

# Spinal Coadministration of Ketamine Reduces the Development of Tolerance to Visceral As Well As Somatic Antinociception During Spinal Morphine Infusion

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This study was designed to investigate the effects of ketamine, an *N*-methyl-D-aspartate receptor antagonist, on the development of tolerance to morphine and morphine antinociception during intrathecal infusion. Two intrathecal catheters were implanted in the subarachnoid space in male rats under pentobarbital anesthesia. One catheter was used for the intrathecal infusion with the following solutions: morphine  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1) and  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5); ketamine  $250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (K250); morphine plus ketamine,  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  plus  $250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1 + K250) and  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  +  $250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5 + K250); or saline. The other catheter was used for morphine challenge tests. The responses to noxious somatic and visceral stimuli were measured by tail flick (TF) and colorectal distension (CD) tests, respectively. Measurements were performed once a day for 7 days. Challenge tests with intrathecal morphine were performed to assess the magnitude of tolerance on Day 5 and Day 7. The antinociceptive effect was evaluated by using the percent of maximal possible effect (%MPE). Morphine infusion produced significant increases in

%MPEs in TF and CD tests, while the saline and K250 infusions did not show any changes. The M1 + K250 infusion significantly increased the %MPEs in TF and CD tests, although the M1 and K250 infusions alone showed no changes. M5 + K250 enhanced the increases of %MPEs in TF and CD tests compared with the M5 infusion alone. In the challenge tests, the M1 + K250 infusion showed no significant decrease in %MPEs and TF and CD tests. The M5 + K250 infusion significantly inhibited those decreases in %MPEs, although the M5 infusion showed significant decreases in TF and CD tests. We concluded that ketamine attenuated the development of morphine tolerance to antinociceptive effects and increased the somatic and visceral antinociception of morphine. **Implications:** Intrathecally co-infused ketamine attenuated morphine tolerance to somatic and visceral antinociception and increased morphine antinociception at the spinal level. These results suggest that a combination of morphine with ketamine may have an advantage in long-term use of opioids for controlling visceral as well as somatic pain.

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**T**olerance-induced decreased analgesia is one of major problems with the chronic use of opioids. *N*-methyl-D-aspartate (NMDA) receptor antagonists inhibit the development of opioid tolerance (1–4). These results support the concept that NMDA receptor antagonists control the development of tolerance. Because epidural or spinal opioids are popular for treating chronic pain, the regulation of tolerance at the spinal level may have advantages, such as reduced doses of opioids and fewer side effects when an NMDA antagonist is given (5). Recent studies demonstrate that both intrathecally and systemically administered ketamine, a noncompetitive NMDA receptor

antagonist, attenuates the development of morphine tolerance in rodents (2,6).

Those studies regarding the effects of NMDA receptor antagonists focused only on the tolerance to somatic, but not visceral, analgesia (2,3,6,7). However, chronic pain from organs, including cancer pain, is mostly derived from visceral afferents (8). NMDA receptor antagonists potentiate the antinociceptive effect of morphine at the spinal cord in rats (9). Furthermore, Wong et al. (10) reported that epidural coadministration of ketamine potentiates the analgesic effect of morphine on postoperative pain.

This study was designed to investigate the effects of ketamine on the development of tolerance to somatic and visceral antinociception of morphine during the intrathecal infusion of morphine. The second aim was to evaluate the effects of ketamine on the somatic and visceral antinociception of morphine.

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## Methods

This protocol was approved by our animal research and use committee. Male Sprague-Dawley rats, weighing 300–350 g, were used for this study. To reduce the influences of handling on nociceptive responses, all animals were handled and trained in the test situation for at least 4 to 6 days before intrathecal catheterization and testing.

Under pentobarbital anesthesia, intrathecal catheters (double-stretched PE-10 joined to PE-10, PE-10 joined to PE-20, in osmotic pumps cases, PE-20 joined to PE-60) were implanted in the subarachnoid space with the tips facing in the rostral direction at the level of L4-5. Each rat was implanted with two catheters. The first catheter was implanted in the subarachnoid space through the dura window punctured by a 26-gauge needle; the tip was dulled to avoid spinal cord injury. The second catheter was implanted through the same window guided by the first catheter. Osmotic pumps were used for continuous infusion and connected to the catheter. The pumps were filled with the drug(s) or saline and attached to the PE-60 end of the catheter and implanted subcutaneously. The other catheter was used for the challenge test on Day 5 and Day 7 after the intrathecal infusion.

