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Inhibitory effect of low-dose pentazocine on the development of antinociceptive tolerance to morphine

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Abstract
Purpose. The development of antinociceptive tolerance to morphine is one of the major problems in its clinical use. Therefore, exploring effective measures to prevent morphine tolerance is of great clinical relevance. We evaluated whether pentazocine could prevent morphine tolerance in mice.
Methods. Five groups of male ICR mice received repeated subcutaneous (s.c.) injections of morphine at a high dose (10 mg·kg^{-1}) or saline, concomitantly with s.c. injections of pentazocine at low, subanalgesic doses (0.1, 0.3, or 1.0 mg·kg^{-1}) or saline, respectively, once daily for 14 days. On day 15, mice received co-injections of morphine and pentazocine 120 min after pretreatment with nor-binaltorphimine (5 mg·kg^{-1}), a selective κ-opioid receptor antagonist. The tail pressure threshold was measured before and 60 min after the daily drug co-injections.
Results. Repeated s.c. co-injections of morphine and saline resulted in a progressive decrease in morphine-induced antinociception, due to the development of morphine tolerance. Co-injections of pentazocine (0.1, 0.3, and 1.0 mg·kg^{-1}) with morphine potentiated the morphine-induced antinociception dose-dependently by preventing the development of morphine tolerance. Nor-binaltorphimine completely inhibited the chronic antinociception maintained by co-injections of morphine and pentazocine.
Conclusion. When chronically co-administered with morphine, pentazocine at low, subanalgesic doses dose-dependently potentiated morphine-induced antinociception in morphine-tolerant mice, through its κ-opioid-receptor-mediated tolerance-preventing activity. Because pentazocine is the only agonist-antagonist analgesic that has an effective oral formulation suitable for chronic administration, the results of the present study warrant clinical trials of pentazocine to assess its tolerance-preventing activity in patients with cancer pain.

Key words Pentazocine · Morphine · Tolerance · Agonist-antagonist · Tail pressure test

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Introduction

Morphine is a widely used drug for the treatment of moderate to severe cancer pain, and in its long-term use, peroral administration is advocated for patients' convenience [1]. Despite the extremely high therapeutic utility of morphine in such use, the development of antinociceptive tolerance to morphine is still one of the most troublesome problems associated with its long-term use for months or years. Therefore, exploring effective measures to prevent morphine tolerance is of critical clinical significance.

Abundant preclinical data suggest that N-methyl D-aspartate (NMDA) receptor antagonists and κ-opioid receptor agonists can prevent morphine tolerance [2,3]. At present, however, clinical data are lacking regarding the effect of the long-term use of ketamine, an NMDA receptor antagonist, on morphine tolerance in humans, because of the absence of oral formulations suitable for chronic administration, the high incidence of its psychocognitive adverse effects (dysphoria, drowsiness, and hallucination), and impaired semantic memory and schizophreniform psychosis associated with its long-term use (addiction) [4]. Although an oral tablet of another NMDA receptor antagonist, dextromethorphan (DM), is clinically available, it did not enhance morphine analgesia or modulate morphine tolerance, while producing more adverse effects such as dizziness, compared with placebo, in patients with cancer pain [5]. Therefore, no effective NMDA antagonists are currently available for long-term use for the prevention of morphine tolerance in cancer patients.

In our previous study, a selective κ-opioid receptor agonist, U50488H, not only prevented the development of morphine tolerance but also reversed already-developed morphine tolerance, while an NMDA receptor antagonist, MK-801, prevented, but did not reverse, morphine tolerance [6]. In this regard, κ-opioid receptor agonists may be superior to NMDA antagonists for

the treatment of morphine tolerance. At present, however, no selective κ-opioid receptor agonists are available for therapeutic use [7], because these drugs produce annoying dysphoric and psychotomimetic effects in humans [7–10]. We have recently demonstrated that processed *Aconiti* tuber (Shuchi Bushi), an oriental herbal medicine that has been widely used as an analgesic since ancient times and has recently been found to act as an indirectly-acting κ-opioid receptor agonist via the increased release of dynorphins [11], could dose-dependently prevent and reverse morphine tolerance in mice [12–14]. These results warranted clinical trials to evaluate its efficacy in preventing tolerance in cancer patients.

Pentazocine is an opioid agonist-antagonist analgesic that is one-sixth to one-third as potent as morphine [15]. Unlike selective κ-opioid receptor agonists, pentazocine has been widely used in the long-term treatment of cancer pain, and it is the only agonist-antagonist analgesic that has a clinically available, effective oral formulation [7,15]. Because its antinociceptive effect is primarily due to its agonistic action on κ-opioid receptors [7,15], pentazocine may have the ability to prevent the development of morphine tolerance, analogously to selective κ-opioid receptors [3]. If so, such ability of pentazocine may be of great clinical relevance, because of the well-established clinical applicability of its oral tablet. To date, however, the tolerance-preventing effect of pentazocine has not been examined in animals or humans. Because pentazocine is not a selective κ-receptor agonist, but a mixed agonist-antagonist analgesic possessing partial κ-agonistic and some μ-antagonistic or weak partial μ-agonistic properties [7], its effects on morphine-induced antinociception or antinociceptive tolerance to morphine may differ from those of selective κ-opioid receptor agonists. Therefore, it seemed necessary to evaluate whether pentazocine could prevent morphine tolerance in a preclinical study, before going on to clinical trials to test this possibility in cancer patients. Therefore, in the present study, as the first step toward a clinical trial, we investigated the effect of pentazocine on the development of morphine tolerance in a mouse tail pressure pain model.

