

Antinociceptive Effect of the Combination of Pentazocine With Morphine in the Tail-Immersion and Scald-Pain Tests in Rats

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Received December 16, 1999 Accepted May 10, 2000

ABSTRACT—We investigated the antinociceptive effect of pentazocine hydrochloride (pentazocine) in combination with morphine hydrochloride (morphine) using two antinociceptive tests; i.e., the tail-immersion and scald-pain tests, in rats. In the tail-immersion test, the rat's tail was immersed in warm water at 47°C, and the latency to a nociceptive response was measured. In the scald-pain test, the right hind foot was scalded by immersion into hot water at 57°C. Two hours later, additional thermal stimulus was applied to the same foot, and the latency to a nociceptive response was measured. Subcutaneous treatment with either pentazocine (6, 12, 24 mg/kg) or morphine (1.5, 3, 6 mg/kg) alone dose-dependently showed antinociceptive effects in both tests. The ED₅₀ values (95% confidence limit) of pentazocine and morphine were 13.0 (5.4–31.5) and 2.4 (1.6–3.7) mg/kg in the tail-immersion test and 11.0 (4.5–26.6) and 3.8 (1.8–7.2) mg/kg in the scald-pain test, respectively. Simultaneous treatment with pentazocine at the similar dose augmented the morphine (1.5 mg/kg)-induced antinociception, but did not diminish the morphine (6 mg/kg)-induced antinociception in both tests. These results suggest that the simultaneous administration of pentazocine at the antinociceptive dose and morphine exerts additional antinociceptive activity against thermal and scald-induced inflammatory pain.

Keywords: Pentazocine, Morphine, Antinociceptive effect, Tail-immersion test, Scald-pain test

Pentazocine, as opioid analgesic, is widely used for the management of pain in humans (1–6), and it acts as an agonist to μ and κ opioid receptors and as a weak antagonist to μ opioid receptor (7–10). It has been reported that pentazocine dose-dependently potentiated morphine-induced analgesia in non-opioid-tolerant patients with chronic pain (11) and that the combination of pentazocine with morphine produced a significantly greater level of analgesia than each opioid analgesic alone in patients undergoing surgery for the removal of impacted wisdom teeth (12). On the other hand, pentazocine antagonized morphine-induced analgesia in opioid-tolerant patients with chronic pain (11). It has also been reported that pentazocine did not influence the analgesic effect of morphine on abdominal operative pain (13) and that of pethidine, an opioid analgesic, on pressure-induced somatic pain (14).

In experimental animal studies, pretreatment with pentazocine antagonized morphine- or pethidine-induced antinociception in the rat tail-flick test (15, 16), but the relationship between antinociceptive dose and antagonistic dose of pentazocine in these reports was not clear. In

another experimental study, simultaneous treatment with pentazocine had a synergistic effect in the mouse tail-pressure and acetic acid writhing tests and an antagonistic effect in the mouse tail pinch test, depending upon the dose of each drug (17). Since it is thought that pentazocine at high doses exhibits an antinociception even against those severe noxious stimuli, the effect of pentazocine on morphine-induced antinociception may be affected by the differences of experimental procedures including drug dosages.

In the mouse tail-immersion test, the subcutaneous administration of pentazocine has a potent antinociceptive action toward noxious thermal stimuli at temperatures of 45°C and 50°C, and morphine has a similar effect at the same temperatures (18). It has also been reported that the subcutaneous administration of pentazocine or morphine has a potent antinociceptive effect on scald-induced persistent inflammatory pain in rats (19). Moreover, the i.c.v. administration of pentazocine showed both antagonistic and additive effects on the antinociception induced by the i.c.v. administration of morphine in the mouse warm plate test

(20). These studies suggest that the tail-immersion and scald-pain tests, using the moderate thermal stimulus like the mouse warm plate test, might be useful for determining the potency and drug interaction of agonist-antagonist analgesics such as pentazocine. Therefore, the purpose of the present study was to investigate the combined effects of pentazocine on morphine-induced antinociception using the tail-immersion and scald-pain tests in rats.