One of the following regimens was intrathecally infused: morphine hydrochloride  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1),  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5); ketamine hydrochloride  $250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (K250); morphine hydrochloride plus ketamine hydrochloride  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1} + 250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1 + K250),  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1} + 250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5 + K250); or saline. Drugs were dissolved in sterile saline. Each animal received only one dose of drug or saline.

The tail flick (TF) test was used to measure responses to noxious somatic stimuli by monitoring latency to withdrawal from a heat source focused on the dorsal surface of the tail approximately 5 cm from the tip. Lack of occurrence of the TF response in 10 s resulted in termination of the stimulus, and the 10-s interval was assigned as the cut-off time to avoid damage to the tail.

The colorectal distension (CD) test was used to measure noxious visceral stimulus. The CD test involves an 8-cm long, flexible, latex balloon. The system consists of two parts: a proximal stimulating balloon and a distal sensing balloon. Both stimulating and sensing balloon pressures were continuously monitored with in-line pressure transducers. The balloons were inserted intra-anally into the colon and rectum under light halothane anesthesia. Animals were tested awake after a minimal 20-min recovery from anesthesia. Pressure within the intracolonic stimulating balloon was increased at a rate of 2.5 mm Hg/s. The pressure in the stimulating balloon at which the increase of the pressure in the sensing balloon was triggered was defined

as the threshold response for CD. A cut-off distension pressure of 60 mm Hg was used to prevent tissue damage.

Measurements were performed daily to assess each animal's response to noxious somatic and visceral stimuli for 7 days during the intrathecal infusion. On Days 5 and 7, the challenge test with morphine was performed to assess the development of tolerance to morphine. In the challenge test, morphine  $5 \mu\text{g}/10 \mu\text{L}$  was intrathecally administered after the determination of baseline values, and measurements from the TF and CD tests were repeated at 5, 10, 15, 20, 30, 60, 90, 120, and 180 min after injection.

TF and CD tests were performed sequentially at the same time point with a 2-min interval between each test sequence. This study was performed in a double-blinded fashion until all measurements were completed.

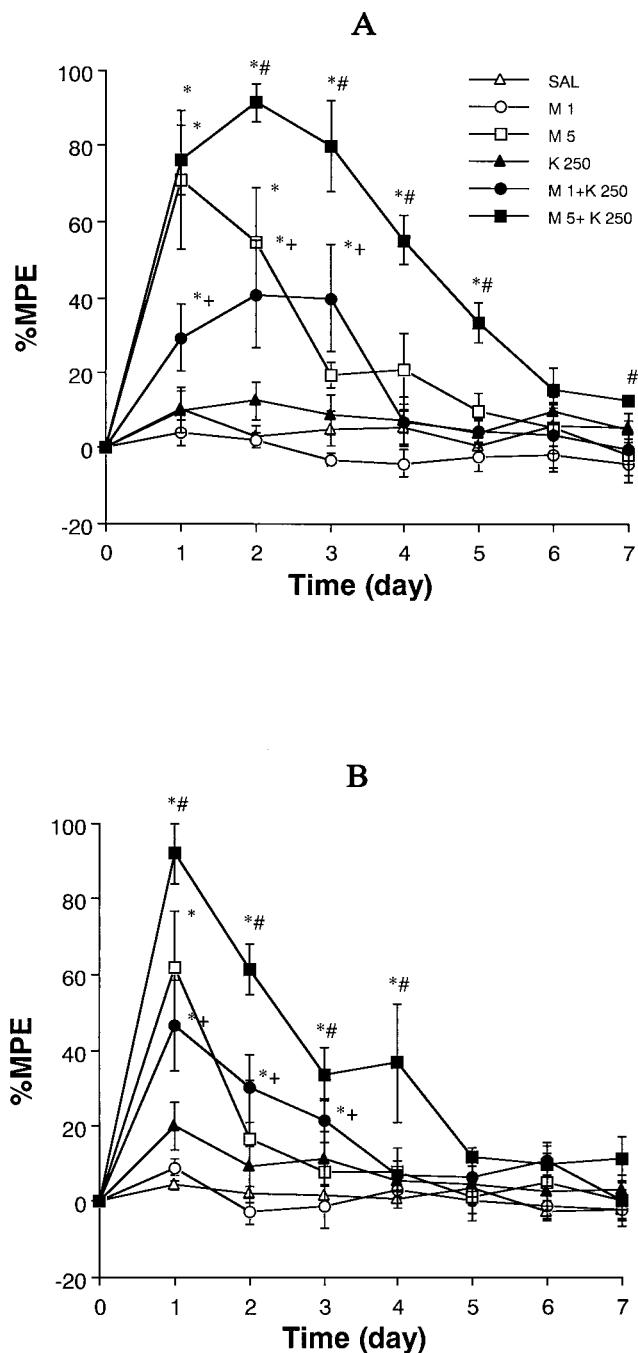
The effectiveness of the catheter was confirmed by the injection of  $10 \mu\text{L}$  of 2% lidocaine, and the injection of indigo carmine dye confirmed catheter placement after completion of measurements. Rats that had motor deficits caused by catheter placement, infection, or other problems were excluded.