Materials and methods

Animals and drugs

The present study was approved by the Institutional Laboratory Animal Care and Use Committee, Graduate School of Medicine, the University of Tokyo. Male ICR mice (SLC Japan, Hamamatsu, Japan), weighing 24–28 g were used. The mice were housed under controlled temperature (23 ± 2°C) and humidity (55 ± 10%), and habituated to the environment for at least 3 days before experiments. They were allowed to access food and water ad libitum. Drugs were purchased from the following sources: morphine hydrochloride (Takeda Pharmaceutical, Osaka, Japan), pentazocine (Sankyo Pharmaceutical, Tokyo, Japan), nor-binaltorphimine (nor-BNI), a selective κ-opioid receptor antagonist (Sigma Japan, Tokyo, Japan) [16,17], and clocinnamox mesylate (C-CAM), a selective μ-opioid receptor antagonist (Tocris Cookson, Ellisville, MO, USA) [18,19]. Morphine hydrochloride, pentazocine, and nor-BNI were dissolved in saline for subcutaneous (s.c.) injections. C-CAM was dissolved in dimethyl sulfoxide (DMSO) diluted with normal saline for s.c. injections. For co-administration, pentazocine (or saline) was injected just before the injection of morphine (or saline). Nor-BNI or C-CAM was s.c. injected 120 min before the injection of pentazocine, morphine, or both [13].

Tail pressure test

The tail pressure threshold was measured with an Analgesy Meter (Ugo Basile, Comerio, Italy) as described previously [12,13]. With this device, the distal part of the tail was supported by a plinth, and pressure, increasing linearly at a rate of 16 $g \cdot s^{-1}$, was applied to the proximal 2 cm of the tail with a wedge-shaped pusher. The end-point was defined as the first motor response of struggling against the pressure. Prior to drug injection, the tail pressure threshold was measured three times, at intervals of 30 min. The first measurement was omitted and the mean of the next two measurements was employed as the baseline tail pressure threshold. The cutoff point was set at 250 *g* to avoid tissue damage. The percentage of maximal possible effect (%MPE) for each animal at each time was calculated using the following formula: %MPE = [(test point – baseline point) / (cutoff point – baseline point)] ×100.

Statistical analyses

All values are reported as means with SEM. When a significant difference among the %MPE data during the experiment after the drug administration was obtained in a two-way (drugs and time) repeated-measures analysis of variance (ANOVA), Fisher's protected least significant difference test (PLSD) was applied to determine significant differences between time points in each group. When a significant difference among the groups was obtained in one-way (drugs) ANOVA, Fisher's PLSD was applied to define which test drug groups contributed to these differences. The level of statistical significance was set at $P < 0.05$.

Results

Dose-response of the antinociceptive effect of pentazocine alone

In the six groups ($n = 8$ in each group), the tail pressure threshold was measured before and 30, 60, 90, 120, 150, and 180 min after the s.c. injection of pentazocine (0.1, 0.3, 1.0, 3.0, or 10 mg·kg^{-1}) or saline, respectively.

Antinociceptive effects, as indicated by significant increases in %MPE compared with saline, were observed after s.c. pentazocine at 1.0, 3.0, and 10 mg·kg^{-1}, but not at 0.1 or 0.3 mg·kg^{-1} (Fig. 1). However, pentazocine at 1.0 mg·kg^{-1} did not produce significant antinociception statistically at 60 min after its injection, the time at which the tail pressure threshold was measured in the following experiments.

Effects of pentazocine in a single dose on acute morphine-induced antinociception in morphine-naïve mice

The tail pressure threshold was measured before and 60 min after co-injections of pentazocine (0.1, 0.3, or 1.0 mg·kg^{-1}) (or saline) and morphine (1.0, 3.0, or 10 mg·kg^{-1}) (or saline) ($n = 6$ in each of the 16 test drug groups).

Compared with saline, pentazocine at 0.1, 0.3, and 1.0 mg·kg^{-1} significantly reduced the antinociception induced by morphine at 1.0 and 3.0 mg·kg^{-1} in most of the groups treated with these dose combinations of the drugs (Fig. 2). However, such antagonizing effects of pentazocine on morphine-induced antinociception were not dose-dependent, and were not observed in animals co-treated with pentazocine at 0.3 mg·kg^{-1} and morphine at 1.0 mg·kg^{-1} (Fig. 2). A significant effect of pentazocine on antinociception induced by morphine at 10 mg·kg^{-1} could not be detected, because in all groups receiving morphine at 10 mg·kg^{-1}, %MPE reached 100% (Fig. 2).

Effects of pentazocine in repeated doses on acute morphine-induced antinociception in morphine-naïve mice

Initially in the four groups, pentazocine (0.1, 0.3, or 1.0 mg·kg^{-1}) or saline, respectively, was s.c. injected once daily for 4 days ($n = 16$ in each group). The tail pressure threshold was measured before and 60 min after s.c. pentazocine. Subsequently, each pentazocine-treated group was divided into four subgroups, which were co-treated with pentazocine at each dose and morphine at 0, 1.0, 3.0, and 10 mg·kg^{-1}, respectively, on day 5 ($n = 4$ in each subgroup). The tail pressure threshold was measured before and 60 min after co-injections of pentazocine and morphine on day 5.

