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**Pentazocine-induced antinociception is mediated mainly by mu-opioid receptors
and compromised by kappa-opioid receptors in mice**

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a) Running title: Mechanism of biphasic dose-response of pentazocine antinociception

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c) This article includes: 26 text pages: 1 table, 6 figures, 36 references. The words in the Abstract were 249, in Introduction were 412, and in Discussion were 1769.

d) List of nonstandard abbreviations: C-CAM, clocinnamox mesylate; nor-BNI, nor-binaltorphimine; KOR, kappa-opioid receptor; MOR, mu-opioid receptor; DMSO, dimethyl sulfoxide; AUC, area under the response-versus-time curve

e) A recommended section: Neuropharmacology

Abstract

Pentazocine is a widely used mixed agonist–antagonist opioid. Previous animal studies demonstrated that pentazocine-induced antinociception displayed a ceiling effect characterized by biphasic dose response with a increasing and then descending analgesia which like a bell-shaped curve. This study attempted to clarify the mechanisms underlying such dose-response relationships. ddY or C57BL/6J Mice received subcutaneous injection of saline or pentazocine 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after subcutaneous injection of saline, a mu-opioid receptor antagonist clocinnamox mesylate (C-CAM) 5 mgkg⁻¹, a kappa-opioid receptor antagonist nor-binaltorphimine (nor-BNI) 10 mgkg⁻¹, or the combination of C-CAM and nor-BNI, respectively. The antinociceptive effects of pentazocine were evaluated with 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 mgkg⁻¹. C-CAM completely and partly antagonized the antinociception induced by pentazocine at low doses (3-30 mgkg⁻¹) and high doses (56-100 mgkg⁻¹), 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

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primarily via activation of mu-opioid receptors, but at high doses, this mu-opioid receptor mediated antinociception is antagonized by concomitant activation of kappa-opioid receptors. This provides the first reasonable hypothesis to explain the ceiling effects of pentazocine analgesia characterized by a biphasic dose-response.

Introduction

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

Previous dose-response studies demonstrate that pentazocine exhibits a biphasic dose response curve that at low to moderate doses it produces dose-dependent antinociception while that at high doses produces diminished antinociception (Fürst and Hosztafi, 2008; Hoskin and Hanks, 1991). Although buprenorphine exhibits a similar biphasic dose-response curve probably via concomitant activation of opioid

receptor-like (ORL-1) receptors (Lutfy et al, 2003; Lutfy and Cowan, 2004), mechanisms underlying the biphasic dose-response curve for pentazocine antinociception remains unknown. While a partial opioid receptor agonist commonly exhibit a ceiling effect that maximal analgesia was observed at a certain dose and neither increased nor decreased with higher doses, 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 (Chiba et al., 2009; Meng et al., 2005; Pan, 1998; Shu et al., 2006b and 2008), one may hypothesize that co-activation 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 acting as an insurmountable, long-acting MOR antagonist in behavioral experiments (Paronis and Woods, 1997; Zernig et al., 1996), and nor-binaltorphimine (nor-BNI), a selective KOR antagonist exerting 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 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 at 23-28 g and Male C57BL/6J mice (Animal center in Sun Yat-sen University, Guangzhou, China) weighing at 18-22 g were used. Mice were housed in a controlled temperature (23 ± 2 °C) and humidity ($55 \pm 10\%$) environment with a 12 h-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 a.m.-7:00 p.m.). All behavior tests were performed by a single investigator blinded to the treatment groups. In each of the tail pressure, hot plate, and tail flick tests, the test was repeated three times at intervals of 30 min prior to 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 Randall-Sellito Analgesy Meter (Ugo

Basile, Comerio, Italy), as described previously (Shu et al., 2006a). Briefly, the distal part of the tail was supported by a plinth while pressure, linearly increasing at a rate of 16 gs^{-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 employed 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., Tokyo, Japan), as described previously (Shu et al., 2007). Briefly, mice were placed on an aluminum hot plate thermo-controlled at $52 \pm 0.5 \text{ }^{\circ}\text{C}$. The latency to a positive response, defined as licking of the fore- or hind-paws, flicking of the hind-paws, or jumping, was measured. A cutoff latency of 30 s was employed to avoid tissue damage.

Tail flick test

The tail flick test was performed using an automated tail flick analgesimeter (Model MK-330A, Muromachi Kikai, Tokyo, Japan), as described previously (Shu et al., 2008). Briefly, a light beam was focused on the dorsal surface of the tail 2.5cm 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 employed 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). Briefly, mice received intraperitoneal (i.p.) injection of 0.6% acetic acid solution (10 mlkg⁻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 the 10-min period from 5 to 15 min after the i.p. injection of acetic acid.

Drugs and chemicals

Pentazocine Hydrochloride (C₁₉H₂₇NO·HCl) (Pentagin, dl-pentazocine; Sankyo Pharmaceutical, Tokyo, Japan) and nor-binaltorphimine (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 mlkg⁻¹ body weight) for subcutaneous (s.c.) injection. clocinnamox mesylate (C-CAM; 14 beta-(p- chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphin one mesylate) (Tocris Cookson Inc., Ellisville, MO, USA) was dissolved in dimethyl

sulfoxide (DMSO) and diluted with normal saline (10 mlkg⁻¹ body weight) for s.c. injection. Acetic acid (Kyowa Hakko Chemical, Tokyo, Japan) was dissolved in normal saline (0.6%, 10 mlkg⁻¹ body weight) for i.p 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 s.c. saline or pentazocine 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120min after pretreatment with s.c. placebo saline, s.c. C-CAM (5 mgkg⁻¹), s.c. nor-BNI (10 mgkg⁻¹), or the combination of s.c. C-CAM and nor-BNI, respectively (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 s.c. 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 s.c. saline or pentazocine 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120min after pretreatment with s.c. placebo saline, s.c. C-CAM

(5 mgkg⁻¹), s.c. nor-BNI (10 mgkg⁻¹), or the combination of s.c. C-CAM and nor-BNI, respectively (n = 8 for each group). Fifty min after the s.c. pentazocine injection, mice received i.p. injection of acetic acid. The number of writhing in each mouse was counted over the 10-min period from 5 to 15 min after the i.p. 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 s.c. saline or pentazocine 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with s.c. placebo saline, s.c. C-CAM (5 mgkg⁻¹), s.c. nor-BNI (10 mgkg⁻¹), or the combination of s.c. C-CAM and nor-BNI, respectively (n = 8 for each group). The tail pressure were performed before, and at 30, 60, 90, 120, 150 and 180 min after, the s.c. 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, however, we considered the AUC the best summary of the antinociceptive effect of pentazocine. The AUC was calculated according to the following formula based on Trapezoid rule:

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$$\text{AUC} = 0.5 h \times (\text{the sum of data measured at set time points}) / 2$$

Data are expressed as mean \pm S.E.M or median (range) according to data types.

Changes in continuous variables over time were analyzed with repeated measures analysis of variance (ANOVA), followed by Fisher's protected least significance difference test (PLSD). Inter-group comparisons were made with ANOVA and Fisher's PLSD test. 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 mgkg⁻¹, at 10, 30, and 56 mgkg⁻¹, and at 100 mgkg⁻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 mgkg⁻¹ than with saline, with pentazocine 10 mgkg⁻¹ than 3 mgkg⁻¹, and with pentazocine 30 mgkg⁻¹ than 10 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD) (Fig. 5A), while the AUCs were less with pentazocine 56 and 100 mgkg⁻¹ than 10 and 30 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD) (Fig. 5A). These results indicated that pentazocine 3 to 100 mgkg⁻¹ produced significant antinociception to mechanical pain, demonstrating a biphasic dose response which like bell-shaped curve, peaking at 30 mgkg⁻¹.

In the hot plate test, only pentazocine at 30 mgkg⁻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 30 mgkg⁻¹ than with saline ($p < 0.05$ by Fisher's PLSD) (Fig. 5B). These results indicated that only pentazocine 30 mgkg⁻¹ produced significant antinociception to thermal pain in the hot plate test, and that the dose-response curve was biphasic, peaking at 30 mgkg⁻¹.

In the tail flick test, pentazocine at 10 mgkg⁻¹ and at 30 mgkg⁻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 10 and 30 mgkg^{-1} than with saline, and with pentazocine 30 mgkg^{-1} than 10 mgkg^{-1} ($p < 0.05$ by Fisher's PLSD), whereas they were not more with pentazocine 3, 56, and 100 mgkg^{-1} than with saline ($p > 0.05$ by Fisher's PLSD) (Fig. 5C). These results indicated that pentazocine 10 and 30 mgkg^{-1} , but not 3, 56, or 100 mgkg^{-1} , produced significant antinociception to thermal pain in the tail flick test, and that the dose-response curve was biphasic, peaking at 30 mgkg^{-1} .