MATERIALS AND METHODS

The present study was conducted in strict accordance with the Guiding Principles for the Care and Use of Laboratory Animals adopted and promulgated by The Japanese Pharmacological Society (Tokyo).

Animals

Male Sprague-Dawley rats, weighing 140–260 g, were purchased from Charles River Japan, Inc. (Kanagawa). Rats were housed in plastic home cages in a room with a 12 h light/dark cycle and a temperature and humidity of $23 \pm 3^\circ\text{C}$ and $50 \pm 10\%$, respectively. The animals were allowed free access to laboratory chow and tap water for the experimental period.

Drugs

Pentazocine hydrochloride was purchased from Sterling-Winthrop Research Institute (New York, NY, USA), morphine hydrochloride was from Takeda Chemical Industries Ltd. (Osaka), and diethyl ether was from Wako Pure Chemical Industries Ltd. (Tokyo). Pentazocine and morphine were dissolved in and diluted with saline, respectively.

Determination of antinociceptive activity using the tail-immersion test

The tail-immersion test used in the present study was a modification of one described previously by Janssen et al. (21). Baseline latency was defined as the time from immersion of the tail in warm water (47°C) until the appearance of nociceptive responses (tail-flicking, vocalization or struggling). Rats were divided into several groups of 6 animals each on the basis of the baseline latency. In the study regarding the antinociceptive effects of individual drugs, pentazocine was administered subcutaneously at doses ranging from 6 to 24 mg/kg. Morphine was administered subcutaneously at doses ranging from 1.5 to 6 mg/kg. In the combination study, pentazocine was administered simultaneously with morphine. Fifteen minutes after the drug treatments, latency (test latency) was measured at 15-min intervals for 1.5 h. A cut-off time of 30 s was used.

Determination of antinociceptive activity using the scald-pain test

The scald-pain test used in the present study was a modification of one described in the previous studies (19, 22). Initially, with rats under ether anesthesia, the right hind foot was scalded by immersing it for 6 s into hot water maintained at 57°C . Two hours later, to induce inflammatory pain, the scalded hind foot was immersed again for 5 s into warm water maintained at 40°C , and then the rats were immediately returned to their home cage. Baseline latency was defined as the time from return to their cage until the appearance of nociceptive responses (foot-lifting or vocalization). Rats were divided into several groups of 6 animals each on the basis of the baseline latency to be equalizing mean value. The drug treatment regimen and the measurements of test latency were performed as with the tail-immersion test. A cut-off time of 30 s was used.

Statistical analyses

The drug-induced antinociceptive effect was expressed as the maximum test latency during the experimental period and was converted to % maximum possible effect (%MPE) by the following formula:

$$\% \text{MPE} = 100 \times (\text{maximum test latency} - \text{baseline latency}) / (\text{cut-off time} - \text{baseline latency})$$

The results are presented as means \pm S.E.M. The ED_{50} and its 95% confidence limit values were calculated by the method of Litchfield and Wilcoxon (23). To analyze the dose-response relationship in the study regarding the antinociceptive effects of individual drugs and their combined effect, the significance of differences was evaluated by a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The Aspin-Welch *t*-test was used to compare the data between saline plus saline- and saline plus morphine-treated groups in the combination study. A probability value of $P < 0.05$ was considered significant.

RESULTS

Determination of antinociceptive activity using the tail-immersion test

Pentazocine and morphine dose-dependently increased %MPE, and the peak of %MPE by each drug was observed at 45 min after the administration (Fig. 1: a and b). The %MPEs ($55.9 \pm 14.1\%$, $64.3 \pm 7.7\%$) of pentazocine at more than 12 mg/kg and that ($63.3 \pm 11.4\%$, $98.1 \pm 1.9\%$) of morphine at more than 3 mg/kg were significantly different from those ($1.9 \pm 1.3\%$, $2.6 \pm 0.8\%$) in the saline-treated groups, respectively. The ED_{50} values (95% confidence limit) of pentazocine and morphine were 13.0