Pentazocine (0.1, 0.3, and 1.0 mg·kg^{-1}) alone, given repeatedly for 4 days, did not produce significant antinociception at 60 min after the daily s.c. pentazocine injections (Fig. 3A). Compared with saline, pentazocine at 0.1, 0.3, and 1.0 mg·kg^{-1} significantly reduced the acute antinociception induced by morphine at 1.0 and 3.0 mg·kg^{-1} in most of the groups treated with these dose combinations of the drugs (Fig. 3B). However, such antagonizing effects of pentazocine on morphine-induced antinociception were not dose-dependent, and were not observed in animals co-treated with pentazo-

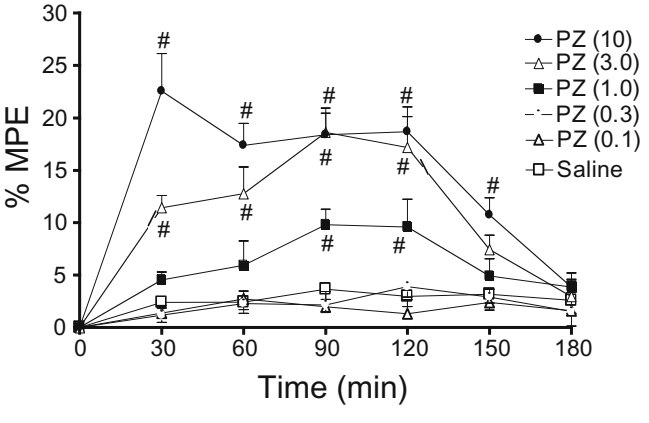

Fig. 1. Dose-response of the antinociceptive effect of pentazocine. Antinociceptive effects induced by pentazocine 0.1, 0.3, 1.0, 3.0, and 10 mg·kg^{-1} in mice ($n = 8$ in each group) are shown as means ± SEM of percentage of maximal possible effect (*%MPE*), which was defined as %MPE = [(test point − baseline point)/(cutoff point − baseline point)] × 100. $^{\#}P < 0.05$ vs saline group, indicating a significant antinociceptive effect. *PZ*, pentazocine

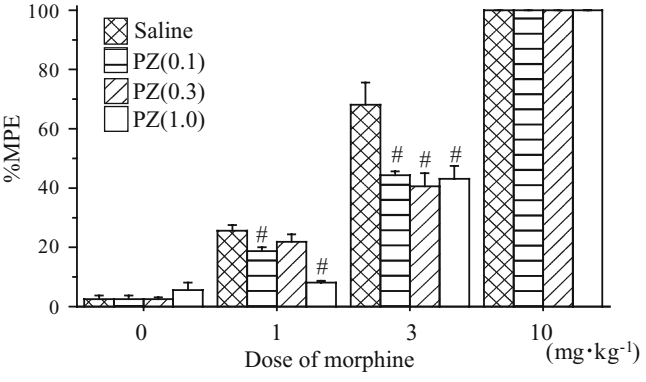

Fig. 2. Effects of pentazocine in a single dose on acute morphine-induced antinociception in morphine-naïve mice. Effects of a single administration of pentazocine (0.1, 0.3, and 1 mg·kg^{-1}) on the antinociceptive effect of morphine (1, 3, and 10 mg·kg^{-1}; $n = 6$ in each group) are shown as means ± SEM of %MPE at 60 min after the co-injections of morphine and pentazocine. $^{\#}P < 0.05$ vs saline group. *PZ*, pentazocine

