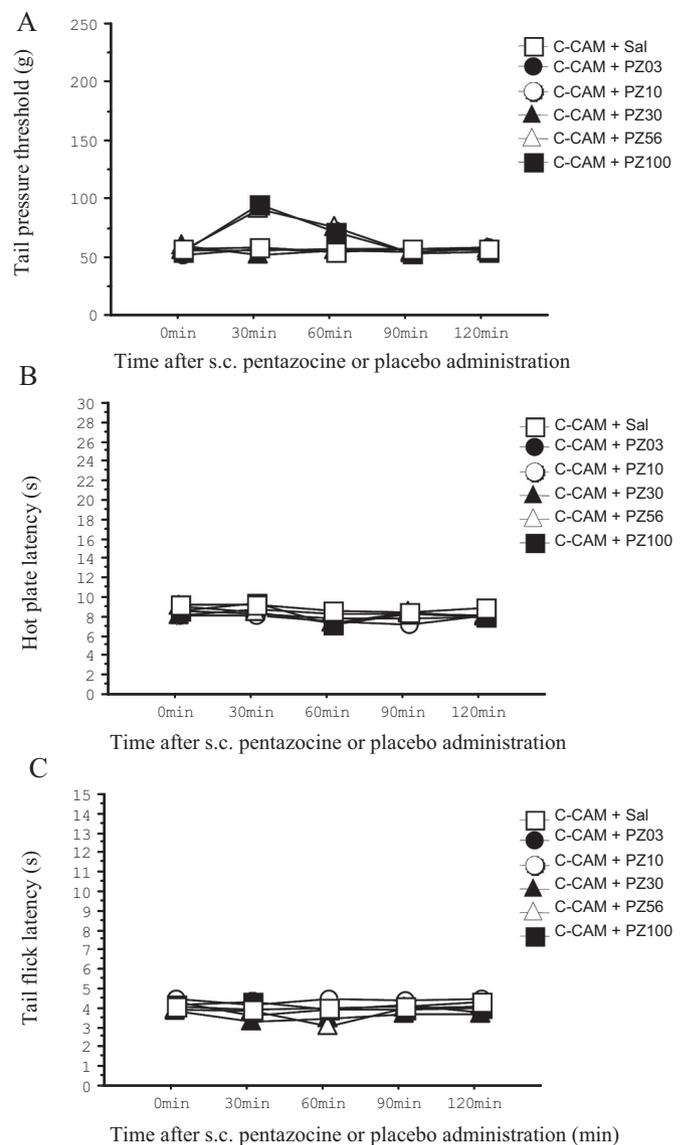
Fig. 3. Effects of C-CAM on antinociceptive effects to somatic pain of pentazocine. Six groups of mice received subcutaneous normal saline or pentazocine at 3, 10, 30, 56, or 100 mg·kg^{-1} at 120 min after pretreatment with subcutaneous C-CAM (5 mg·kg^{-1}) (C-CAM + Sal, C-CAM + PZ03, C-CAM + PZ10, C-CAM + PZ30, C-CAM + PZ56, and Sal + PZ100 groups, $n = 8$ for each group). The tail pressure threshold (g) (A), hot plate latency (s) (B), and tail flick latency (s) (C) immediately before (0 min) and at 30, 60, 90, and 120 min after the subcutaneous pentazocine injection are shown as mean ± S.E.M.

ent between the C-CAM- and saline-treated mice ($p > 0.05$ by Fisher's PLSD) (Fig. 2A). These results indicated that C-CAM completely antagonized antinociception to mechanical pain by pentazocine at 3, 10, and 30 mg·kg^{-1}, while not antagonizing that induced by pentazocine at 56 and 100 mg·kg^{-1} at all.

After treatment with C-CAM, pentazocine at any dose did not produce significant antinociception to thermal pain, compared with saline, in the hot plate or tail flick tests ($p > 0.05$ by Fisher's PLSD) (Fig. 3, B and C). In the C-CAM-treated mice, the AUCs with pentazocine at any dose were not more than the AUC with saline in the hot plate or tail flick tests (Fig. 2, B and C). These results in the C-CAM-treated mice, in comparison with the saline-treated mice, indicated that C-CAM completely antagonized antinociception to thermal pain by pentazocine (10 and/or 30 mg·kg^{-1}) in the hot plate and tail flick tests.