The analgesic effect was evaluated by transforming the response threshold to the percent of maximal possible effect (%MPE = [postdrug value – baseline value]/[cut-off value – baseline value]  $\times$  100). All data were presented as mean  $\pm$  SEM. Analysis of variance for repeated measures followed by Fisher's protected least significant difference was used to evaluate statistical significance. Differences were considered to be significant at  $P < 0.05$ .

## Results

We prepared 45 animals, and 9 animals were excluded from the study (6 because of catheter failure, 2 because of pump failure, and 1 because of infection). Thirty-six animals (six animals in each group) were analyzed in the results.

Baseline values of the %MPEs in the TF and CD tests were not statistically different among the groups. The intrathecal infusion of morphine produced significant increases in %MPEs in both the TF and CD tests, while saline and K250 infusions did not show any significant changes (Fig. 1). The increases of %MPEs gradually returned to the baseline values from the peak effects on Day 1. The M1 + K250 infusion significantly increased the %MPEs in the TF and CD tests for 3 days (Fig. 1), although the M1 and K250 infusions alone did not show significant changes in the %MPEs in the TF and CD tests. The M5 infusion significantly increased %MPEs in the TF and CD tests for 2 days and 1 day, respectively, compared with the saline infusion. The M5 + K250 infusion enhanced the increases of %MPEs in the TF and CD tests caused by the M5 infusion and prolonged their duration.



**Figure 1.** Time course effects in percent of maximal possible effect (%MPE) in the tail flick (TF) test (A) and colorectal distension (CD) test (B) during infusion of morphine  $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1) and  $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5), ketamine  $250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (K250), morphine plus ketamine  $1 + 250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1 + K250) and  $5 + 250 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5 + K250), and saline (SAL). \* $P < 0.05$  versus SAL. + $P < 0.05$  versus M1. ## $P < 0.05$  versus M5.

In the challenge test on Day 5, there was no significant difference in the time course changes in %MPEs in the TF tests among the three groups (M1, M1 + K250, and saline groups) (Fig. 2A). However, the %MPE in the CD test decreased from 90 min in the M1