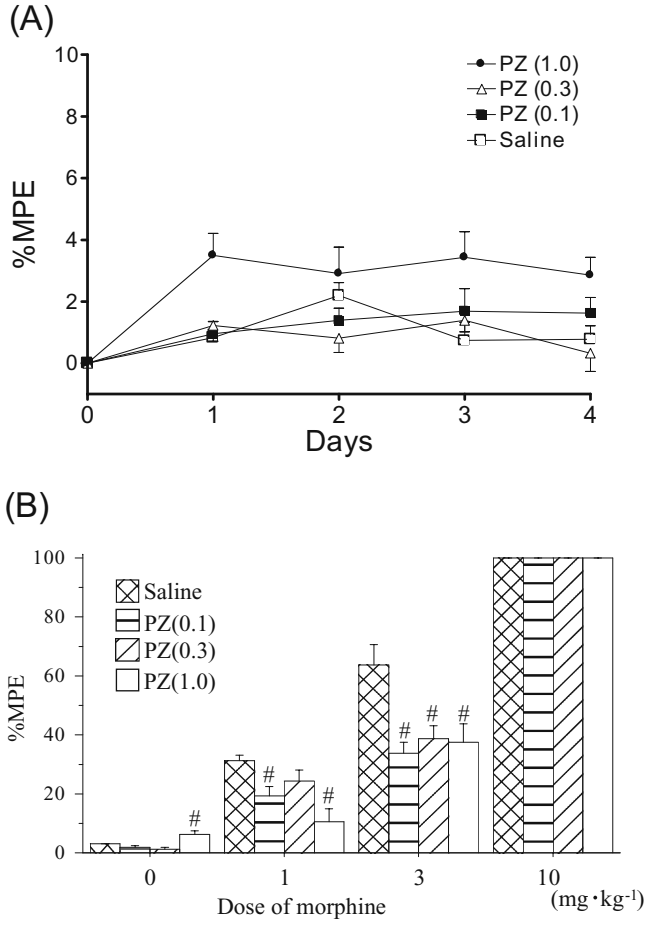

Fig. 3. A Antinociceptive effects of s.c. pentazocine (0, 0.1, 0.3, and 1 mg·kg^{-1}) in repeated doses and **B** effects of pentazocine in repeated doses on acute morphine-induced antinociception in morphine-naïve mice. **A** Antinociceptive effects of repeated s.c. injections of pentazocine (0, 0.1, 0.3, and 1 mg·kg^{-1}) once daily for 4 days (n = 16 in each group) are shown as means ± SEM of %MPE at 60 min after the daily pentazocine injections. %MPE did not change significantly in any group, indicating no antinociceptive effects of pentazocine in repeated doses. Subsequently, each pentazocine group was subdivided into four subgroups and they received co-injections of pentazocine at each dose and morphine at 0, 1.0, 3.0, and 10 mg·kg^{-1} on day 5 (n = 4 in each subgroup). **B** Effects of pentazocine in repeated doses (0, 0.1, 0.3, and 1 mg·kg^{-1} once daily for 5 days) on acute antinociception induced by morphine (0, 1, 3, and 10 mg·kg^{-1}) in morphine-naïve mice are shown as means ± SEM of %MPE at 60 min after the co-injections of pentazocine and morphine on day 5. $^{\#}P < 0.05$ vs saline group. *PZ*, pentazocine

cine at 0.3 mg·kg^{-1} and morphine at 1.0 mg·kg^{-1} (Fig. 3B). A significant effect of pentazocine on antinociception induced by morphine at 10 mg·kg^{-1} could not be detected, because in all groups receiving morphine at 10 mg·kg^{-1}, %MPE reached 100% (Fig. 3B). The effect of pentazocine in repeated doses on morphine-induced antinociception was not significantly different from that of pentazocine in a single dose (Figs. 2 and 3B).

Effects of nor-BNI and C-CAM on acute morphine-induced antinociception

To evaluate the effect of C-CAM and nor-BNI on acute antinociception induced by morphine at 10 mg·kg^{-1}, four groups of mice were co-treated with s.c. injections of saline and diluted DMSO; morphine and diluted DMSO; morphine and C-CAM (0.5 mg·kg^{-1}); and saline and C-CAM, respectively (n = 8 in each group), and another four groups of mice were co-treated with s.c. injections of saline and saline; morphine and saline; morphine and nor-BNI (5 mg·kg^{-1}); and saline and nor-BNI (5 mg·kg^{-1}), respectively (n = 8 in each group). The tail pressure threshold was measured before and 30, 60, 90, and 120 min after s.c. injections of morphine or saline.

C-CAM completely inhibited acute morphine-induced antinociception (Fig. 4A), while nor-BNI did not inhibit it at all (Fig. 4B).

Effects of nor-BNI and C-CAM on acute pentazocine-induced antinociception

To evaluate the effects of C-CAM and nor-BNI on acute antinociception induced by pentazocine at an analgesic dose (10 mg·kg^{-1}), four groups of mice were co-treated with s.c. injections of saline and saline; pentazocine and saline; pentazocine and C-CAM (0.5 mg·kg^{-1}); and pentazocine and nor-BNI (5 mg·kg^{-1}), respectively (n = 8 in each group). The tail pressure threshold was measured before and 30, 60, 90, and 120 min after s.c. injections of pentazocine or saline.

Nor-BNI completely inhibited acute pentazocine-induced antinociception, while pretreatment with C-CAM did not inhibit it at all (Fig. 5).

Effects of low-dose pentazocine on the development of antinociceptive tolerance to morphine

To evaluate the development of antinociceptive tolerance to morphine (10 mg·kg^{-1}) and also to evaluate the effects of pentazocine at low, subanalgesic doses (0.1, 0.3, and 1.0 mg·kg^{-1}) on the development of morphine tolerance, five groups of mice received repeated s.c. co-injections of saline and saline; saline and morphine (10 mg·kg^{-1}); pentazocine (0.1 mg·kg^{-1}) and morphine; pentazocine (0.3 mg·kg^{-1}) and morphine; and pentazocine (1.0 mg·kg^{-1}) and morphine, respectively, once daily for 14 days (n = 8 in each group). The tail pressure threshold was measured before and 60 min after the daily s.c. co-injections of morphine (or saline) and pentazocine (or saline).