Effects of C-CAM on antinociception to somatic pain in ddY mice by pentazocine

After pretreatment with C-CAM 5 mgkg^{-1} , pentazocine 3, 10, or 30 mgkg^{-1} did not produce significant antinociception to mechanical pain, compared with saline, in the tail pressure test ($p > 0.05$ by Fisher's PLSD), whereas pentazocine 56 and 100 mgkg^{-1} produced significant antinociception to mechanical pain at 30 and 60 min ($p < 0.05$ by Fisher's PLSD) (Fig. 2A). The AUCs with pentazocine 3, 10, and 30 mgkg^{-1} were not more than the AUC with saline in the C-CAM-treated mice, and the AUCs with pentazocine 3, 10, and 30 mgkg^{-1} were significantly less in the C-CAM-treated than the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 5A). On the other hand, the AUCs with pentazocine 56 and 100 mgkg^{-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 mgkg^{-1} were not different between the C-CAM-treated and the

saline-treated mice ($p > 0.05$ by Fisher's PLSD) (Fig. 5A). These results indicated that C-CAM completely antagonized antinociception to mechanical pain by pentazocine 3, 10, 30 mgkg^{-1} , while not antagonizing that induced by pentazocine 56 and 100 mgkg^{-1} at all.

After treatment with C-CAM, pentazocine at any doses did not produce significant antinociception to thermal pain, compared with saline, in the hot plate or tail flick test ($p > 0.05$ by Fisher's PLSD) (Figs. 2B and 2C). In the C-CAM-treated mice, the AUCs with pentazocine at any doses were not more than the AUC with saline in the hot plate or tail flick test (Figs. 5B and 5C). 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 mgkg^{-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 mgkg^{-1}), pentazocine 3, 10, 30, 56, and 100 mgkg^{-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. 3A). In the nor-BNI-treated mice, the AUCs were significantly more with pentazocine 3, 10, 30, 56, and 100 mgkg^{-1} than with saline, and with pentazocine 30, 56, and 100 mgkg^{-1} than with pentazocine 3 and 10 mgkg^{-1} ($p < 0.05$ by Fisher's PLSD), while the AUCs

were not different between pentazocine 3 and 10 mgkg⁻¹, or among pentazocine 30, 56, and 100 mgkg⁻¹, respectively ($p > 0.05$ by Fisher's PLSD) (Fig. 5A). The AUCs with pentazocine 56 and 100 mgkg⁻¹ were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 5A). These results indicated that nor-BNI enhanced antinociception to mechanical pain induced by pentazocine 56 and 100 mgkg⁻¹ in the tail pressure test, and changed the biphasic bell-shaped dose-response curve peaking at 30 mgkg⁻¹ to a sigmoid curve peaking about 30 mg/kg without diminishment at higher doses.

After treatment with nor-BNI, pentazocine at 30 mgkg⁻¹, at 56 mgkg⁻¹, and at 100 mgkg⁻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. 3B). In the nor-BNI-treated mice, the AUCs were significantly more with pentazocine 30, 56, and 100 mgkg⁻¹ than with saline, and with pentazocine 56 and 100 mgkg⁻¹ than with pentazocine 30 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD), while they were not different between pentazocine 56 and 100 mgkg⁻¹ ($P > 0.05$ by Fisher's PLSD) (Fig. 5B). The AUCs with pentazocine 56 and 100 mgkg⁻¹ were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 5B). These results indicated that nor-BNI enhanced antinociception to thermal pain induced by pentazocine 56 and 100 mgkg⁻¹ in the hot plate test, and changed the biphasic bell-shaped dose-response curve peaking at 30

mgkg⁻¹ to a sigmoid curve peaking about 30 mg/kg without diminishment at higher doses.

After treatment with nor-BNI, pentazocine at 3 mgkg⁻¹, at 10 mgkg⁻¹, and at 30, 56, and 100 mgkg⁻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. 3C). In the nor-BNI-treated mice, the AUCs were more with pentazocine 10, 30, 56, and 100 mgkg⁻¹ than with saline and pentazocine 3 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD), while they were not different among pentazocine 10, 30, 56 and 100 mgkg⁻¹ ($p > 0.05$ by Fisher's PLSD) (Fig. 5C). The AUCs with pentazocine 10, 56, and 100 mgkg⁻¹ were more in the nor-BNI-treated mice than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig. 5C). These results indicated that nor-BNI enhanced antinociception to thermal pain induced by pentazocine 10, 56, and 100 mgkg⁻¹ in the tail flick test, and changed the biphasic bell-shaped dose-response curve peaking at 30 mgkg⁻¹ to a sigmoid curve peaking about 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 mgkg⁻¹) and C-CAM (5 mgkg⁻¹), pentazocine at any doses did not produce significant antinociception to mechanical or

thermal pain, compared with saline, in the tail pressure, hot plate, or tail flick test (Fig.4), and the AUCs were not more with pentazocine at any doses than with saline (Figs.5A, 5B, and 5C). 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 number of writhing was less with pentazocine 3 mgkg⁻¹ than saline, pentazocine 10 mgkg⁻¹ than 3 mgkg⁻¹, and pentazocine 30, 56, and 100 mgkg⁻¹ than 10 mgkg⁻¹ ($P < 0.05$ by Mann-Whitney test), while it was not different among pentazocine 30, 56, and 100 mgkg⁻¹ ($P > 0.05$ by Mann-Whitney test) (Table). These results indicated that pentazocine inhibited acetate-induced writhing in a dose-dependent manner that maximal analgesia was observed at 30 mgkg⁻¹ and neither increased nor decreased with higher doses (Table).

In the C-CAM-treated mice, pentazocine 3, 10, or 30 mgkg⁻¹ did not significantly decrease the number of writhing, compared with saline ($P > 0.05$ by Mann-Whitney test), while pentazocine 56 and 100 mgkg⁻¹ significantly decreased the number 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). These results indicated that C-CAM completely antagonized the inhibition of

acetate-induced writhing by pentazocine 3, 10, and 30 mgkg⁻¹, but only partly antagonized the inhibition by pentazocine 56 and 100 mgkg⁻¹.

In mice pretreated with both C-CAM and nor-BNI, pentazocine at any doses did not significantly decrease the number of acetic acid-induced writhing, compared with saline ($P > 0.05$ by Mann-Whitney test) (Table). 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 mgkg⁻¹, at 10, 30, and 56 mgkg⁻¹, and at 100 mgkg⁻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. 6 A). The AUCs were more with pentazocine 3, 10, 30, 56, and 100 mgkg⁻¹ than with saline, with pentazocine 10 mgkg⁻¹ than 3 mgkg⁻¹, and with pentazocine 30 mgkg⁻¹ than 10 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD) (Fig. 6B), while the AUCs were less with pentazocine 56 and 100 mgkg⁻¹ than 10 and 30 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD) (Fig. 6B). These results indicated that pentazocine 3 to 100 mgkg⁻¹ produced significant antinociception to mechanical pain in C57BL/6J mice,

similar to in ddY mice which 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 mgkg⁻¹ were not more than the AUC with saline, and the AUCs with pentazocine 3, 10, and 30 mgkg⁻¹ were significantly less in the C-CAM-treated than in the saline-treated mice ($p < 0.05$ by Fisher's PLSD) (Fig.6B). On the other hand, the AUCs with pentazocine 56 and 100 mgkg⁻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 mgkg⁻¹ were not different between the C-CAM-treated and the saline-treated mice ($p > 0.05$ by Fisher's PLSD) (Fig. 6B). These results indicated that in C57BL/6J mice C-CAM completely antagonized antinociception to mechanical pain by pentazocine 3, 10, 30 mgkg⁻¹, while not antagonizing that induced by pentazocine 56 and 100 mgkg⁻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 3, 10, 30, 56, and 100 mgkg⁻¹ than with saline, and with pentazocine 30, 56, and 100 mgkg⁻¹ than with pentazocine 3 and 10 mgkg⁻¹ ($p < 0.05$ by Fisher's PLSD), with pentazocine 10 mgkg⁻¹ than 3 mgkg⁻¹ while the AUCs were not different among pentazocine 30, 56, and 100 mgkg⁻¹ ($p > 0.05$ by Fisher's

PLSD) (Fig. 6B). The AUCs with pentazocine 56 and 100 mgkg⁻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 indicated that nor-BNI enhanced antinociception to mechanical pain induced by pentazocine 56 and 100 mgkg⁻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 doses did not produce significant antinociception to mechanical pain, the AUCs were not more with pentazocine at any doses than with saline (Fig 6B). These results indicated 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, named (+)-pentazocine and (-)-pentazocine. These two optically pure compounds usually demonstrate different even opposing pharmacological properties, e.g., (-)-pentazocine is a KOR agonist, while (+)-pentazocine is not (Kinouchi et al. 1986; Chien and Pasternak, 1995). In the present study, we used clinical practice one which was dl-pentazocine (racemic form). It was 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 characterized as biphasic dose response with a increasing and then descending analgesia which like a bell-shaped curve, peaking at 30 mgkg⁻¹ using the tail pressure, hot plate, and tail flick tests, while exhibiting a ceiling effect on antinociception to visceral pain in a dose-dependent manner that maximal analgesia was observed at 30 mgkg⁻¹ and neither increased nor decreased with higher doses using the acetic acid writhing test. The similar antinociception could be seen in C57BL/6J mice when assessed by the tail pressure test.