Effects of nor-BNI on Antinociception to Somatic Pain in ddY Mice by Pentazocine. After treatment with nor-BNI (10 mg·kg^{-1}), pentazocine at 3, 10, 30, 56, and 100

mg·kg^{-1} produced significant antinociception to mechanical pain, compared with saline, at 30, 60, and 90 min in the tail pressure test ($p < 0.05$ by Fisher's PLSD) (Fig. 4A). In the nor-BNI-treated mice, the AUCs were significantly more with pentazocine (3, 10, 30, 56, and 100 mg·kg^{-1}) than with saline, and with pentazocine at 30, 56, and 100 mg·kg^{-1} compared with pentazocine at 3 and 10 mg·kg^{-1} ($p < 0.05$ by Fisher's PLSD), whereas the AUCs were not different between pentazocine at 3 and 10 mg·kg^{-1} or among pentazocine at 30, 56, and 100 mg·kg^{-1} ($p > 0.05$ by Fisher's PLSD) (Fig. 2A). The AUCs with pentazocine (56 and 100 mg·kg^{-1}) were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 2A). These results indicated that nor-BNI enhanced antinociception to
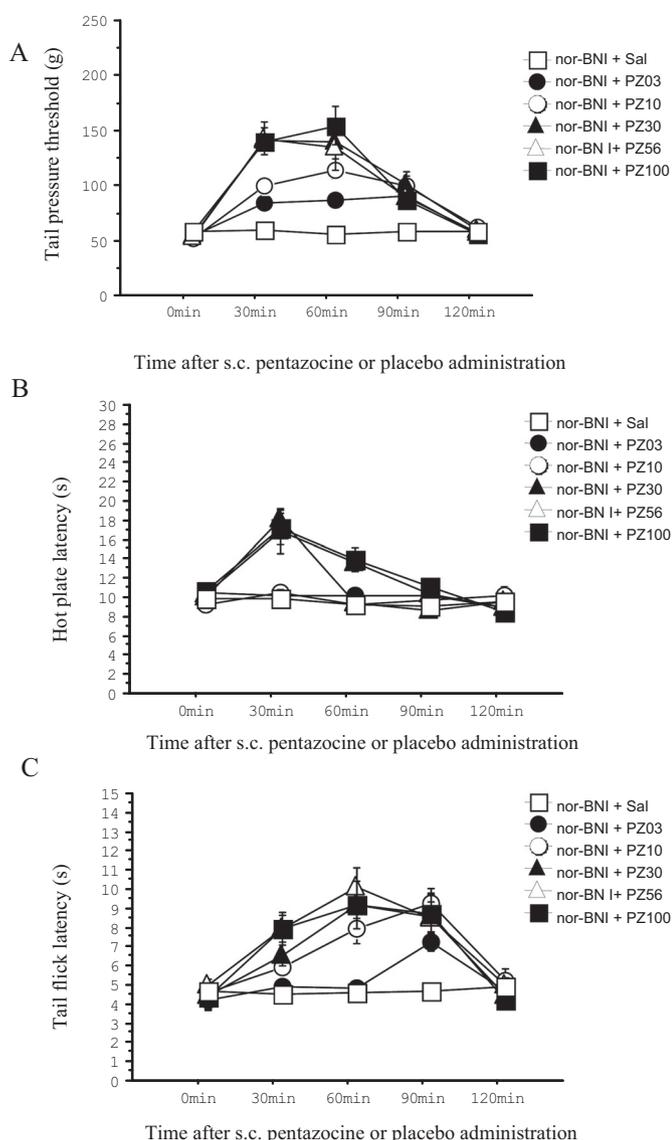
Fig. 4. Effects of nor-BNI on antinociceptive effects to somatic pain of pentazocine. Six groups of mice received subcutaneous normal saline or pentazocine at 3, 10, 30, 56, or 100 mg · kg^{-1} at 120 min after pretreatment with subcutaneous nor-BNI (10 mg · kg^{-1}) (nor-BNI + Sal, nor-BNI + PZ03, nor-BNI + PZ10, nor-BNI + PZ30, nor-BNI + PZ56, and nor-BNI + PZ100 groups, n = 8 for each group). The tail pressure threshold (g) (A), hot plate latency (s) (B), and tail flick latency (s) (C) immediately before (0 min) and at 30, 60, 90, and 120 min after the subcutaneous pentazocine injection are shown as mean ± S.E.M.

mechanical pain induced by pentazocine at 56 and 100 mg · kg^{-1} in the tail pressure test and changed the biphasic bell-shaped dose-response curve, peaking at 30 mg · kg^{-1}, to a sigmoid curve, peaking approximately at 30 mg/kg without diminishment at higher doses.

After treatment with nor-BNI, pentazocine at 30, 56, and 100 mg · kg^{-1} produced significant antinociception to thermal pain, compared with saline, at 30 min, at 30 and 60 min, and at 30, 60, and 90 min, respectively, in the hot plate test ($p < 0.05$ by Fisher's PLSD) (Fig. 4B). In the nor-BNI-treated mice, the AUCs were significantly more with pentazocine (30, 56, and 100 mg · kg^{-1}) than with saline and with pentazocine at 56 and 100 mg · kg^{-1} compared with pentazocine at 30 mg · kg^{-1} ($p < 0.05$ by Fisher's PLSD), whereas they were not different between pentazocine at 56 and 100 mg · kg^{-1} ($P >$

0.05 by Fisher's PLSD) (Fig. 2B). The AUCs with pentazocine (56 and 100 mg · kg^{-1}) were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 2B). These results indicated that nor-BNI enhanced antinociception to thermal pain induced by pentazocine (56 and 100 mg · kg^{-1}) in the hot plate test and changed the biphasic bell-shaped dose-response curve, peaking at 30 mg · kg^{-1}, to a sigmoid curve, peaking at approximately 30 mg/kg without diminishment at higher doses.