On days 1 and 2, morphine (10 mg·kg^{-1}) produced remarkable antinociception, indicated by %MPE of 100% in morphine-naïve mice in all four of the

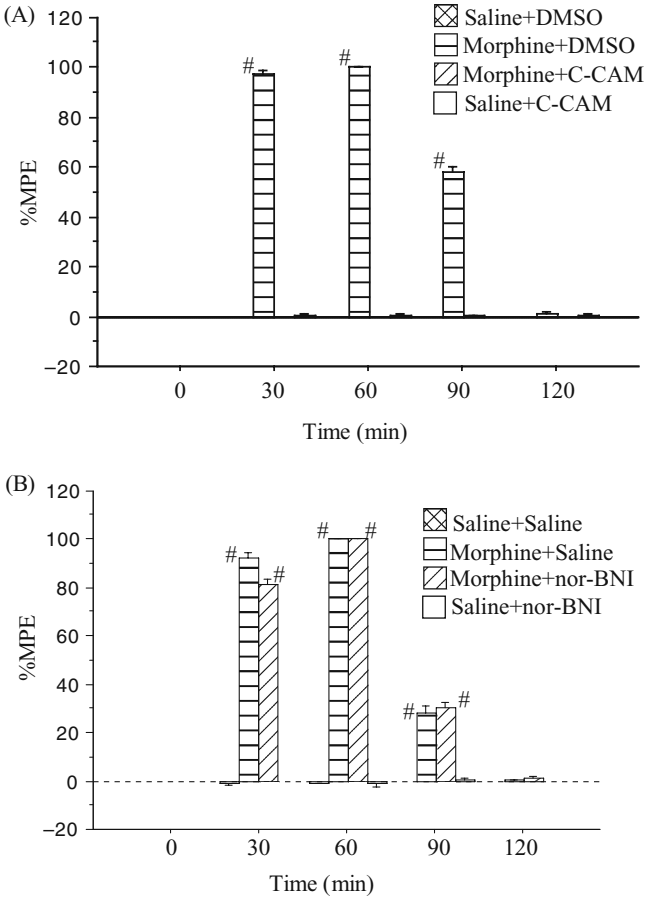

Fig. 4A,B. Effects of clocinnamox mesylate (*C-CAM*; **A**) and nor-binaltorphimine (*nor-BNI*; **B**) on acute morphine-induced antinociception. Data values are shown as means ± SEM of %MPE before, and 30, 60, 90, and 120 min after s.c. injections of morphine (10 mg·kg^{-1}), given 120 min after pretreatment with s.c. nor-BNI (5 mg·kg^{-1}), s.c. C-CAM (0.5 mg·kg^{-1}), or s.c. placebo. $^{\#}P < 0.05$ vs negative control (Saline+dimethyl sulfoxide [*DMSO*] or Saline+Saline) group, indicating significant antinociception

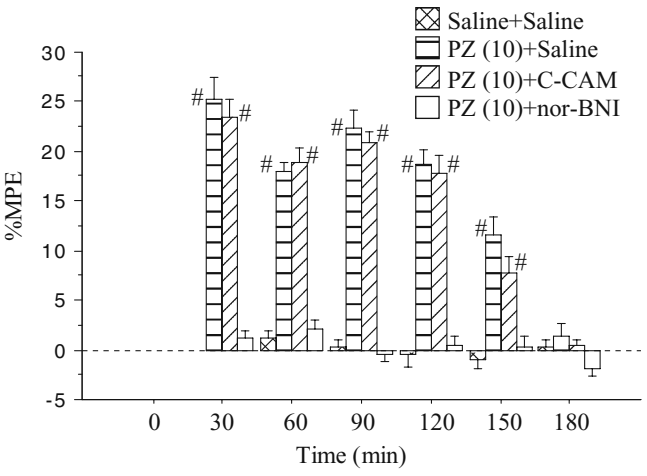

Fig. 5. Effects of nor-BNI and C-CAM on acute antinociception induced by pentazocine at an analgesic dose (10 mg·kg^{-1}). Data values are shown as means ± SEM of %MPE before, and 30, 60, 90, 120, 150, and 180 min after s.c. injections of pentazocine at an analgesic dose (10 mg·kg^{-1}), given 120 min after pretreatment with s.c. nor-BNI (5 mg·kg^{-1}), s.c. C-CAM (0.5 mg·kg^{-1}), or s.c. placebo saline. $^{\#}P < 0.05$ vs negative control (Saline+Saline) group, indicating significant antinociception. *PZ*, pentazocine