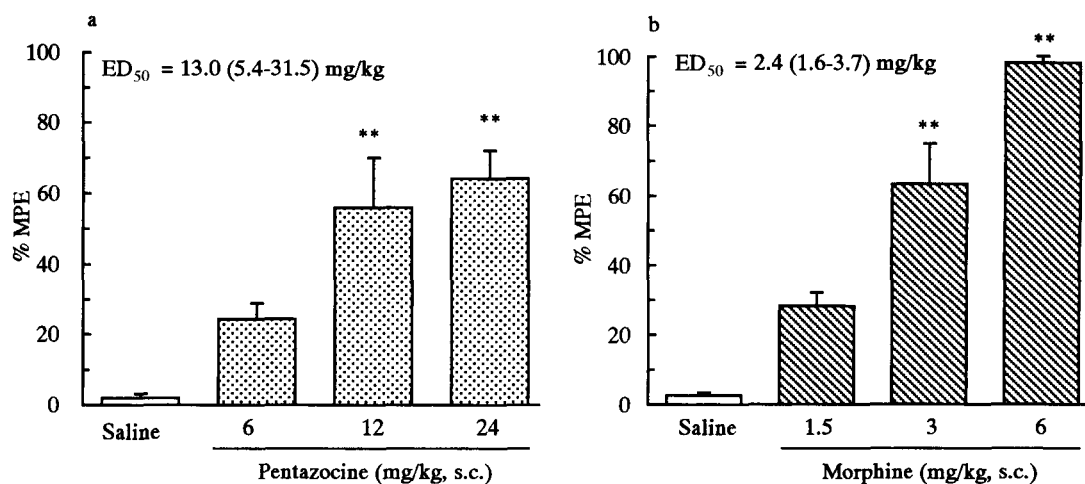


Fig. 1. Antinociceptive effects of pentazocine (a) and morphine (b) using the tail-immersion test in rats. After pentazocine or morphine was administered, the latency of the nociceptive response was measured at 15-min intervals for 1.5 h. The maximum latency was observed at 45 min after each drug administration, and converted to %MPE. Each point represents a mean %MPE \pm S.E.M. (n=6). ** P <0.01, significantly different from the saline-treated group (Dunnett's multiple comparison test).

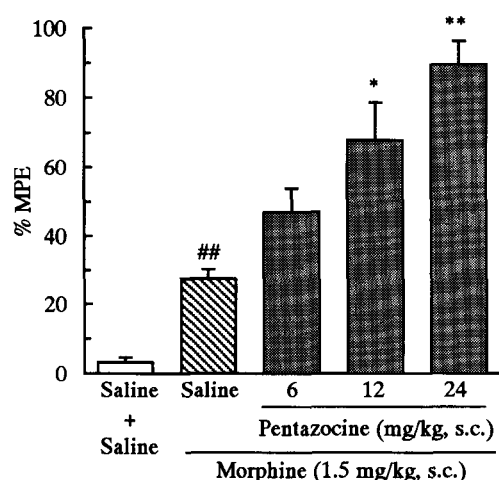


Fig. 2. Antinociceptive effect of the combination of pentazocine with a low dose (1.5 mg/kg) of morphine using the tail-immersion test in rats. After pentazocine was administered simultaneously with morphine, the latency of the nociceptive response was measured at 15-min intervals for 1.5 h. The maximum latency was observed at 45 min after the drug administrations and converted to %MPE. Each column represents a mean %MPE \pm S.E.M. (n=6). ## P <0.01, significantly different from the saline plus saline-treated group (Aspin-Welch t -test). * P <0.05, ** P <0.01, significantly different from the saline plus morphine-treated group (Dunnett's multiple comparison test).