It may wonder that the later descending portion of pentazocine biphasic dose-response curve may 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 mgkg⁻¹) increased

to the level of 30 mgkg⁻¹ when pretreated with nor-BNI, a selective kappa opioid receptor 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 mgkg⁻¹ produced the maximum antinociceptive effect, while pentazocine at higher doses produced reduced antinociception; (2) a selective MOR antagonist C-CAM (5 mgkg⁻¹) completely antagonized antinociception by pentazocine at low doses (30 mgkg⁻¹ or less) while not antagonizing that by pentazocine at higher doses; (3) a selective KOR antagonist nor-BNI (10 mgkg⁻¹) enhanced, rather than antagonized, antinociception by high-dose pentazocine and thus changed the later descending portion of the biphasic dose response curve to 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 primarily mediated by MORs but not by KORs, since 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 mgkg⁻¹, seemed complex, since it was not antagonized by C-CAM alone, enhanced by nor-BNI alone, and completely antagonized by C-CAM + nor-BNI. It was shown that C-CAM acted as an high

selective and insurmountable mu antagonist, but not delta or kappa opioid agonists in behavioral experiments, although it displayed only moderate mu selectivity (29:6:1 for mu:delta:kappa) in radioligand displacement experiments (Zernig et al., 1996). As a synthesized MOR antagonist, like Methocinnamox (M-CAM) rather than other MOR antagonists such as β -funaltrexamine (β -FNA), C-CAM is devoid of agonist effects in vivo (Butelman et al., 1996; Moynihan et al., 2009; Zernig et al., 1996). In addition, it was unlikely that the dose of C-CAM (5 mgkg^{-1}) was not large enough to completely antagonize MOR-mediated antinociception by pentazocine at doses up to 100 mgkg^{-1} , since studies show that only 0.5 mgkg^{-1} of C-CAM can completely antagonize antinociception to mechanical pain by morphine 10 mgkg^{-1} (Shu et al., 2006b), and that 10 mgkg^{-1} of C-CAM can eliminate antinociception by morphine at doses up to 1000 mgkg^{-1} (Paronis and Woods, 1997). Taken together, it was likely that antinociception to mechanical pain by high-dose pentazocine was mediated not by MORs but by KORs, since it was not antagonized by C-CAM alone while being completely antagonized by C-CAM + nor-BNI. However, this assumption seemed apparently contradictory to our finding that nor-BNI alone did not antagonize, or 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 mgkg^{-1} , might be mediated not by MORs but by KORs, since 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 already self-antagonized by concurrent activation of KORs, and therefore, C-CAM did not antagonize antinociception to mechanical pain by high-dose pentazocine, as were 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, since 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 30 mgkg^{-1} produced the maximum thermal antinociceptive effect, while 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 as was applied to explain the bell-shaped dose-response curve for pentazocine-induced antinociception to mechanical pain might be applied also 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 non-thermal nociceptive stimuli, i.e. they do not inhibit thermal nociceptive responses while inhibiting mechanical as well as visceral chemical nociceptive responses (Fürst, 1991; Schmauss et al., 1983; Schmauss, 1987; ; Yaksh, 1997), probably due to differences in neuronal pathways involved in thermal and non-thermal 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 co-activated 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 using 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 mgkg^{-1} in antinociception to somatic pain, while exhibiting a sigmoid curve peaking at 30 to 100 mgkg^{-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 suggested 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 (Cervero et al, 2004; Ren et al, 2009; Tanimoto et al, 2003).

However, the results of this study are not in consist with the previous report (Chien and Pasternak, 1995) which 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 caused these difference was unclear. The pentazocine used in Chien and Pasternak's study was optically pure (-)-isomer, however, the drug used in our study was racemic form which containing (+)- and (-)-isomers. As it described above, these two isomers exhibit quite different pharmacological effects, e.g., (-)-pentazocine exerts an agonistic effect on KOR, while (+)-pentazocine does not, instead displaying a ten-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 form and racemic form brings significant problems with 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 study, CD-1 mice was selected which was sensitive to KOR drugs (Chien and Pasternak, 1995), while in our study two mouse strains including ddY mice and C57BL/6J mice were investigated and the 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 mgkg⁻¹, while producing antinociception to visceral pain characterized by a sigmoid dose-response curve peaking at 30 to 100 mgkg⁻¹ in ddY mice. Our data suggested that pentazocine at low doses (30 mgkg⁻¹ or less) produced antinociceptive effects to somatic and visceral pain dose-dependently via activation of MORs almost exclusively, while pentazocine at high doses (> 30 mgkg⁻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 a KOR antagonist nor-BNI enhanced antinociception to somatic pain

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by high-dose pentazocine and changed dose-response curves from bell-shaped to sigmoid ones by inhibiting KOR-mediated antagonism against MOR-mediated antinociception, and thus by unmasking MOR-mediated pentazocine antinociception. Our conclusions that pentazocine-induced antinociception is mediated by MORs but compromised by co-activation 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 (GPCRs) is important subject of analgesic activity and tolerance development.

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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

Other: Shu and Hanaoka acquired funding for the research.

References

- Bidlack JM, McLaughlin JP, Wentland MP (2000). Partial opioids. Medications for the treatment of pain and drug abuse. *Ann N Y Acad Sci* 909: 1-11.
- Butelman ER, Negus SS, Lewis JW and Woods JH (1996). Clocinnamox antagonism of opioid suppression of schedule-controlled responding in rhesus monkeys. *Psychopharmacology* 123:320–324.
- Cervero F, Laird JM (2004). Understanding the signaling and transmission of visceral nociceptive events. *J Neurobiol* 61: 45-54.
- Chiba S, Hayashida M, Yoshikawa M, Shu H, Nishiyama T, Yamada Y (2009). Inhibitory effect of low-dose pentazocine on the development of antinociceptive tolerance to morphine. *J Anesth* 23:99-107.
- Chien CC, Pasternak GW (1995). (-)-Pentazocine analgesia in mice: interactions with a sigma receptor system. *Eur J Pharmacol* 294:303-308
- Delaney KM, Rourke JD, Shaw JS (1986). Sensitivity of antinociceptive tests to opioid agonists and partial agonists. *NIDA Res Monogr* 75: 438-441.
- Endoh T, Matsuura H, Tanaka C, Nagase H (1992). Nor-binaltorphimine: a potent and selective kappa-opioid receptor antagonist with long-lasting activity in vivo. *Arch Int Pharmacodyn Ther* 316: 30–42.
- Fudala PJ, Johnson RE (2006). Development of opioid formulations with limited diversion and abuse potential. *Drug Alcohol Depend* 83 (Suppl 1): S40-47.

Fürst S (1991). Pharmacological interaction of opiates with various classes of centrally acting dopaminergic drugs. *Drug Metabol Drug Interact* 9: 77-102.

Fürst S, Hosztafi S (2008). The chemical and pharmacological importance of morphine analogues. *Acta Physiol Hung* 95: 3-44.

Gutstein HB, Akil H (2006). Opioid analgesics. In: Brunton LL, Lazo JS, Parker KL, (ed). *Goodman and Gilman's: the pharmacological basis of therapeutics*. McGrawHill/Hardman: New York, pp. 547–590.

Hayes AG, Tyers MB (1983). Determination of receptors that mediate opiate side effects in the mouse. *Br J Pharmacol* 79:731-736.

Hoskin PJ, Hanks GW (1991). Opioid agonist-antagonist drugs in acute and chronic pain states. *Drugs* 41: 326–344.

Kinouchi K, Fukumitsu K, Takezawa J, Maeda S, Enomoto H, Kakuhari J, Yoshiya I, Inoki R (1986). Stereospecific effects of d- and l-pentazocine on contractions of the mouse vas deferens. *Life Sci* 39:127-133.

Kurihara T, Nonaka T, Tanabe T (2003). Acetic acid conditioning stimulus induces long-lasting antinociception of somatic inflammatory pain. *Pharmacol Biochem Behav* 74: 841-849.

Lutfy K, Eitan S, Bryant CD, Yang YC, Saliminejad N, Walwyn W, Kieffer BL, Takeshima H, Carroll FI, Maidment NT, Evans CJ (2003). Buprenorphine-induced antinociception is mediated by mu-opioid receptors and

- compromised by concomitant activation of opioid receptor-like receptors. *J Neurosci* 23: 10331-10337.
- Lutfy K, Cowan A (2004). Buprenorphine: a unique drug with complex pharmacology. *Curr Neuropharmacol* 2: 395-402.
- Meng ID, Johansen JP, Harasawa I, Fields HL (2005). Kappa opioids inhibit physiologically identified medullary pain modulating neurons and reduce morphine antinociception. *J Neurophysiol* 93: 1138–1144.
- Moynihan H, Jales AR, Greedy BM, Rennison D, Broadbear JH, Purington L, Traynor JR, Woods JH, Lewis JW, Husbands SM (2009). 14 beta-O-cinnamoylnaltrexone and related dihydrocodeinones are mu opioid receptor partial agonists with predominant antagonist activity. *J Med Chem* 52:1553-7
- Pan ZZ (1998). Mu-opposing actions of kappa-opioid receptor. *Trends Pharmacol Sci* 19: 94–98.
- Paronis CA, Woods JH (1997). Clocinnamox dose-dependently antagonizes morphine analgesia and [3H]DAMGO binding in rats. *Eur J Pharmacol* 337: 27–34.
- Preston KL, Bigelow GE (1993). Differential naltrexone antagonism of hydromorphone and pentazocine effects in human volunteers. *J Pharmacol Exp Ther* 264: 813-823.
- Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI, Reisine T (1994). Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid

receptors. *Mol Pharmacol* 45: 330-334.