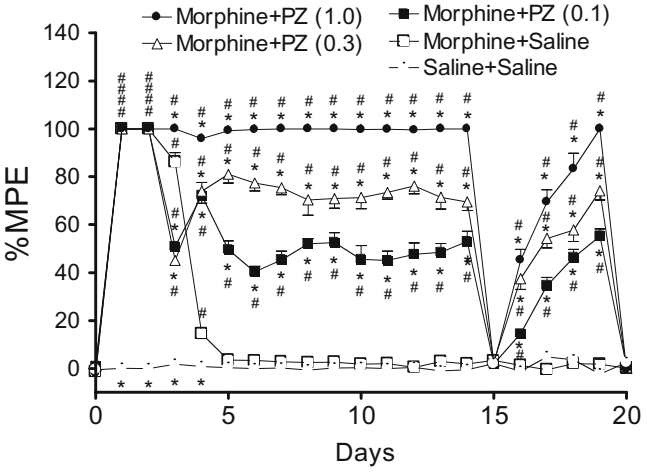

Fig. 6. Effects of low-dose pentazocine on the development of antinociceptive tolerance to morphine. The antinociceptive effects induced by co-injections of morphine (10 mg·kg^{-1}) and pentazocine (0.1, 0.3, and 1.0 mg·kg^{-1}) given once daily for 20 days (n = 8 in each group) are shown as means ± SEM of %MPE at 60 min after the daily drug co-injections. On day 15, nor-BNI (5.0 mg·kg^{-1}) was s.c. injected 120 min before the co-injections of morphine and pentazocine. On day 20, C-CAM (0.5 mg·kg^{-1}) was s.c. injected 120 min before the co-injections of morphine and pentazocine. $^{\#}P < 0.05$ vs Saline plus Saline group, indicating significant antinociception. $^{*}P < 0.05$ vs Morphine plus Saline group, indicating significant antagonism or potentiation of morphine-induced antinociception. *PZ*, pentazocine

morphine-treated groups (Fig. 6). In the group co-treated with morphine and saline, however, the antinociceptive effect of morphine progressively decreased and was completely abolished by day 5 due to the establishment of morphine tolerance (Fig. 6). On the other hand, in all three of the groups co-treated with morphine (10 mg·kg^{-1}) and pentazocine at low, subanalgesic doses (0.1, 0.3, or 1.0 mg·kg^{-1}), the significant antinociception was maintained throughout days 1–14 (Fig. 6). On day 3, in the initial phase of tolerance development, the effect of pentazocine on the morphine-induced antinociception was inconsistent; compared with saline, pentazocine at 0.1 and 0.3 mg·kg^{-1} significantly reduced, while pentazocine at 1.0 mg·kg^{-1} significantly potentiated, the antinociception on day 3 (Fig. 6). In contrast, on days 4–14, after the establishment of morphine tolerance, pentazocine significantly potentiated the chronic

antinociception in a dose-dependent manner, although the effect of pentazocine at 1.0 mg·kg^{-1} on the chronic antinociception could not be fully elucidated, because %MPE continued to reach 100% in mice co-treated with morphine at 10 mg·kg^{-1} and pentazocine at 1.0 mg·kg^{-1} until day 14, reflecting the remarkable antinociception-potentiating effect of pentazocine 1.0 mg·kg^{-1} (Fig. 6).

Effects of nor-BNI and C-CAM on chronic antinociception maintained by chronic co-administration of high-dose morphine and low-dose pentazocine

To evaluate the effects of nor-BNI and C-CAM on the chronic antinociception maintained by chronic co-treatment with morphine and pentazocine, the chronic co-treatment, described in the previous section, was continued until day 20. On day 15, morphine (10 mg·kg^{-1}) (or saline) and pentazocine (0.1, 0.3, or 1.0 mg·kg^{-1}) (or saline) were s.c. injected 120 min after pretreatment with s.c. nor-BNI (5.0 mg·kg^{-1}). Likewise, on day 20, when the effect of nor-BNI was completely abolished [13], morphine and pentazocine were s.c. injected 120 min after pretreatment with s.c. C-CAM (0.5 mg·kg^{-1}). The tail pressure threshold was measured before and 60 min after the daily s.c. co-injections of morphine and pentazocine on days 14–20.

On day 15, nor-BNI completely inhibited the significant antinociception that had been maintained until day 14 in the three groups co-treated with morphine and pentazocine (Figs. 6 and 7A). This inhibiting effect of nor-BNI completely disappeared by day 19. On day 20, C-CAM also completely inhibited the significant antinociception that had been present on day 19 in these three groups (Figs. 6 and 7B).