After treatment with nor-BNI, pentazocine at 3 mg · kg^{-1}, at 10 mg · kg^{-1}, and at 30, 56, and 100 mg · kg^{-1} produced significant antinociception to thermal pain, compared with saline, at 90 min, at 60 and 90 min, and at 30, 60, and 90 min, respectively, in the tail flick test ($p < 0.05$ by Fisher's PLSD) (Fig. 4C). In the nor-BNI-treated mice, the AUCs were more with pentazocine at 10, 30, 56, and 100 mg · kg^{-1} than with saline and pentazocine at 3 mg · kg^{-1} ($p < 0.05$ by Fisher's PLSD), whereas they were not different among pentazocine at 10, 30, 56, and 100 mg · kg^{-1} ($p > 0.05$ by Fisher's PLSD) (Fig. 2C). The AUCs with pentazocine (10, 56, and 100 mg · kg^{-1}) were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 2C). These results indicated that nor-BNI enhanced antinociception to thermal pain induced by pentazocine at 10, 56, and 100 mg · kg^{-1} in the tail flick test and changed the biphasic bell-shaped dose-response curve, peaking at 30 mg · kg^{-1}, to a sigmoid curve, peaking at approximately 30 mg/kg without diminishment at higher doses.

Effects of nor-BNI and C-CAM on Antinociception to Somatic Pain in ddY Mice by Pentazocine. After treatment with both nor-BNI (10 mg · kg^{-1}) and C-CAM (5 mg · kg^{-1}), pentazocine at any dose did not produce significant antinociception to mechanical or thermal pain, compared with saline, in the tail pressure, hot plate, or tail flick tests (Fig. 5), and the AUCs were not more with pentazocine at any dose than with saline (Fig. 2). These results indicated that treatment with both nor-BNI and C-CAM completely antagonized antinociception to somatic pain by pentazocine at all doses examined.

Antinociceptive Effect to Visceral Pain of Pentazocine in ddY Mice. In the saline-treated mice, the amount of writhing was less with pentazocine (3 mg · kg^{-1}) than saline, pentazocine at 10 mg · kg^{-1} compared with 3 mg · kg^{-1}, and pentazocine at 30, 56, and 100 mg · kg^{-1} compared with 10 mg · kg^{-1} ($P < 0.05$ by Mann-Whitney test), whereas it was not different among pentazocine at 30, 56, and 100 mg · kg^{-1} ($P > 0.05$ by Mann-Whitney test) (Table 1). These results indicated that pentazocine inhibited acetate-induced writhing in a dose-dependent manner, and maximal analgesia was observed at 30 mg · kg^{-1} and neither increased nor decreased with higher doses (Table 1).

In the C-CAM-treated mice, pentazocine at 3, 10, or 30 mg · kg^{-1} did not significantly decrease the amount of writhing, compared with saline ($P > 0.05$ by Mann-Whitney test), whereas pentazocine at 56 and 100 mg · kg^{-1} significantly decreased the amount of writhing, compared with saline ($P < 0.05$ by Mann-Whitney test), although the decreases were significantly less in the C-CAM-treated mice than the saline-treated mice (Table 1). These results indicated that C-CAM completely antagonized the inhibition of acetate-induced writhing by pentazocine at 3, 10, and 30 mg · kg^{-1}, but only
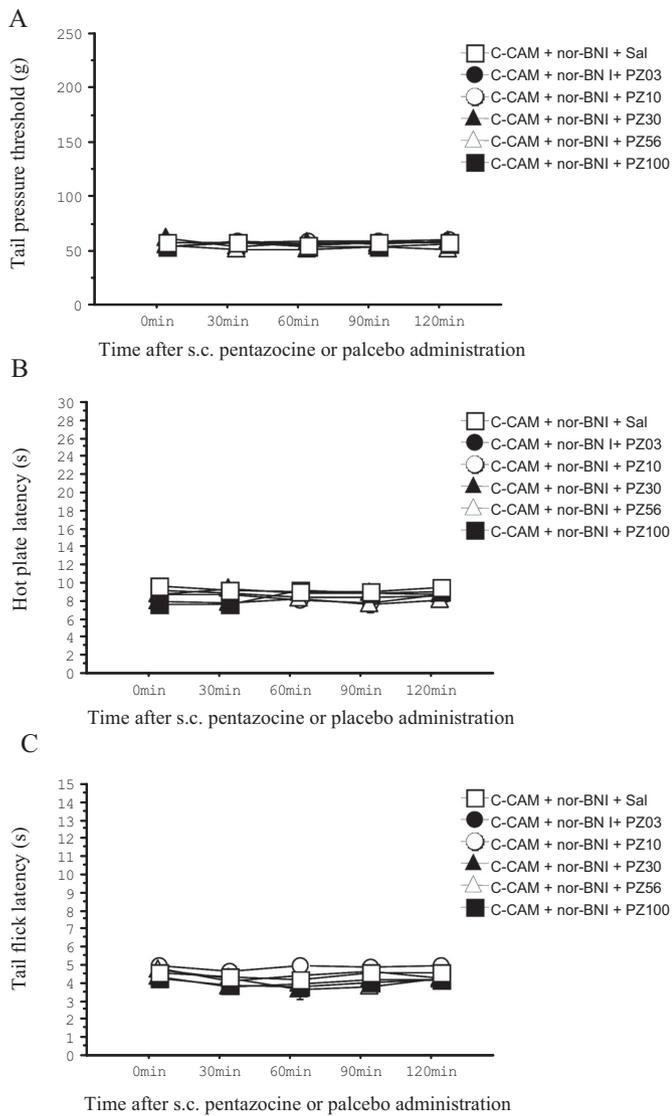
A