Ren Y, Zhang L, Lu Y, Yang H, Westlund KN (2009). Central lateral thalamic neurons receive noxious visceral mechanical and chemical input in rats. *J Neurophysiol* 102: 244-258.

Schmauss C, Yaksh TL, Shimohigashi Y, Harty G, Jensen T, Rodbard D (1983). Differential association of spinal mu, delta and kappa opioid receptors with cutaneous thermal and visceral chemical nociceptive stimuli in the rat. *Life Sci* 33: 653–656.

Schmauss C (1987). Spinal kappa-opioid receptor-mediated antinociception is stimulus-specific. *Eur J Pharmacol* 137: 197–205.

Shaw JS, Rourke JD, Burns KM (1988). Differential sensitivity of antinociceptive tests to opioid agonists and partial agonists. *Br J Pharmacol* 95: 578-84.

Shu H, Arita H, Hayashida M, Sekiyama H, Hanaoka K (2006a). Effects of processed Aconiti tuber and its ingredient alkaloids on the development of antinociceptive tolerance to morphine. *J Ethnopharmacol* 103: 398–405.

Shu H, Arita H, Hayashida M, Chiba S, Sekiyama H, Hanaoka K (2006b). Inhibition of morphine tolerance by processed Aconiti tuber is mediated by kappa-opioid receptors. *J Ethnopharmacol* 106(2):263-71.

Shu H, Hayashida M, Chiba S, Sekiyama H, Kitamura T, Yamada Y, Hanaoka K, Arita H (2007). Inhibitory effect of processed Aconiti tuber on the development of

antinociceptive tolerance to morphine: evaluation with a thermal assay. *J Ethnopharmacol* 113: 560-563.

Shu H, Hayashida M, Arita H, Huang W, Xiao L, Chiba S, Sekiyama H, Hanaoka K (2008). High doses of processed Aconiti tuber inhibit the acute but potentiate the chronic antinociception of morphine. *J Ethnopharmacol* 119: 276-283.

Suzuki T, Narita M, Misawa M, Nagase H (1991). Pentazocine-induced biphasic analgesia in mice. *Life Sci* 48:1827-1835.

Tanimoto S, Nakagawa T, Yamauchi Y, Minami M, Satoh M (2003). Differential contributions of the basolateral and central nuclei of the amygdala in the negative affective component of chemical somatic and visceral pains in rats. *Eur J Neurosci* 18: 2343-2350.

Tyers MB (1980). A classification of opiate receptors that mediate antinociception in animals. *Br J Pharmacol* 69: 503-512.

Yaksh TL (1997). Pharmacology and mechanisms of opioid analgesic activity. *Acta Anaesthesiol Scand* 41: 94-111

Zacny JP, Hill JL, Black ML, Sadeghi P (1998). Comparing the subjective, psychomotor and physiological effects of intravenous pentazocine and morphine in normal volunteers. *J Pharmacol Exp Ther* 286: 1197-1207.

Zernig G, Burke T, Lewis JW, Woods JH (1996). Mechanism of clocinnamox blockade of opioid receptors: evidence from in vitro and ex vivo binding and behavioral

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assays. J Pharmacol Exp Ther 279: 23–31.

Footnotes

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Figure legends

Fig.1 Dose response of antinociceptive effects to somatic pain of pentazocine

Six groups of mice received s.c normal saline or pentazocine at 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with s.c. 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) (Fig. 1A), hot plate latency (s) (Fig. 1B), and tail flick latency (s) (Fig. 1C) immediately before (0 min), and at 30, 60, 90, and 120 min after, the s.c. pentazocine injection are shown in mean \pm S.E.M.

Fig.2 Effects of clocinnamox mesylate (C-CAM) on antinociceptive effects to somatic pain of pentazocine

Six groups of mice received s.c normal saline, or pentazocine at 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with s.c. C-CAM (5 mgkg⁻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) (Fig. 2A), hot plate latency (s) (Fig. 2B), and tail flick latency (s) (Fig. 2C) immediately before (0 min), and at 30, 60, 90, and 120 min after, the s.c. pentazocine injection are shown in mean \pm S.E.M.

Fig.3 Effects of nor-binaltorphimine (nor-BNI) on antinociceptive effects to somatic

pain of pentazocine

Six groups of mice received s.c normal saline, or pentazocine at 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with s.c. nor-BNI (10 mgkg⁻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) (Fig. 3A), hot plate latency (s) (Fig. 3B), and tail flick latency (s) (Fig. 3C) immediately before (0 min), and at 30, 60, 90, and 120 min after, the s.c. pentazocine injection are shown in mean ± S.E.M.

Fig.4 Effects of both clocinnamox mesylate (C-CAM) and nor-binaltorphimine (nor-BNI) on antinociceptive effects to somatic pain of pentazocine

Six groups of mice received s.c normal saline, or pentazocine at 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with s.c. C-CAM (5 mgkg⁻¹) and s.c. nor-BNI (10 mgkg⁻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) (Fig. 4A), hot plate latency (s) (Fig. 4B), and tail flick latency (s) (Fig. 4C) immediately before (0 min), and at 30, 60, 90, and 120 min after, the s.c. pentazocine injection are shown in mean ± S.E.M.

Fig.5 The calculated Area Under Curve (AUC) (antinociception-over-time) 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 (Fig. 5A); Hot plate AUC (s·h) represent the antinociception assessed by the hot plate test (Fig. 5B); and Tail flick AUC (s·h) represent the antinociception assessed by the tail flick test (Fig. 5C). Closed circles (Sal + PZ) indicate pretreatment with saline (as a placebo for C-CAM and/or nor-BNI). Open circles (C-CAM + PZ) indicate pretreatment with clocinnamox mesylate (C-CAM) (5 mgkg^{-1}). Closed triangles (nor-BNI + PZ) indicate pretreatment with nor-binaltorphimine (nor-BNI) (10 mgkg^{-1}). Open triangles (C-CAM + nor-BNI + PZ) indicate pretreatment with both C-CAM and nor-BNI. Data are expressed as mean \pm S.E.M.

Fig 6 Antinociceptive effects of pentazocine in C57BL/6J mice

24 groups of C57BL/6J received s.c. saline or pentazocine 3, 10, 30, 56 or 100 mgkg^{-1} , respectively, at 120 min after pretreatment with s.c. placebo saline, s.c. C-CAM (5 mgkg^{-1}), s.c. nor-BNI (10 mgkg^{-1}), or the combination of s.c. C-CAM and nor-BNI, respectively (n = 8 for each group). The tail pressure thresholds (Fig. 6A), immediately before (0 min), and at 30, 60, 90, 120, 150 and 180 min after, the s.c. pentazocine injection are shown in mean \pm S.E.M. Fig. 6B shows the calculated Area

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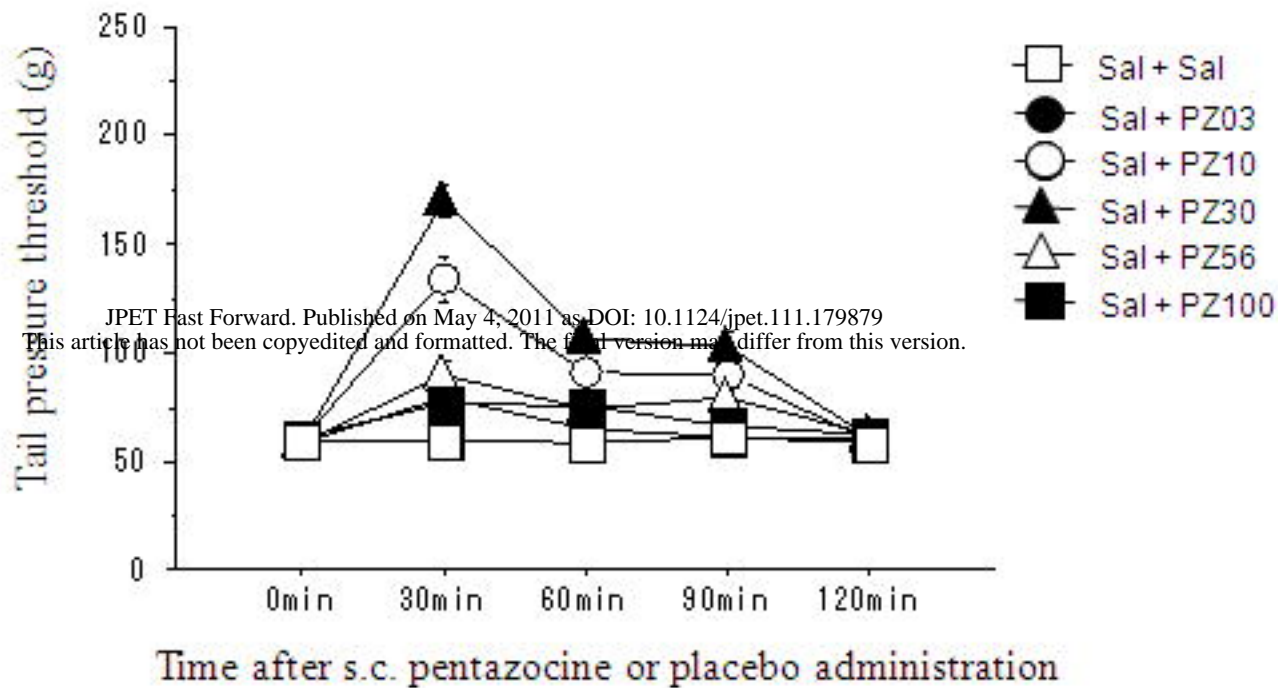
Under Curve (AUC) (antinociception-over-time) representing the antinociceptive effect to mechanical pain of pentazocine. Abscissa: Doses of pentazocine (Sal, PZ03, PZ10, PZ30, PZ56, PZ100), Ordinate: Tail pressure AUC (g·h) represent the antinociception assessed by the tail pressure test. Closed circles (Sal + PZ) indicate pretreatment with saline (as a placebo for C-CAM and/or nor-BNI). Open circles (C-CAM + PZ) indicate pretreatment with clocinnamox mesylate (C-CAM) (5 mgkg^{-1}). Closed triangles (nor-BNI + PZ) indicate pretreatment with nor-binaltorphimine (nor-BNI) (10 mgkg^{-1}). Open triangles (C-CAM + nor-BNI + PZ) indicate pretreatment with both C-CAM and nor-BNI. Data are expressed as mean \pm S.E.M.