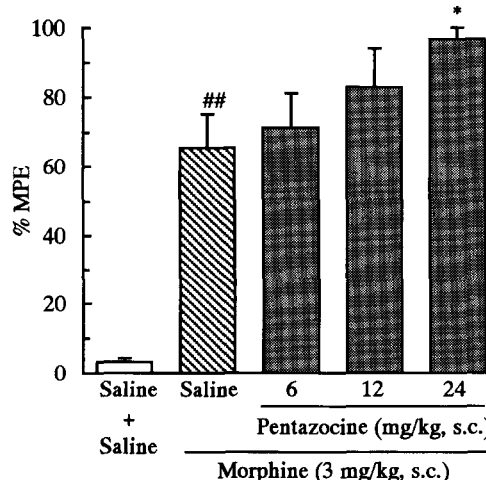


Fig. 3. Antinociceptive effect of the combination of pentazocine with a middle dose (3 mg/kg) of morphine using the tail-immersion test in rats. The protocol for drug administration and measurement of %MPE was the same as for a low dose of morphine (Fig. 2). The maximum latency was observed at 45 min after the drug administrations. Each column represents the mean %MPE \pm S.E.M. (n=6). ## P <0.01, significantly different from the saline plus saline-treated group (Aspin-Welch t -test). * P <0.05, significantly different from the saline plus morphine-treated group (Dunnett's multiple comparison test).

(5.4–31.5) and 2.4 (1.6–3.7) mg/kg. Simultaneous treatment with pentazocine and morphine (1.5 and 3 mg/kg) increased %MPE in a dose-dependent manner, and the peak %MPE by each combination was observed at 45 min after the administration (Figs. 2 and 3). The combination of pentazocine at more than 12 mg/kg with morphine at 1.5

mg/kg ($68.0 \pm 10.6\%$, $89.6 \pm 6.8\%$) and the combination of pentazocine at 24 mg/kg with morphine at 3 mg/kg ($96.8 \pm 3.2\%$) significantly increased %MPEs compared to each saline plus morphine-treated group ($3.2 \pm 1.5\%$, $3.2 \pm 1.1\%$), respectively. Furthermore, pentazocine in combination with morphine at 6 mg/kg did not change %MPE

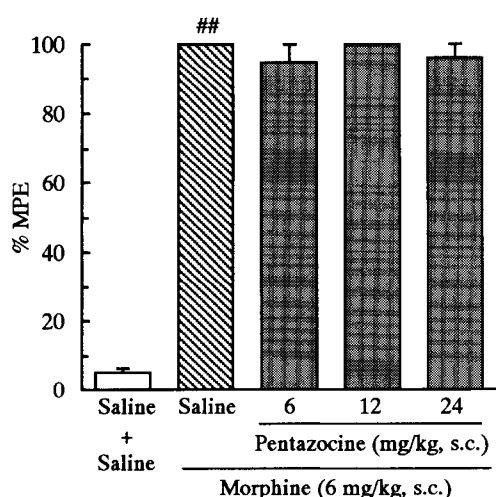


Fig. 4. Antinociceptive effect of the combination of pentazocine with a high dose (6 mg/kg) of morphine using the tail-immersion test in rats. The protocol for drug administration and measurement of %MPE was the same as for a low and middle dose of morphine (Figs. 2 and 3). The maximum latency was observed at 45 min after the drug administrations. Each column represents a mean %MPE \pm S.E.M. ($n=6$). $^{###}P<0.01$, significantly different from the saline plus saline-treated group (Aspin-Welch t -test).

($96.2 \pm 3.9\%$ – $100.0 \pm 0.0\%$), the peak values at 45 min after the administration, compared to the saline plus morphine-treated group ($100.0 \pm 0.0\%$) (Fig. 4).