B

C

Fig. 5. Effects of both C-CAM and nor-BNI on antinociceptive effects to somatic pain of pentazocine. Six groups of mice received subcutaneous normal saline or pentazocine at 3, 10, 30, 56, or 100 mg · kg^{-1} at 120 min after pretreatment with subcutaneous C-CAM (5 mg · kg^{-1}) and subcutaneous nor-BNI (10 mg · kg^{-1}) (C-CAM + nor-BNI + Sal, C-CAM + nor-BNI + PZ03, C-CAM + nor-BNI + PZ10, C-CAM + nor-BNI + PZ30, C-CAM + nor-BNI + PZ56, and C-CAM + nor-BNI + PZ100 groups, $n = 8$ for each group). The tail pressure threshold (g) (A), hot plate latency (s) (B), and tail flick latency (s) (C) immediately before (0 min) and at 30, 60, 90, and 120 min after the subcutaneous pentazocine injection are shown as mean ± S.E.M.

partly antagonized the inhibition by pentazocine at 56 and 100 mg · kg^{-1}.

In mice pretreated with both C-CAM and nor-BNI penta-

zocine at any dose did not significantly decrease the amount of acetic acid-induced writing, compared with saline ($P >$ 0.05 by Mann-Whitney test) (Table 1). These results indicated that treatment with both C-CAM and nor-BNI completely antagonized the inhibition of acetate-induced writhing by pentazocine at all doses.

Antinociceptive Effects of Pentazocine in C57BL/6J Mice. The antinociception of pentazocine in C57BL/6J mice was assessed only by the tail pressure test. Pentazocine at 3 mg · kg^{-1}, at 10, 30, and 56 mg · kg^{-1}, and at 100 mg · kg^{-1} produced significant antinociception to mechanical pain, compared with saline, at 30, 60, 90, and 120 min, at 30, 60, 90, and 120 min, at 30, 60, 90, 120, and 150 min, at 30, 60, 90 and 120 min, and at 30, 60, 90 and 120 min, respectively ($p <$ 0.05 by Fisher's PLSD) (Fig. 6A). The AUCs were more with pentazocine at 3, 10, 30, 56, and 100 mg · kg^{-1} compared with saline, pentazocine at 10 mg · kg^{-1} compared with 3 mg · kg^{-1}, and pentazocine at 30 mg · kg^{-1} compared with 10 mg · kg^{-1} ($p <$ 0.05 by Fisher's PLSD) (Fig. 6B), whereas the AUCs were less with pentazocine at 56 and 100 mg · kg^{-1} than at 10 and 30 mg · kg^{-1} ($p <$ 0.05 by Fisher's PLSD) (Fig. 6B). These results indicated that pentazocine (3–100 mg · kg^{-1}) produced significant antinociception to mechanical pain in C57BL/6J mice, similar to that in ddY mice, demonstrating a biphasic dose-response curve, but the duration of antinociception by pentazocine in C57BL/6J mice was longer than in ddY mice.

When the C57BL/6J mice were pretreated with C-CAM, the AUCs with pentazocine (3, 10, and 30 mg · kg^{-1}) were not more than the AUC with saline, and the AUCs with pentazocine (3, 10, and 30 mg · kg^{-1}) were significantly less in the C-CAM-treated compared with the saline-treated mice ($p <$ 0.05 by Fisher's PLSD) (Fig. 6B). On the other hand, the AUCs with pentazocine at 56 and 100 mg · kg^{-1} were significantly more than the AUC with saline in the C-CAM-treated mice ($p <$ 0.05 by Fisher's PLSD), and the AUCs with pentazocine at 56 and 100 mg · kg^{-1} were not different between the C-CAM- and saline-treated mice ($p >$ 0.05 by Fisher's PLSD) (Fig. 6B). These results indicate that in C57BL/6J mice C-CAM completely antagonized antinociception to mechanical pain by pentazocine at 3, 10, and 30 mg · kg^{-1}, while not antagonizing that induced by pentazocine at 56 and 100 mg · kg^{-1} at all, similar to that in ddY mice.