Table Antinociceptive effects to visceral pain of pentazocine.

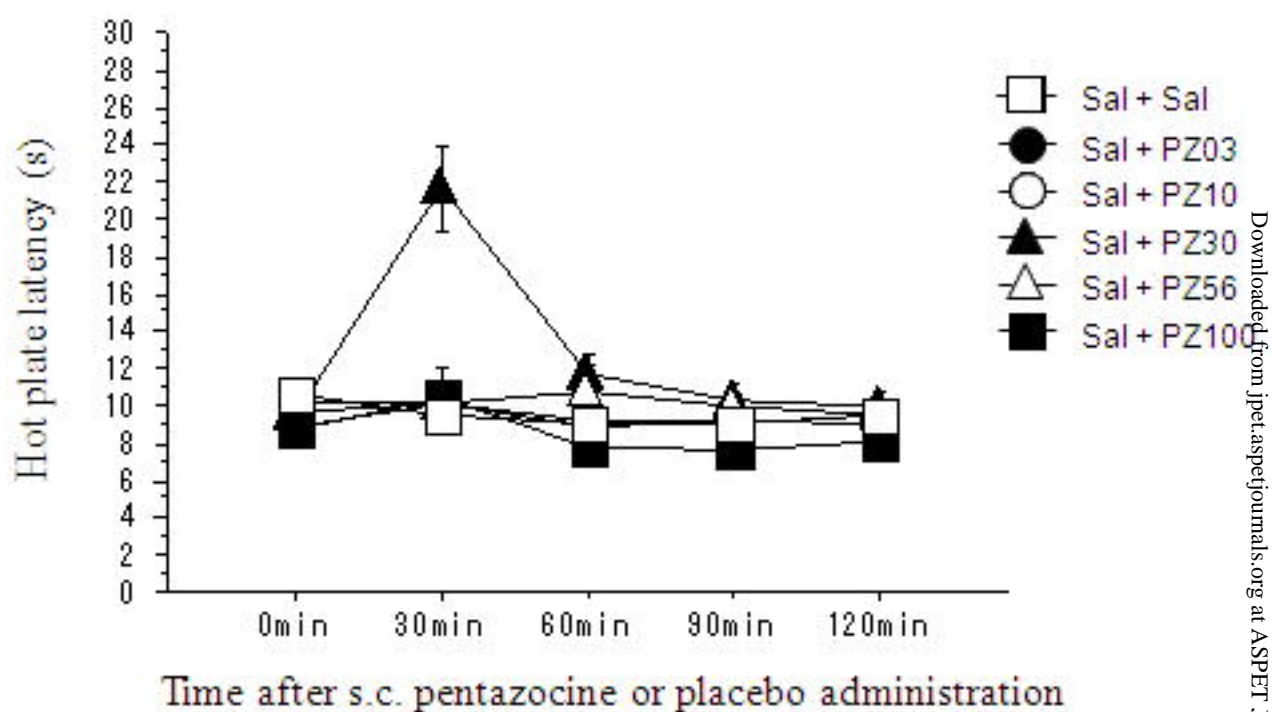
	Saline	PZ03	PZ10	PZ30	PZ56	PZ100
Placebo saline	41.5 (39.5, 44.3)	32.5 (29.8, 35.0) *	18.5 (16.8, 19.3) *	2.0 (0.0, 3.3) *	0.5 (0.0, 2.0) *	0.0 (0.0, 0.3) *
C-CAM	42.0 (39.5, 43.3)	42.5 (40.3, 45.0)#	41.5 (38.8, 42.5)#	41.0 (38.0, 42.8)#	20.5 (16.0, 24.3) *#	27.5 (18.3, 31.5) *#
nor-BNI	42.0 (39.8, 45.3)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

Twenty-four groups of mice received s.c normal saline, or s.c. pentazocine at 3, 10, 30, 56 or 100 mgkg⁻¹, respectively, at 120 min after pretreatment with placebo saline, clocinnamox mesylate (C-CAM) (5 mgkg⁻¹), nor-binaltorphimine (nor-BNI) (10 mgkg⁻¹), or both nor-BNI and C-CAM, respectively (n = 8 for each group). The number of writhing per mouse was counted over 10 min from 5 to 15 min after the intraperitoneal acetic acid injection. The numbers of writhing are expressed as median (25 and 75 percentiles). * 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 antagonist(s).