Determination of antinociceptive activity using the scald-pain test

Pentazocine and morphine dose-dependently increased

%MPE, and the peak %MPE by each drug was observed at 30 min after the administration. The %MPEs ($58.9 \pm 13.3\%$, $78.4 \pm 8.2\%$) of pentazocine at more than 12 mg/kg and that ($44.2 \pm 5.9\%$, $78.0 \pm 11.7\%$) of morphine at more than 3 mg/kg were significantly different from those in the saline-treated group ($6.4 \pm 1.8\%$, $5.4 \pm 1.3\%$), respectively (Fig. 5). The ED_{50} values (95% confidence limit) of pentazocine or morphine were 11.0 (4.5–26.6) and 3.6 (1.8–7.2) mg/kg. Simultaneous treatment with pentazocine and morphine at 1.5 and 3 mg/kg increased %MPE in a dose-dependent manner, and the peak %MPE by each combination was observed at 30 min after the administration (Figs. 6 and 7). The combinations of pentazocine at more than 12 mg/kg with morphine at 1.5 mg/kg ($73.1 \pm 9.8\%$, $84.2 \pm 7.7\%$) and with morphine at 3 mg/kg ($80.9 \pm 8.4\%$, $92.9 \pm 6.5\%$) significantly increased %MPEs compared to each saline plus morphine-treated group ($4.7 \pm 1.6\%$, $4.5 \pm 1.2\%$), respectively. Furthermore, the combination of pentazocine with morphine at 6 mg/kg did not change %MPE ($82.0 \pm 9.8\%$ – $90.1 \pm 6.6\%$), the peak values at 45 min after the administration, compared to the saline plus morphine-treated group ($78.0 \pm 11.7\%$) (Fig. 8).

DISCUSSION

In the present study, the antinociception induced by morphine alone increased in a dose-dependent manner in both the tail-immersion and scald-pain tests. Although the antinociceptive potencies of pentazocine were ca one-fifth and one-third those of morphine in the tail-immersion and scald-pain tests respectively, the ED_{50} value (13.0 mg/kg) of pentazocine using the tail-immersion test was not greater

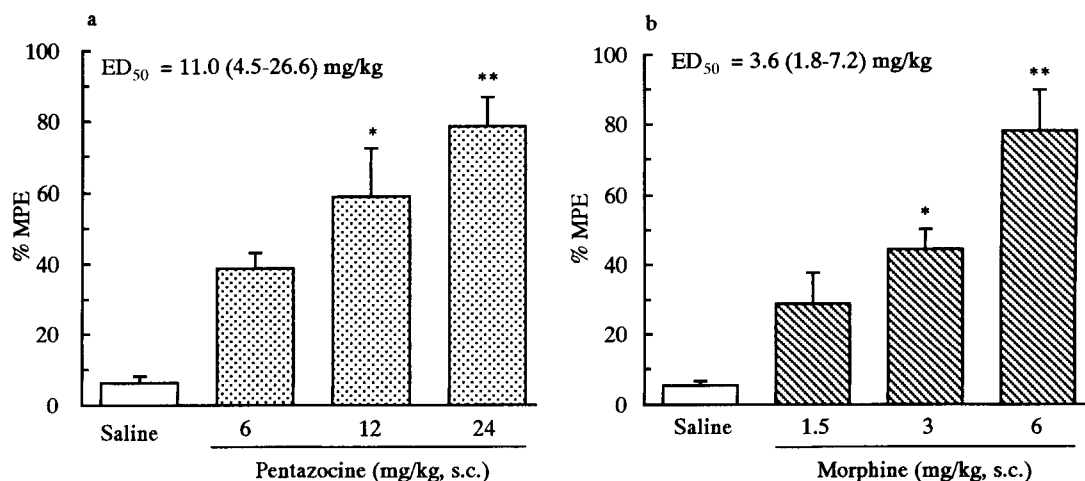


Fig. 5. Antinociceptive effects of pentazocine (a) and morphine (b) using the scald-pain test in rats. After pentazocine or morphine was administered, the latency of the nociceptive response was measured at 15-min intervals for 1.5 h. The maximum latency was observed at 30 min after each drug administration and converted to %MPE. Each point represents a mean %MPE \pm S.E.M. ($n=6$). $^{*}P<0.05$, $^{**}P<0.01$, significantly different from the saline-treated group (Dunnett's multiple comparison test).