When the C57BL/6J mice were pretreated with nor-BNI, the AUCs were significantly more with pentazocine at 3, 10, 30, 56, and 100 mg · kg^{-1} than with saline, pentazocine at 30, 56, and 100 mg · kg^{-1} compared with pentazocine at 3 and 10 mg · kg^{-1} ($p <$ 0.05 by Fisher's PLSD), and pentazocine at 10 mg · kg^{-1} compared with 3 mg · kg^{-1}, whereas the AUCs

TABLE 1

Antinociceptive effects to visceral pain of pentazocine

Twenty-four groups of mice received subcutaneous normal saline or subcutaneous pentazocine at 3, 10, 30, 56, or 100 mg · kg^{-1} at 120 min after pretreatment with placebo saline, C-CAM (5 mg · kg^{-1}), nor-BNI (10 mg · kg^{-1}), or both nor-BNI and C-CAM ($n = 8$ for each group). The amount of writhing per mouse was counted over 10 min from 5 to 15 min after the intraperitoneal acetic acid injection. The amount of writhing is expressed as median in parentheses (25 and 75 percentiles).

	Saline	PZ03	PZ10	PZ30	PZ56	PZ100
Placebo saline	41.5 (39.5, 44.3)	32.5 (29.8, 35.0)*	18.5 (16.8, 19.3)*	2.0 (0.0, 3.3)*	0.5 (0.0, 2.0)*	0.0 (0.0, 0.3)*
C-CAM	42.0 (39.5, 43.3)	42.5 (40.3, 45.0)#	41.5 (38.8, 42.5)#	41.0 (38.0, 42.8)#	20.5 (16.0, 24.3)*#	27.5 (18.3, 31.5)*#
nor-BNI	42.0 (39.8, 45.3)	32.5 (30.8, 35.3)*	18.5 (16.8, 21.0)*	2.0 (0.0, 6.5)*	1.0 (0.0, 3.5)*	0.0 (0.0, 0.3)*
C-CAM + nor-BNI	42.0 (39.0, 43.3)	41.5 (40.8, 42.8)#	41.5 (40.5, 42.5)#	41.0 (40.3, 42.5)#	43.5 (40.8, 50.3)#	42.5 (40.5, 45.3)#

* $P <$ 0.05 compared with saline as a placebo for pentazocine by Mann-Whitney test, indicating significant antinociceptive effects to visceral pain of pentazocine.
\# $P <$ 0.05 compared with mice pretreated with saline (as a placebo for C-CAM and/or nor-BNI) by Mann-Whitney test, indicating significant antagonism by the opioid receptor antagonists.
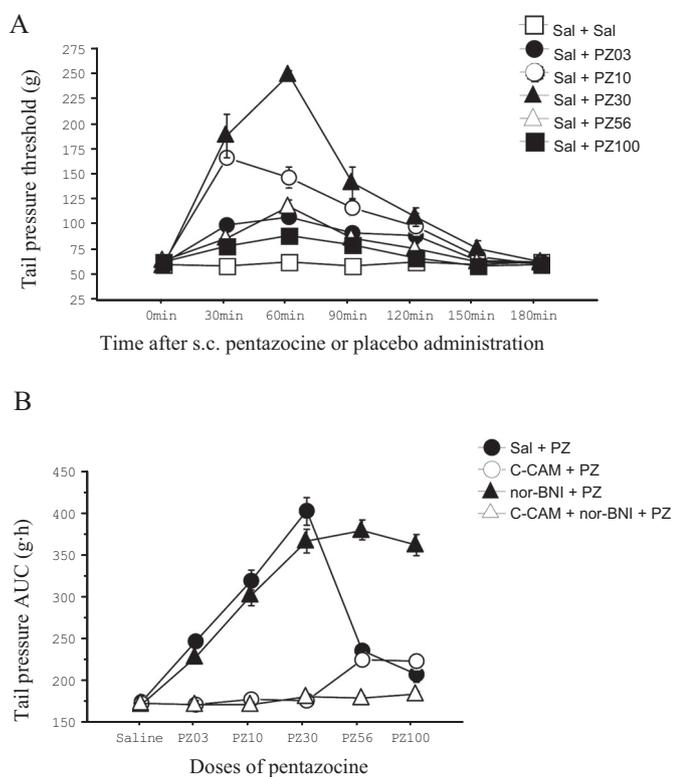
A