A



B



C

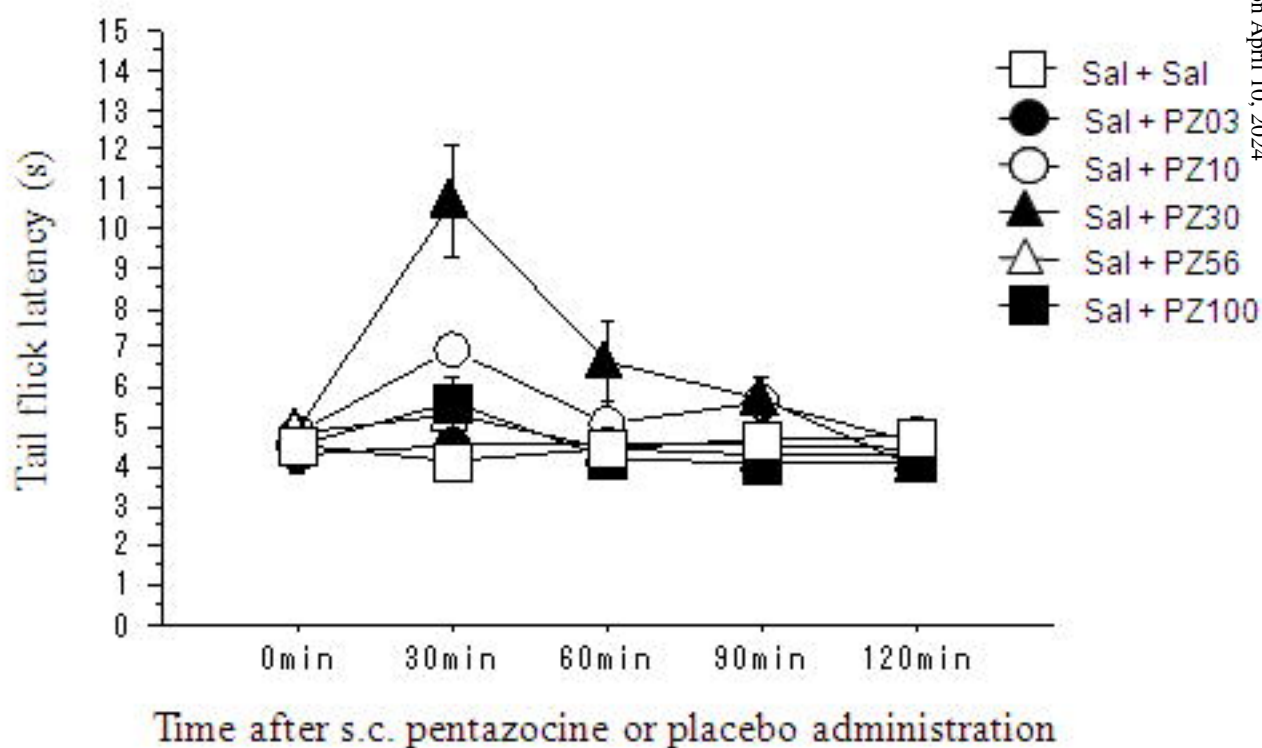


Figure 1

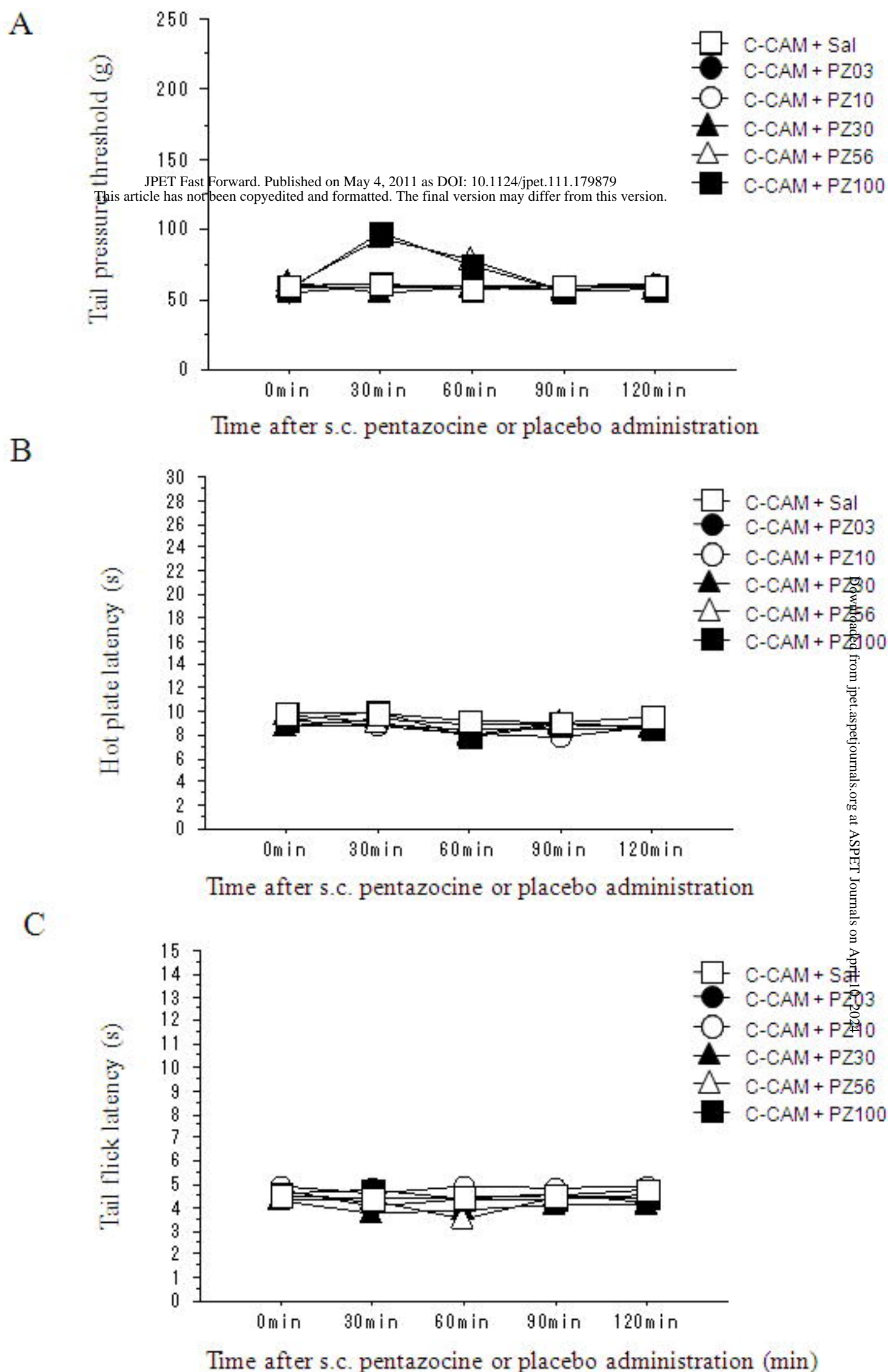


Figure 2