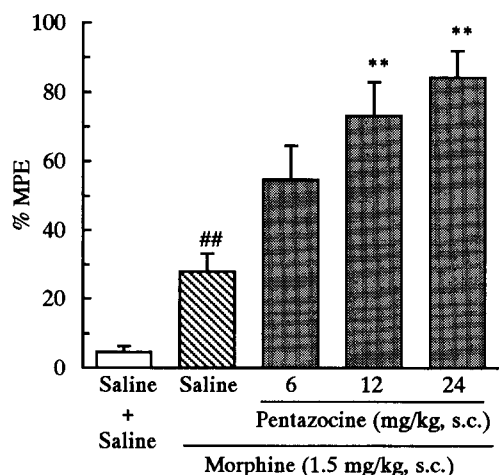


Fig. 6. Antinociceptive effect of the combination of pentazocine with a low dose (1.5 mg/kg) of morphine using the scald-pain test in rats. After pentazocine was administered simultaneously with morphine, the latency of the nociceptive response was measured at 15-min intervals for 1.5 h. The maximum latency was observed at 30 min after the drug administrations, and converted to %MPE. Each column represents a mean %MPE \pm S.E.M. ($n=6$). ## $P<0.01$, significantly different from the saline plus saline-treated group (Aspin-Welch t -test). ** $P<0.01$, significantly different from the saline plus morphine-treated group (Dunnett's multiple comparison test).

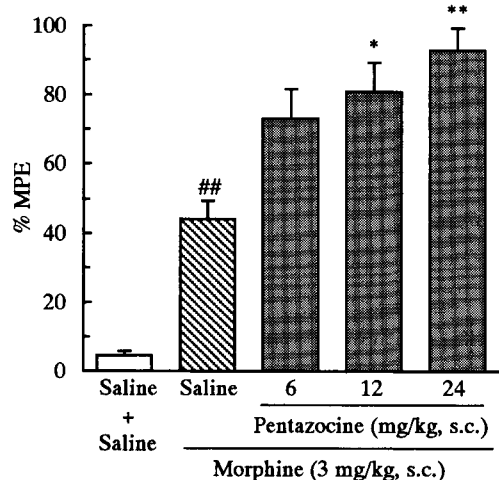


Fig. 7. Antinociceptive effect of the combination of pentazocine with a middle dose (3 mg/kg) of morphine using the scald-pain test in rats. The protocol for drug administration and measurement of %MPE was the same as for a low dose of morphine (Fig. 6). The maximum latency was observed at 30 min after the drug administrations. Each column represents a mean %MPE \pm S.E.M. ($n=6$). ## $P<0.01$, significantly different from the saline plus saline-treated group (Aspin-Welch t -test). * $P<0.05$, ** $P<0.01$, significantly different from the saline plus morphine-treated group (Dunnett's multiple comparison test).

than that (11.0 mg/kg) using the scald-pain test. Since the antinociception induced by pentazocine at a high dose (24

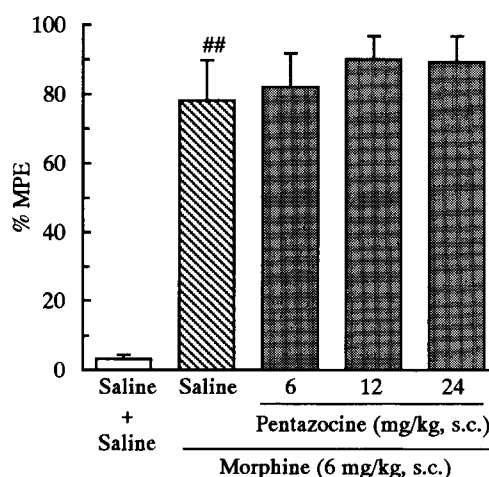


Fig. 8. Antinociceptive effect of the combination of pentazocine with a high dose (6 mg/kg) of morphine using the scald-pain test in rats. The protocol for drug administration and measurement of %MPE was the same as for a low and middle dose of morphine (Figs. 6 and 7). The maximum latency was observed at 30 min after the drug administrations. Each column represents a mean %MPE \pm S.E.M. ($n=6$). ## $P<0.01$, significantly different from the saline plus saline-treated group (Aspin-Welch t -test).

mg/kg) was slightly higher than that at a middle dose (12 mg/kg) in the tail-immersion test, this slight increase in %MPE by pentazocine in this test might be due to a ceiling effect in agreement with the result of a previous study where pentazocine was shown to produce a ceiling effect on respiratory depression (3).