B

Fig. 6. Antinociceptive effects of pentazocine in C57BL/6J mice. Twenty four groups of C57BL/6J received subcutaneous saline or pentazocine 3, 10, 30, 56, or 100 mg · kg^{-1} at 120 min after pretreatment with subcutaneous placebo saline, subcutaneous C-CAM (5 mg · kg^{-1}), subcutaneous nor-BNI (10 mg · kg^{-1}), or a combination of subcutaneous C-CAM and nor-BNI ($n = 8$ for each group). A, the tail pressure thresholds immediately before (0 min) and at 30, 60, 90, 120, 150, and 180 min after the subcutaneous pentazocine injection are shown as mean ± S.E.M. B, the calculated AUC representing the antinociceptive effect to mechanical pain of pentazocine. Abscissa: doses of pentazocine (Sal, PZ03, PZ10, PZ30, PZ56, PZ100). Ordinate: tail pressure AUC (g·h) represent the antinociception assessed by the tail pressure test. ● (Sal + PZ) indicate pretreatment with saline (as a placebo for C-CAM and/or nor-BNI). ○ (C-CAM + PZ) indicate pretreatment with C-CAM (5 mg · kg^{-1}). ▲ (nor-BNI + PZ) indicate pretreatment with nor-BNI (10 mg · kg^{-1}). △ (C-CAM + nor-BNI + PZ) indicate pretreatment with both C-CAM and nor-BNI. Data are expressed as mean ± S.E.M.

were not different among pentazocine at 30, 56, and 100 mg · kg^{-1} ($p > 0.05$ by Fisher's PLSD) (Fig. 6B). The AUCs with pentazocine at 56 and 100 mg · kg^{-1} were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 6B). These results indicate that nor-BNI enhanced antinociception to mechanical pain induced by pentazocine at 56 and 100 mg · kg^{-1} and changed the biphasic dose-response curve in C57BL/6J mice, similar to that in ddY mice.

When the C57BL/6J mice were pretreated with C-CAM and nor-BNI pentazocine at any dose did not produce significant antinociception to mechanical pain, and the AUCs were not more with pentazocine at any dose than with saline (Fig. 6B). These results indicate that treatment with both nor-BNI and C-CAM completely antagonized antinociception to mechanical pain by pentazocine at all doses examined in C57BL/6J mice, similar to that in ddY mice.

Discussion

Pentazocine exists as one of two enantiomers, (+)-pentazocine and (−)-pentazocine. These two optically pure com-

pounds usually demonstrate different, even opposing, pharmacological properties, e.g., (−)-pentazocine is a KOR agonist, whereas (+)-pentazocine is not (Kinouchi et al., 1986; Chien and Pasternak, 1995). In the present study, we used DL-pentazocine (racemic form). It has been reported that the racemic pentazocine produced a moderate effect between the two isomers (Kinouchi et al., 1986).

We found that in ddY mice pentazocine exhibited a ceiling effect on antinociception to somatic pain, which was characterized as a biphasic dose response with a increasing and then descending analgesia, like a bell-shaped curve, peaking at 30 mg · kg^{-1}, in the tail pressure, hot plate, and tail flick tests, while exhibiting a ceiling effect on antinociception to visceral pain in a dose-dependent manner with maximal analgesia observed at 30 mg · kg^{-1} that neither increased nor decreased with higher doses in the acetic acid writhing test. Similar antinociception could be seen in C57BL/6J mice assessed by the tail pressure test.

The later descending portion of pentazocine biphasic dose-response curve may be caused by the motor effects and/or sedation of higher doses of pentazocine (Hayes and Tyers, 1983). However, this possibility could be excluded because the diminished antinociception by higher doses (56 and 100 mg · kg^{-1}) increased to the level of 30 mg · kg^{-1} when pretreated with nor-BNI, a selective KOR antagonist.

Regarding pentazocine-induced antinociception to mechanical pain, we found that in two different mouse strains (the ddY mice and C57BL/6J mice): 1) pentazocine at 30 mg · kg^{-1} produced the maximum antinociceptive effect, whereas pentazocine at higher doses produced reduced antinociception; 2) the selective MOR antagonist C-CAM (5 mg · kg^{-1}) completely antagonized antinociception by pentazocine at low doses (30 mg · kg^{-1} or less) while not antagonizing that by pentazocine at higher doses; 3) the selective KOR antagonist nor-BNI (10 mg · kg^{-1}) enhanced, rather than antagonized, antinociception by high-dose pentazocine and thus changed the later descending portion of the biphasic dose-response curve to a neither increased nor decreased curve; and 4) concurrent treatment with C-CAM and nor-BNI completely antagonized antinociception by pentazocine at low and high doses.