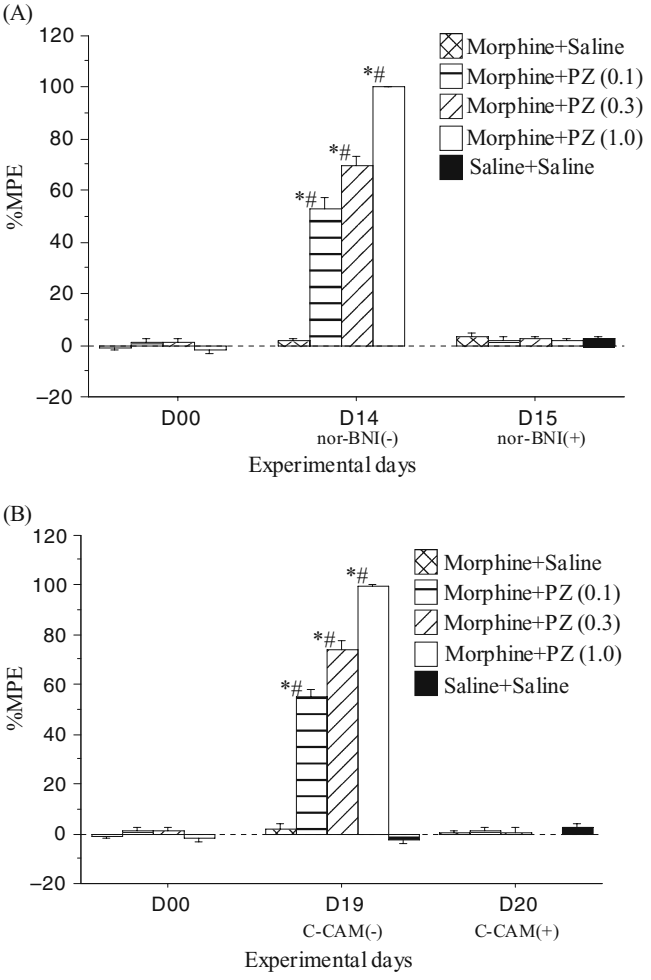

Fig. 7A,B. Effects of nor-BNI (**A**) and C-CAM (**B**) on antinociception maintained by chronic co-administration of high-dose morphine and low-dose pentazocine. **A** Data values are shown as means ± SEM of %MPE before co-injections of morphine and pentazocine on day 0 (baseline), 60 min after the co-injections of morphine and pentazocine on day 14, and 60 min after the co-injections of morphine and pentazocine, given 120 min after pretreatment with nor-BNI (5.0 mg·kg^{-1}) on day 15. *$P < 0.05$ vs Morphine plus Saline group; #$P < 0.05$ vs Saline plus Saline group. **B** Data values are shown as means ± SEM of %MPE before co-injections of morphine and pentazocine on day 0 (baseline), 60 min after the co-injections of morphine and pentazocine on day 19, and 60 min after the co-injections of morphine and pentazocine, given 120 min after pretreatment with C-CAM (0.5 mg·kg^{-1}) on day 15. *$P <$ 0.05 vs Morphine plus Saline group; #$P < 0.05$ vs Saline plus Saline group. *PZ*, pentazocine

Discussion

The present study demonstrated that pentazocine, at doses that were 10- to 100-fold less than that of morphine (0.1, 0.3, and 1.0 mg·kg^{-1} vs 10 mg·kg^{-1}), dose-dependently potentiated morphine-induced antinociception in morphine-tolerant mice. The significant antinociception potentiated by low-dose pentazocine in morphine-tolerant mice persisted for at least 14 or 19 days. Single or repeated administrations of pentazocine at these low doses neither produced antinociception of its own nor potentiated the acute morphine-induced antinociception in morphine-naïve mice. These results indicated that the potentiation of antinociception induced by low-dose pentazocine in morphine-tolerant mice resulted not from an additive or synergistic antinociceptive interaction between the two drugs but from the prevention of morphine tolerance by low-dose pentazocine.

The present study also demonstrated that the acute morphine-induced antinociception and the acute pentazocine-induced antinociception were inhibited only by C-CAM and only by nor-BNI, respectively, whereas the significant chronic antinociception maintained by the co-administration of morphine and pentazocine was

completely inhibited both by C-CAM and by nor-BNI. These results indicated that the acute morphine-induced antinociception and the pentazocine-induced antinociception were mediated only by μ-opioid receptors and only by κ-opioid receptors, respectively, whereas both μ- and κ-opioid receptors were involved in the chronic antinociception maintained by the drug combination. The above-mentioned results most likely suggested that the co-administration of pentazocine at low, subanalgesic doses could potentiate μ-receptor-mediated morphine-induced antinociception in morphine-tolerant mice, through its κ-receptor-mediated tolerance-preventing activity, analogously to selective κ-opioid receptor agonists [20–23]. C-CAM completely inhibited the chronic antinociception, presumably because this antinociception was μ-opioid-receptor-mediated morphine-induced antinociception, while nor-BNI also completely inhibited the chronic antinociception, probably by antagonizing the κ-receptor-mediated tolerance-preventing activity of pentazocine, thus unmasking the reduced antinociceptive efficacy of morphine caused by tolerance.

The activation of κ-opioid receptors opposes a variety of μ-opioid-receptor-mediated actions of morphine, including antinociception and antinociceptive tolerance, although detailed mechanisms underlying such receptor interactions have not been fully elucidated [3]. Recent basic research suggests that antinociceptive tolerance to morphine is the result of two, partially overlapping, processes: a gradual loss of inhibitory opioid signal transduction and an increase in excitatory signaling [24]. It is well established that specific regions of the brain can positively or negatively modulate incoming pain signals at the level of the spinal cord [25]. Morphine application into the midbrain periaqueductal gray, the locus coeruleus, and the rostral ventromedial medulla (RVM) produces robust antinociception by activating inhibitory pathways descending from these regions to inhibit nociceptive inputs at the spinal level. On the other hand, the RVM is also the source of descending tracts that facilitate nociceptive inputs at the spinal level. Prolonged exposure to morphine enhances the descending pain facilitatory pathway from the RVM, and neuroplastic changes that cause the activation of this pathway are considered to play a crucial role in the development of morphine tolerance [25]. It has been suggested that in the nucleus raphe magnus (NRM) included in the RVM, the activation of κ-receptors inhibits the activities of cells of two different types that are thought to, respectively, inhibit and facilitate spinal pain transmission, by presynaptically inhibiting the synaptic release of excitatory neurotransmitters onto these RVM cells of both types [3,26,27]. Therefore, it is probable that κ-opioid receptor agonists oppose morphine tolerance in morphine-tolerant animals, at least in part, through the presynaptic inhibition of chronic-morphine-induced enhancement of the descending pain facilitatory pathway, while opposing morphine-induced antinociception in morphine-naïve animals, at least in part, through the presynaptic inhibition of acute-morphine-induced stimulation of the descending pain inhibitory pathway.