Several studies using the tail-flick tests have shown that pentazocine antagonized morphine- or pethidine-induced antinociception (15, 16). Because the noxious stimuli were very severe in these studies, the relationship between the antagonistic dose and the antinociceptive one of pentazocine was not clear. It has been reported that marked differences exist between μ , δ and κ opioid receptor-mediated antinociception using different stimuli between cutaneous thermal stimuli in the tail-flick/hot plate test and a visceral chemical stimulus, acetic acid, in the writhing test (24). The writhing syndrome is prevented by several drugs, including non-steroid anti-inflammatory drugs, μ , δ and κ opioid receptor agonists. Using the writhing test, pentazocine produced significant antinociception and potentiated morphine-induced antinociception in mice (17, 25). Pentazocine not only potentiated the antinociceptive activity of morphine when the temperature of the noxious stimulus was 50°C but also the antagonistic action was observed when the temperature was 59°C using the mouse hot plate test (26). Such studies indicate that pentazocine might produce a modulation opposite to morphine-induced antinociception, which is dependent upon the strength of noxious stimulus. Moreover, thermal injury precipitates an

increase in vascular permeability, proteolysis, systemic inflammatory response, and release of chemical mediators, which are followed by persistent pain. It is known that several chemical mediators, i.e., bradykinin and prostaglandins, produce pain in thermal injury and that μ , δ and κ opioid receptor agonists mediate potent antinociceptive activity in animals subjected to thermal injury (27, 28). Since pentazocine exhibits high affinity for μ_1 , μ_2 and κ_1 opioid receptors (8), it is proposed that pentazocine may exhibit antinociception against the moderate thermal stimulus via these opioid receptors.

In the present study, simultaneous treatment with pentazocine additionally augmented morphine-induced antinociception when a low dose (1.5 mg/kg) of morphine was used, and pentazocine did not diminish the potent antinociception caused by a high dose (6 mg/kg) of morphine in not only the tail-immersion but also the scald-pain tests. These results demonstrate that pentazocine might have a additive effect on morphine-induced antinociception when a moderate noxious stimulus was used. It has been reported that the subcutaneous administration of (–)-pentazocine, an optical isomer of pentazocine, produced the antinociception via κ opioid receptors, and the antinociception of the subcutaneous administration of morphine was mediated by μ opioid receptors in the mouse tail-flick test (8). Moreover, the antinociception of pentazocine or morphine by the subcutaneous administration against inflammatory pain induced by formalin was mediated via κ and μ opioid receptors, respectively (29). On the other hand, it has also been reported that the i.c.v. administration of pentazocine may predominantly act as a partial μ and/or δ antagonist when the low dose was used in combination with morphine and acts as a μ , δ and κ agonist when the high dose was used in the mouse warm plate test, and that the additional antinociception by the combination was mediated through κ as well as μ and δ opioid receptors (20). The difference of opioid receptor subtype targeted by pentazocine between the former and the latter studies might be dependent upon the route of drug administration; e.g., central action versus systemic action. In the present study, pentazocine was administered through the subcutaneous route, like the former studies (8, 29), so the antinociception induced by pentazocine in response to acute thermal stimulus and scald-induced inflammatory pain may be mainly mediated by κ opioid receptors, and the additive antinociception of the combination of pentazocine with morphine in responses to these pains may be mainly mediated by μ and κ opioid receptors. The combination of μ and κ opioid receptor agonists produced synergistic antinociception in response to somatic noxious stimuli without increasing common deleterious side effects (30), and the activation of κ opioid receptors prevented the development of tolerance to morphine-induced antinocicep-

tion in the rat hot plate test (31). Therefore, the results of the present study support the hypothesis that the combination of pentazocine with morphine may be useful for the clinical management of both moderate somatic and persistent inflammatory pain.

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