Clearly, antinociception to mechanical pain by low-dose pentazocine was mediated primarily by MORs but not by KORs, because it was antagonized completely by C-CAM, but not at all by nor-BNI. On the other hand, mechanisms underlying antinociception to mechanical pain by high-dose pentazocine, which was reduced compared with that by pentazocine at 30 mg · kg^{-1}, seemed complex, because it was not antagonized by C-CAM alone, was enhanced by nor-BNI alone, and was completely antagonized by C-CAM + nor-BNI. It was shown that C-CAM acted as an high selective and insurmountable μ antagonist, but not δ or κ opioid agonists in behavioral experiments, although it displayed only moderate μ selectivity (29:6:1 for μ/δ/κ) in radioligand displacement experiments (Zernig et al., 1996). As a synthesized MOR antagonist, like methocinnamox rather than other MOR antagonists such as β-funaltrexamine, C-CAM is devoid of agonist effects in vivo (Butelman et al., 1996; Zernig et al., 1996; Moynihan et al., 2009). In addition, it was unlikely that the dose of C-CAM (5 mg · kg^{-1}) was large enough to completely antagonize MOR-mediated antinociception by pentazocine at doses up to 100 mg · kg^{-1}, because studies have shown that only 0.5 mg · kg^{-1} of C-CAM can completely

antagonize antinociception to mechanical pain by morphine at 10 mg · kg^{-1} (Shu et al., 2006a), and 10 mg · kg^{-1} of C-CAM can eliminate antinociception by morphine at doses up to 1000 mg · kg^{-1} (Paronis and Woods, 1997). Taken together, it is likely that antinociception to mechanical pain by high-dose pentazocine was mediated not by MORs but by KORs, because it was not antagonized by C-CAM alone but was completely antagonized by C-CAM + nor-BNI. However, this assumption seemed apparently contradictory to our finding that nor-BNI alone did not antagonize, but rather enhanced, antinociception to mechanical pain by high-dose pentazocine.

However, these complex findings might be clearly explained by the following assumptions: 1) low-dose pentazocine might activate MORs almost exclusively, whereas high-dose pentazocine might activate MORs and KORs concurrently; 2) antinociception to mechanical pain by high-dose pentazocine, which was reduced compared with that by pentazocine at 30 mg · kg^{-1}, might be mediated not by MORs but by KORs, because it could be antagonized completely by C-CAM + nor-BNI, but not at all by C-CAM alone; 3) MOR-mediated antinociception by high-dose pentazocine might be self-antagonized by concurrent activation of KORs, and therefore, C-CAM did not antagonize antinociception to mechanical pain by high-dose pentazocine, as was suggested by previous reports showing that KOR agonists can antagonize MOR-mediated morphine antinociception (Pan, 1998; Meng et al., 2005; Shu et al., 2008); 4) pretreatment with nor-BNI alone might unmask MOR-mediated antinociception by high-dose pentazocine by inhibiting KOR-mediated self-antagonism against MOR-mediated pentazocine antinociception, thereby enhancing antinociception to mechanical pain by high-dose pentazocine and changing the dose-response curve from a bell-shaped to sigmoid one; and 5) antinociception by high-dose pentazocine enhanced by nor-BNI might be mediated primarily by MORs, because C-CAM + nor-BNI completely antagonized antinociception to mechanical pain by high-dose pentazocine, indicating that C-CAM could completely antagonize antinociception to mechanical pain by high-dose pentazocine enhanced by nor-BNI.

Regarding pentazocine-induced antinociception to thermal pain, we found that 1) pentazocine at 30 mg · kg^{-1} produced the maximum thermal antinociceptive effect, whereas pentazocine at higher doses produced no antinociception; 2) not only concurrent treatment with C-CAM and nor-BNI but also treatment with C-CAM alone completely antagonized this low-dose pentazocine-induced antinociception, whereas nor-BNI did not antagonize this at all; and 3) pretreatment with nor-BNI resulted in the appearance of antinociception by high-dose pentazocine and thus changed the dose-response curve from a bell-shaped to sigmoid one. The same assumptions applied to explain the bell-shaped dose-response curve for pentazocine-induced antinociception to mechanical pain might be applied to explain the bell-shaped dose-response curve for pentazocine-induced antinociception to thermal pain. Only one difference existed between antinociception to mechanical pain and antinociception to thermal pain in that high-dose pentazocine produced some antinociception to mechanical pain while not producing any significant antinociception to thermal pain. However, several lines of evidence show that KOR agonists or mixed agonist-antagonists with KOR agonistic property exert differential actions on thermal and nonthermal nociceptive stimuli, i.e., they do not inhibit thermal nociceptive responses while inhibiting mechanical as well as visceral chemical nociceptive responses (Schmauss et al., 1983; Schmauss, 1987; Fürst, 1991; Yaksh, 1997), probably because of differences in neuronal pathways involved in thermal and nonthermal nociceptive information possessing different classes of opioid receptors (Tyers, 1980) and/or differences in the intensity of the noxious stimulus and the intrinsic activity of the drug (Delaney et al., 1986; Shaw et al., 1988). Therefore, it seemed plausible that high-dose pentazocine produced some antinociception to mechanical pain mediated by KORs as a result of complex interaction between coactivated MORs and KORs, while producing no antinociception to thermal pain by the same mechanisms.