By such mechanisms, selective κ-opioid receptor agonists such as tifluadom, bremazocine, U-50,488H, and dynorphins dose-dependently antagonize acute morphine-induced antinociception in morphine-naive animals, while dose-dependently potentiating chronic morphine-induced antinociception in morphine-tolerant animals [20–23]. In the present study, however, the antagonism of the morphine-induced antinociception by pentazocine in morphine-naïve mice was not dose-dependent (see Figs. 2 and 3B), unlike the antagonism induced by selective κ-opioid receptor agonists [20–23]. In addition, in the chronic experiment, pentazocine at 0.1 and 0.3 $mg \cdot kg^{-1}$ significantly antagonized, while pentazocine at 1.0 $mg \cdot kg^{-1}$ significantly potentiated, morphine-induced antinociception on day 3, in the initial phase of tolerance development (see Fig. 6). Such complex dose-response interactions between pentazocine and morphine may reflect the differences in the pharmacological properties between the mixed agonist-antagonist pentazocine and selective κ-agonists. Several studies have also reported the complex nature of the antinociceptive interactions between pentazocine and morphine in morphine-naïve animals. For example, Blane and Dugdall [28] showed that antinociception induced by s.c. morphine (0.6 $mg \cdot kg^{-1}$) was antagonized, additively potentiated, and again antagonized, by s.c. pentazocine at a low dose (0.35 $mg \cdot kg^{-1}$), intermediate doses (0.36 and 1.25 $mg \cdot kg^{-1}$), and high doses (2.5 and 5.0 $mg \cdot kg^{-1}$), respectively, in rats. Suzuki et al. [29] showed that antinociception induced by intracerebroventricular (i.c.v.) morphine 5 μg was antagonized, and additively potentiated, by i.c.v. pentazocine 5 μg and 50 μg, respectively, in mice. Likewise, Shimada et al. [30] reported that pentazocine synergized or antagonized morphine-induced antinociception depending on the dose sizes of s.c. morphine (0.69 to 2.75 $mg \cdot kg^{-1}$) and s.c. pentazocine (2.38 to 19.0 $mg \cdot kg^{-1}$). The results of the present study on the complex nature of interactions between morphine and pentazocine in morphine-naïve mice were in good agreement with data in these previous studies [28–30].

One limitation of the present study was that in the chronic experiment employing the daily s.c. injections of morphine at 10 $mg \cdot kg^{-1}$, the effect of pentazocine at 1.0 $mg \cdot kg^{-1}$ on the development of morphine tolerance could not be fully elucidated, because %MPE remained at 100% in animals co-treated with morphine at 10 $mg \cdot kg^{-1}$ and pentazocine at 1.0 $mg \cdot kg^{-1}$, reflecting the

remarkable antinociception-potentiating effect of pentazocine at 1.0 mg·kg^{-1} (see Fig. 6). To define the tolerance-preventing effect of pentazocine at 1.0 mg·kg^{-1} more clearly, morphine at a lower dose (e.g., 5 mg·kg^{-1}) should have been employed in the chronic experiment. In our previous study, however, morphine tolerance developed much more slowly with morphine at 5 mg·kg^{-1}, compared with morphine at 10 mg·kg^{-1}, and morphine at 5 mg·kg^{-1} continued to produce significant antinociception for at least 7 days [12]. We thus employed 10 mg·kg^{-1} as the daily morphine dose to detect the tolerance-preventive effect of the test drugs more rapidly, clearly, and easily, as was done in many of the previous studies employing a similar study design [6,12–14,31,32]. We believe that our data are sufficient to clearly demonstrate that pentazocine at doses 10- to 100-fold less than that of morphine could produce a significant tolerance-preventive effect and that pentazocine at a dose 10-fold less than that of morphine could remarkably potentiate morphine-induced antinociception in morphine-tolerant animals.

Although mounting evidence from preclinical studies suggests that NMDA antagonists and selective κ-opioid receptor agonists can prevent morphine tolerance [2,3], no drugs in these classes have become available for long-term therapeutic use for the prevention of morphine tolerance because of the lack of effective oral formulations and/or because of their annoying side-effect profiles. Given that the effective oral tablet of pentazocine has already been used in clinical practice for the long-term treatment of cancer pain without producing annoying adverse effects, our data suggested that pentazocine could be an attractive pharmacological tool for the prevention of morphine tolerance in patients with cancer pain. Before going on to clinical trials to test this possibility, however, it is clear that further preclinical studies are required to determine whether pentazocine can prevent the development of morphine tolerance in other animal pain models, such as inflammatory pain and neuropathic pain models, because it has been well recognized that not only nociceptive but also inflammatory as well as neuropathic pain components can contribute to cancer pain [33], and that the rate of development of morphine tolerance is changed (delayed or enhanced) in subjects suffering from chronic pain [34,35].

In conclusion, the present study demonstrated that co-administration of the mixed agonist-antagonist analgesic pentazocine at low, subanalgesic doses with morphine dose-dependently prevented the development of antinociceptive tolerance to morphine and thus potentiated morphine-induced antinociception in morphine-tolerant mice. The tolerance-preventing activity of pentazocine was mediated by κ-opioid receptors. Such activity of pentazocine seems to be of great clinical relevance because of the well-established clinical applicability of the oral tablet form of this drug.

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