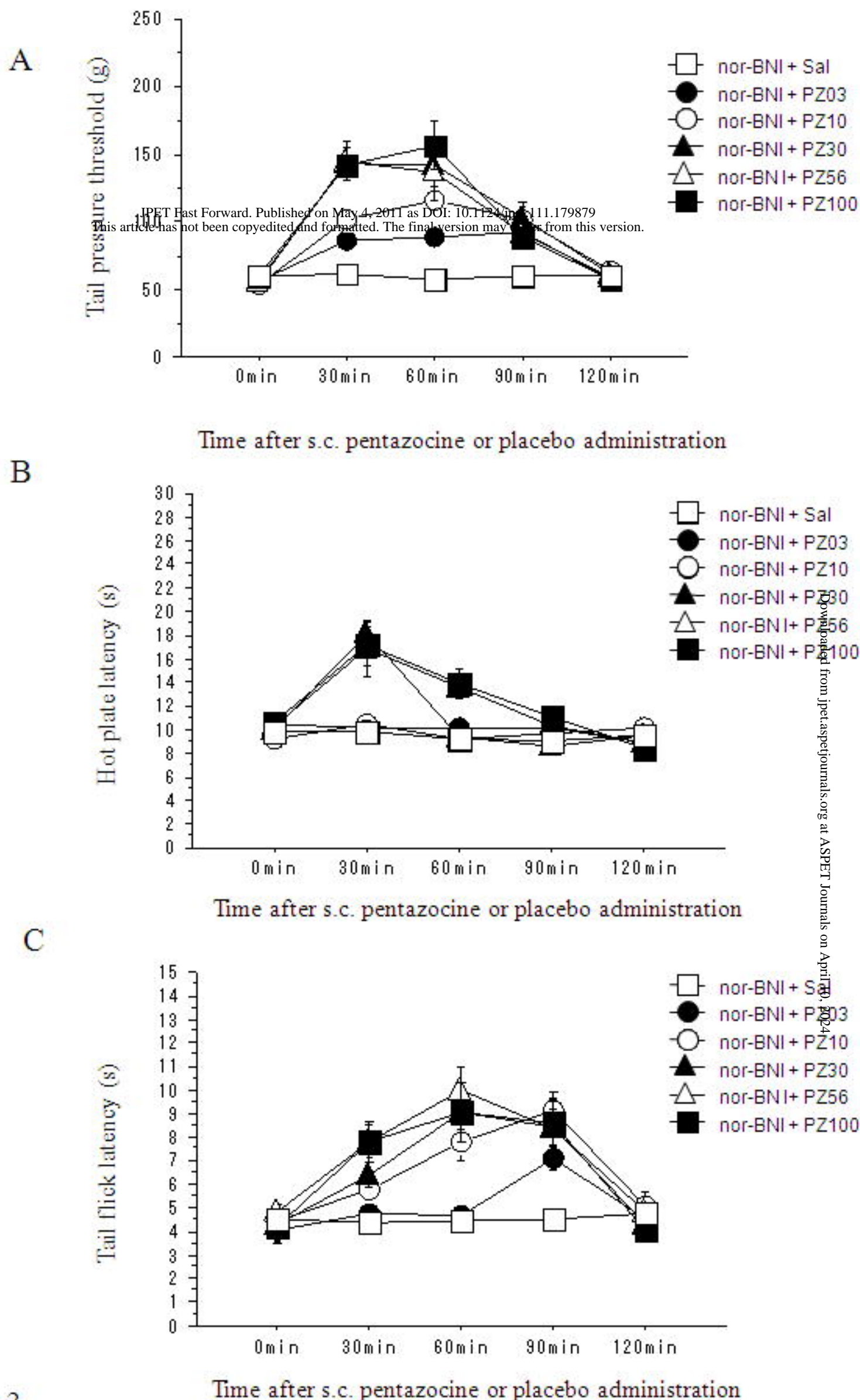
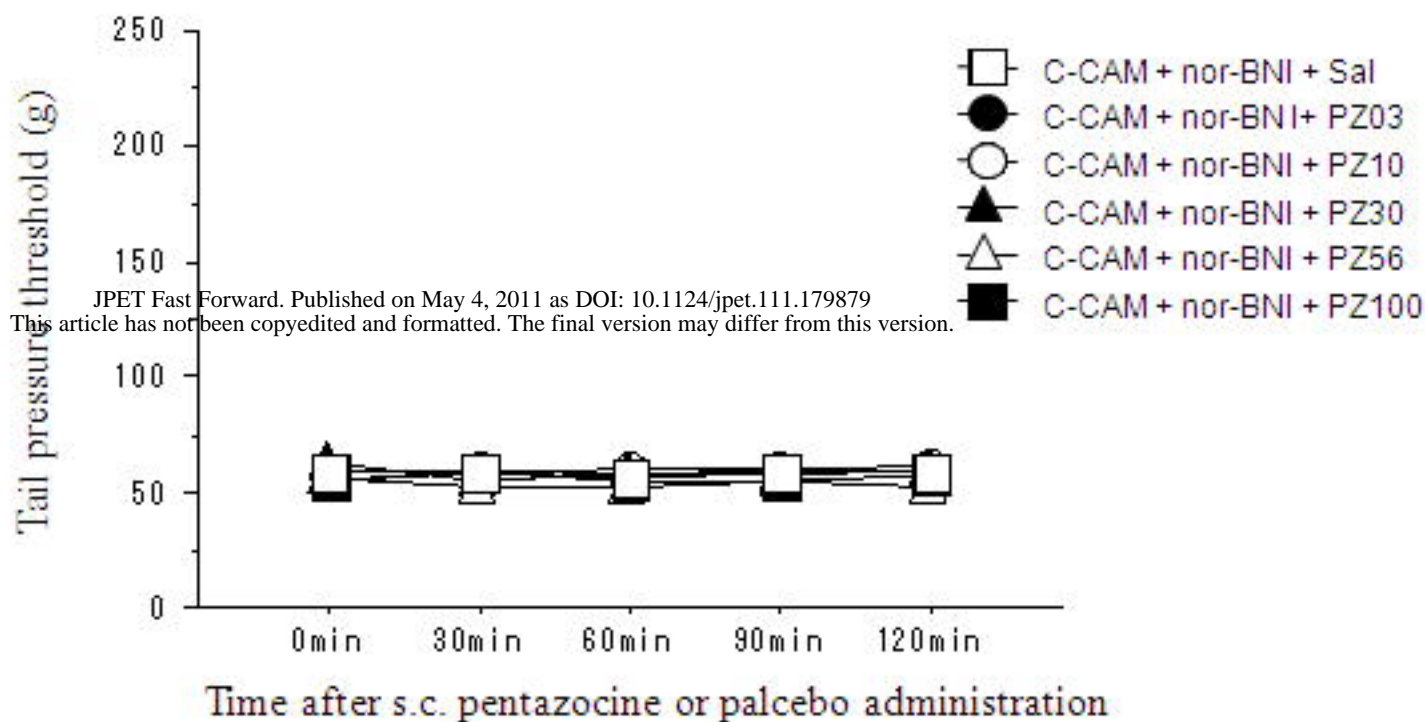
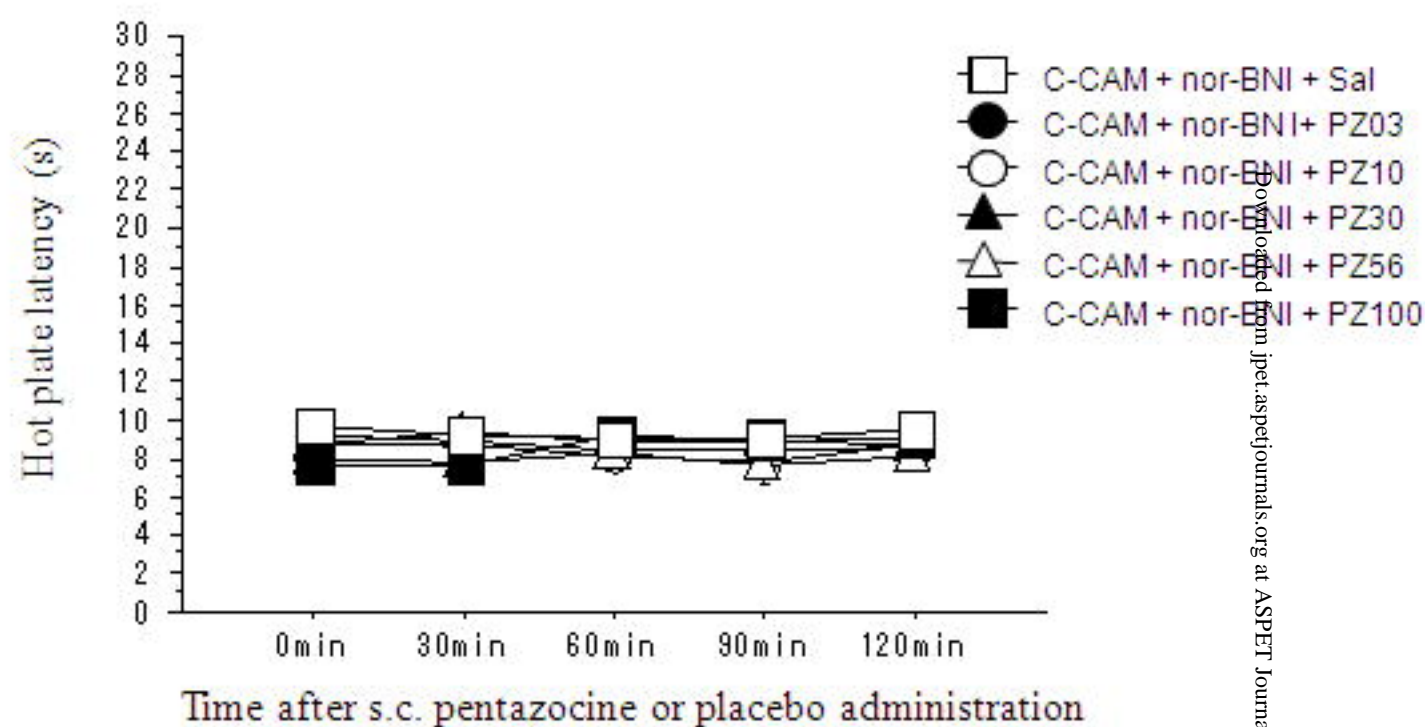