Regarding pentazocine-induced antinociception to visceral pain, we found that 1) pentazocine produced dose-response antinociception in the acetic acid writhing test; 2) C-CAM completely antagonized antinociception by low-dose pentazocine while only partly antagonizing antinociception by high-dose pentazocine; and 3) concurrent treatment with C-CAM and nor-BNI completely antagonized antinociception by high-dose pentazocine. These findings are consistent with our above-mentioned assumptions that low-dose pentazocine activates MORs almost exclusively, whereas high-dose pentazocine activates MORs and KORs concurrently. However, there were some differences between antinociception to somatic pain and antinociception to visceral pain by pentazocine. First, pentazocine exhibited biphasic bell-shaped dose-response curves peaking at 30 mg · kg^{-1} in antinociception to somatic pain, whereas exhibiting a sigmoid curve peaking at 30 to 100 mg · kg^{-1} in antinociception to visceral pain. Second, high-dose pentazocine-induced antinociception to visceral pain, but not somatic antinociception to mechanical pain, was partly antagonized by C-CAM, although both were completely antagonized by C-CAM + nor-BNI. These results suggest that antinociception to mechanical pain by high-dose pentazocine was mediated almost exclusively by KORs as a result of complex interaction between activated MORs and KORs, whereas antinociception to visceral pain by high-dose pentazocine was mediated by both MORs and KORs. These results also suggested that MOR-mediated antinociception to mechanical pain by high-dose pentazocine was completely antagonized by simultaneous activation of KORs, whereas MOR-mediated antinociception to visceral pain was not. Mechanisms underlying such differences were unclear. However, the differences might result from some differences in neuronal pathways involved in visceral nociception and somatic nociception (Tanimoto et al., 2003; Cervero et al., 2004; Ren et al., 2009).

However, the results of this study are not consistent with a previous report (Chien and Pasternak, 1995) that showed that the same biphasic dose-response curve with (−)-pentazocine appeared in CD-1 mice, but increasing doses of nor-BNI dose-dependently antagonized (not enhanced) pentazocine analgesia. Furthermore, they found that the sigma receptor antagonist but not KOR antagonist, potentiated the high-dose pentazocine analgesia. The reason causing these differences is unclear. The pentazocine used in Chien and Pasternak's study was optically pure (−)-isomer; however, the drug used in our study was a racemic form containing (+)- and (−)- isomers. As described above, these two isomers exhibit quite different pharmacological effects, e.g., (−)-pentazocine exerts an agonistic effect on KOR, whereas (+)-pentazocine does not, instead displaying a 10-fold greater

affinity for the sigma receptor than (−)- isomer (Chien and Pasternak, 1995). In addition, the two isomers in the racemic mixture may modulate pharmacological effects of each other (Kinouchi et al., 1986). Thus, it may be the difference between pure (−)- isomer and racemic forms brings significant problems with the influence of sigma receptors on observed antinociceptive effects, thereby resulting in the difference between our study and Chien and Pasternak's study. Another possible explanation is the differences of mouse strains. In Chien and Pasternak's study, CD-1 mice were selected, which were sensitive to KOR drugs, whereas in our study two mouse strains, ddY mice and C57BL/6J mice, were investigated, and similar effects were obtained.

In conclusion, pentazocine produced antinociception to somatic mechanical as well as thermal pain characterized by bell-shaped dose-response curves peaking at 30 mg · kg^{-1}, while producing antinociception to visceral pain characterized by a sigmoid dose-response curve peaking at 30 to 100 mg · kg^{-1} in ddY mice. Our data suggested that pentazocine at low doses (30 mg · kg^{-1} or less) produced antinociceptive effects to somatic and visceral pain dose-dependently via activation of MORs almost exclusively, whereas pentazocine at high doses (> 30 mg · kg^{-1}) activated MORs and KORs concurrently. High-dose pentazocine produced reduced antinociception to mechanical pain and no antinociception to thermal pain not via MORs but via KORs, presumably because activated KORs antagonized MOR-mediated antinociception, while producing unreduced antinociception to visceral pain via activation of both MORs and KORs. Pretreatment with the KOR antagonist nor-BNI enhanced antinociception to somatic pain by high-dose pentazocine and changed dose-response curves from bell-shaped to sigmoid ones by inhibiting KOR-mediated antagonism against MOR-mediated antinociception, thus unmasking MOR-mediated pentazocine antinociception. Our conclusions that pentazocine-induced antinociception is mediated by MORs but compromised by coactivation of KORs provide the first reasonable hypothesis to explain the biphasic bell-shaped dose response curves for antinociception to somatic pain by pentazocine. The present study provides an example of evidence that the cross-talk between opioid G protein-coupled receptors is an important subject of analgesic activity and tolerance development.

Authorship Contributions

Participated in research design: Shu, Hayashida, Arita, and Hanaoka.

Conducted experiments: Shu, Zhang, and Wu.

Performed data analysis: Shu, Hayashida, An, and Huang.

Wrote or contributed to the writing of the manuscript: Shu and Hayashida.

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Address correspondence to: Haihua Shu, Department of Anesthesiology, First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong, 510080, China. E-mail: shuhaihua@gmail.com