Figure 3

A



B



C

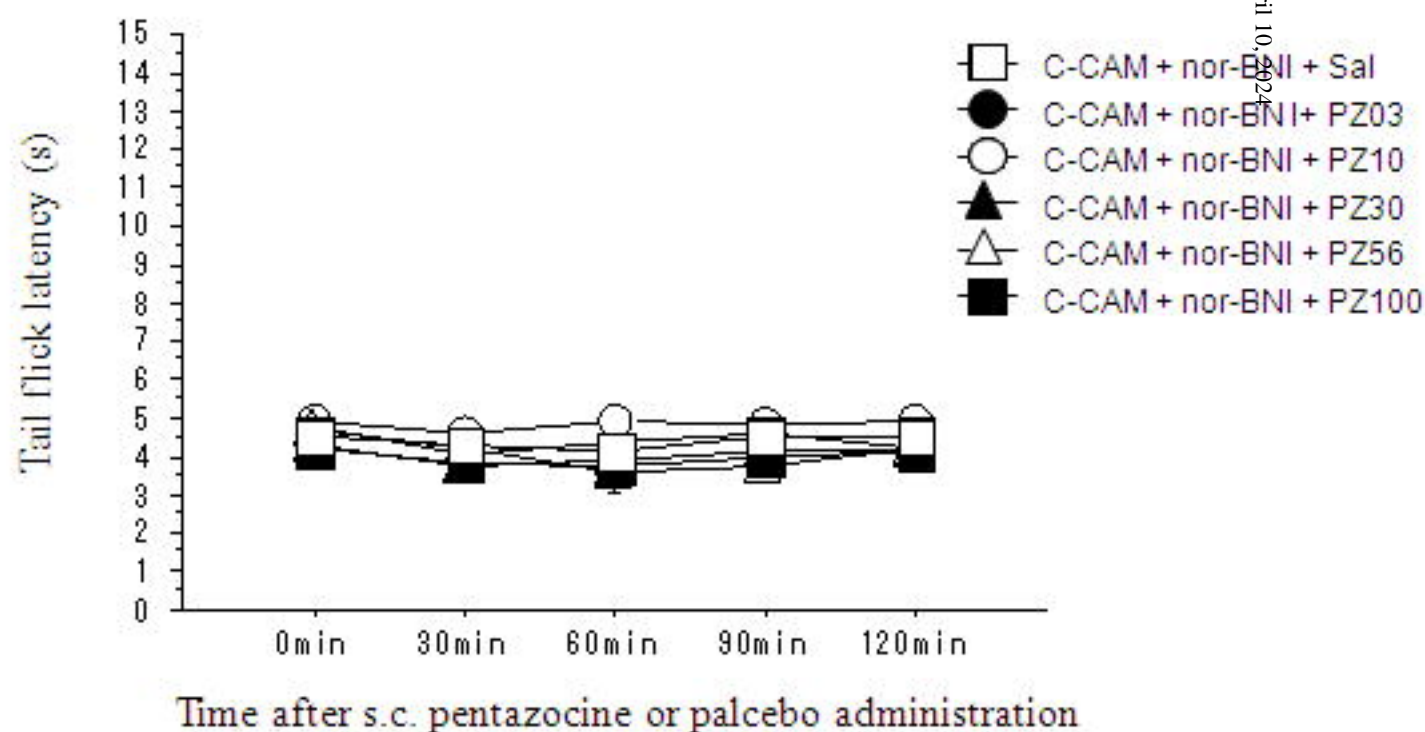


Figure 4

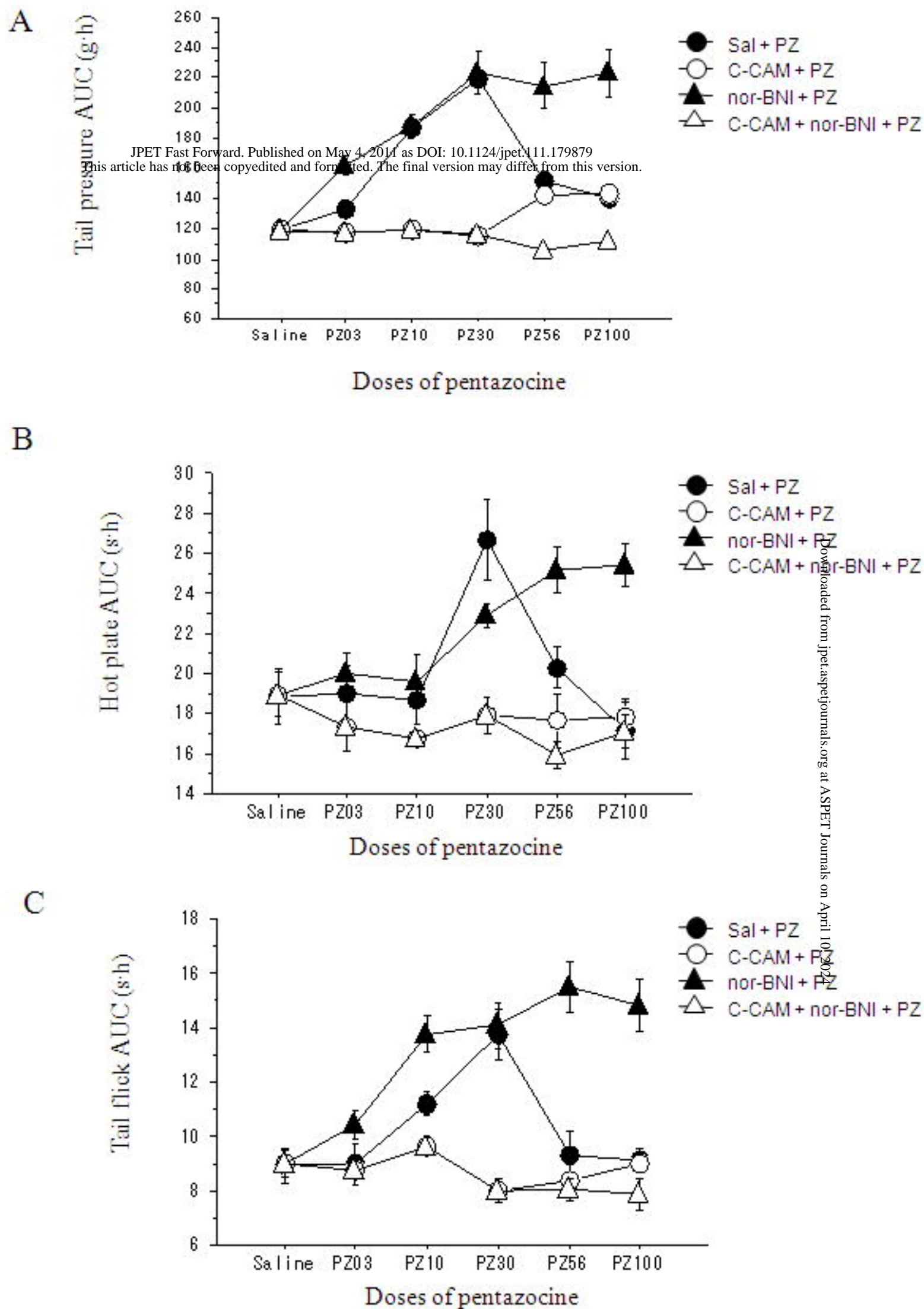
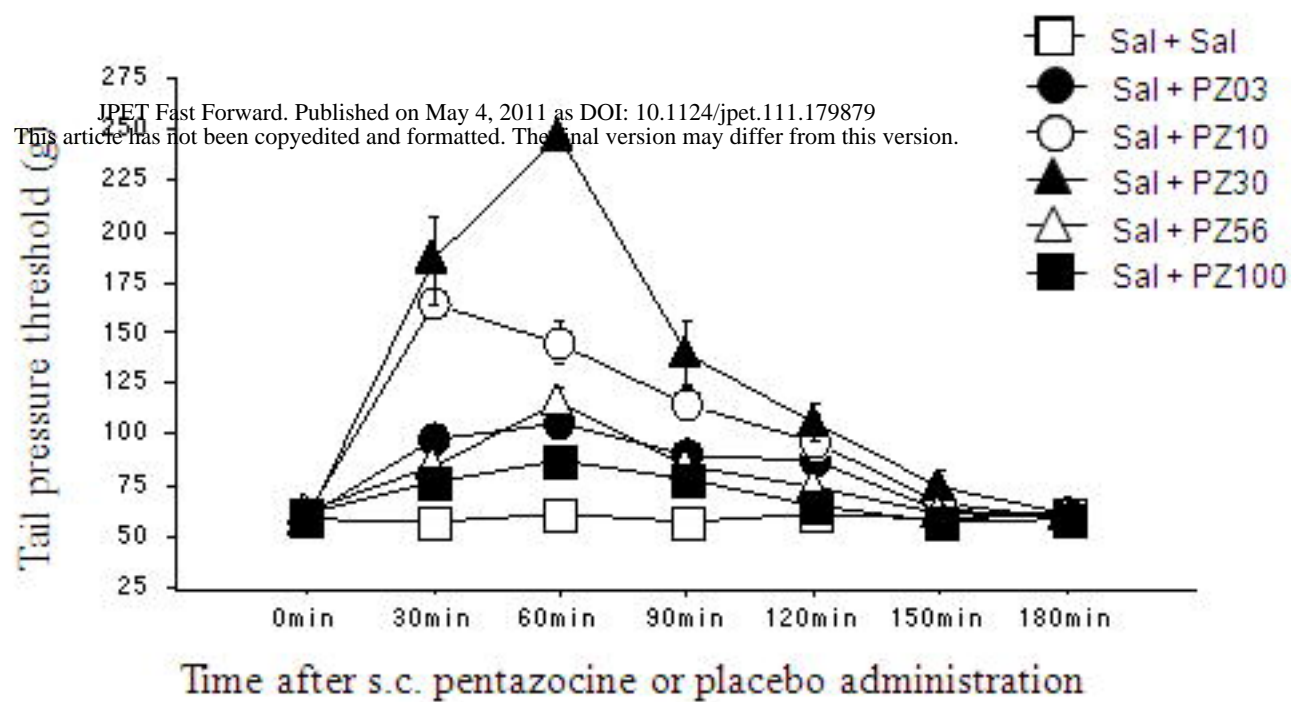


Figure 5

A



B

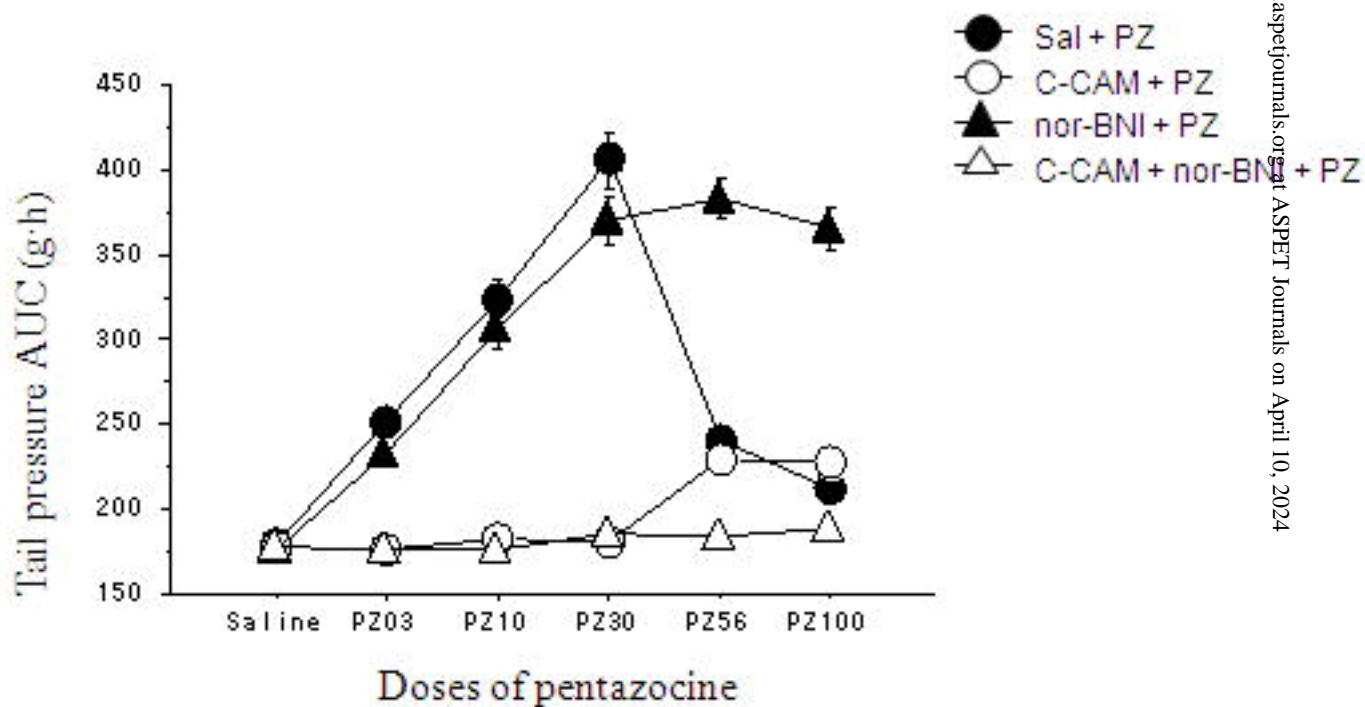


Figure 6