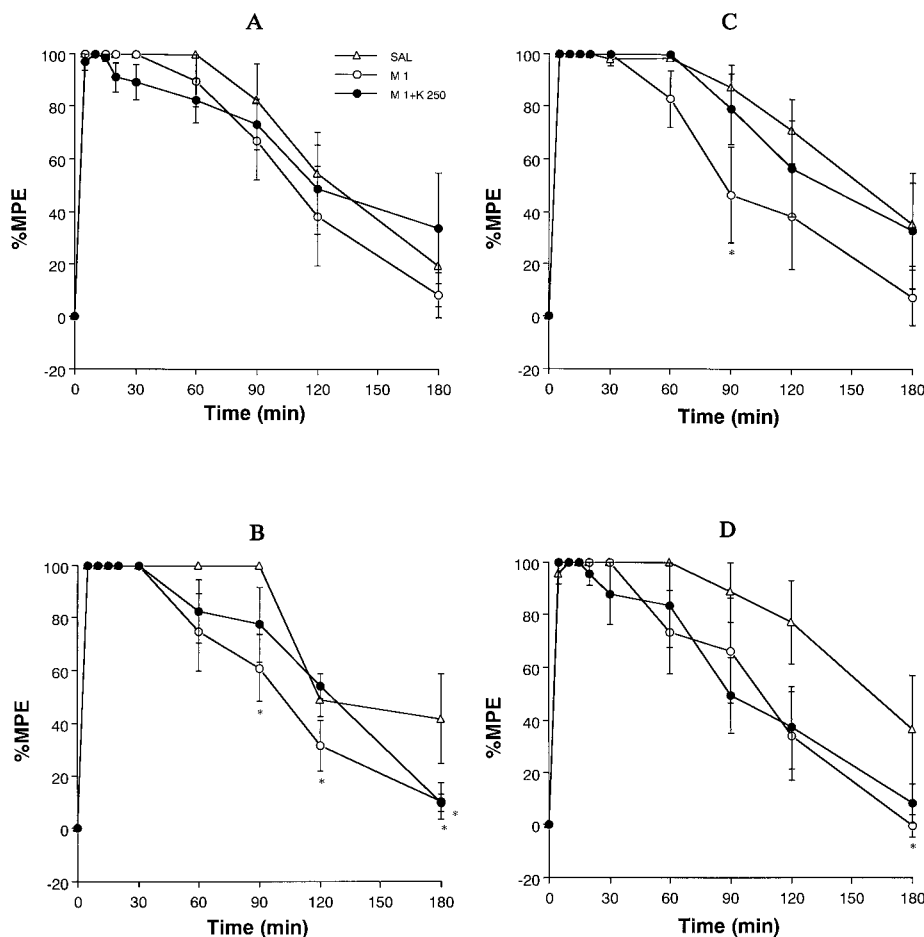
infusion group compared with the saline infusion group (Fig. 2B). The challenge test on Day 7 showed the significant decreases in %MPEs in both the TF and CD tests in the M1 infusion group compared with the saline infusion group (Fig. 2, C and D), indicating the development of tolerance to morphine. However, the M1 + K250 infusion did not produce any significant decreases in %MPEs in both the TF and CD tests in the challenge tests. There is no difference between the M1 and M1 + K250 groups in the challenge tests on Day 5 and Day 7.

The M5 infusion demonstrated significant decreases the %MPEs in the TF and CD tests on both challenge tests on Day 5 and Day 7 compared with saline infusion (Fig. 3), indicating apparent development of tolerance. The M5 + K250 infusion significantly inhibited those decreases in %MPEs in the challenge tests on Day 5 and Day 7.

## Discussion

We demonstrated that intrathecally coadministered ketamine attenuated the development of tolerance to morphine antinociception, both somatic and visceral. The literature documents that NMDA receptor antagonists attenuate the development of tolerance to opioid antinociception (1-4,7). This supports our findings that the attenuation of tolerance to somatic and visceral antinociception is, at least in part, attributed to the inhibition of the NMDA receptor at the spinal cord because ketamine is a noncompetitive NMDA receptor antagonist. However, the exact mechanisms in that phenomenon are not clear from our results, because ketamine acts on a rather heterogenous family of receptors, such as NMDA, non-NMDA, opiate, cholinergic, adrenergic, and  $\gamma$ -aminobutyric acid ( $\text{GABA}_A$ ) receptors (11). One study showed that NMDA antagonists could attenuate the development of tolerance to morphine antinociception, but not to selective  $\mu$ - or  $\delta$ -opioid agonists in mice (12). Therefore, it is not clear that ketamine can be solely attributed to its action as an NMDA receptor antagonists on  $\mu$ -opioid receptor-related antinociception.

Intrathecally administered ketamine significantly shifted the dose-response curve to the left in TF latency and abolished the increase of its 50% effective dose by 46-fold in the rats that received morphine intrathecally three times a day (6). Those results coincide with our results which showed that ketamine attenuated tolerance to morphine antinociception at the spinal level, although there are differences in assessing the development of tolerance between two studies. The challenge test, with using several doses of morphine, provides the dose-response curve which these authors used to evaluate the magnitude of tolerance. However, we constructed the time-response curves in the morphine challenge tests instead of the



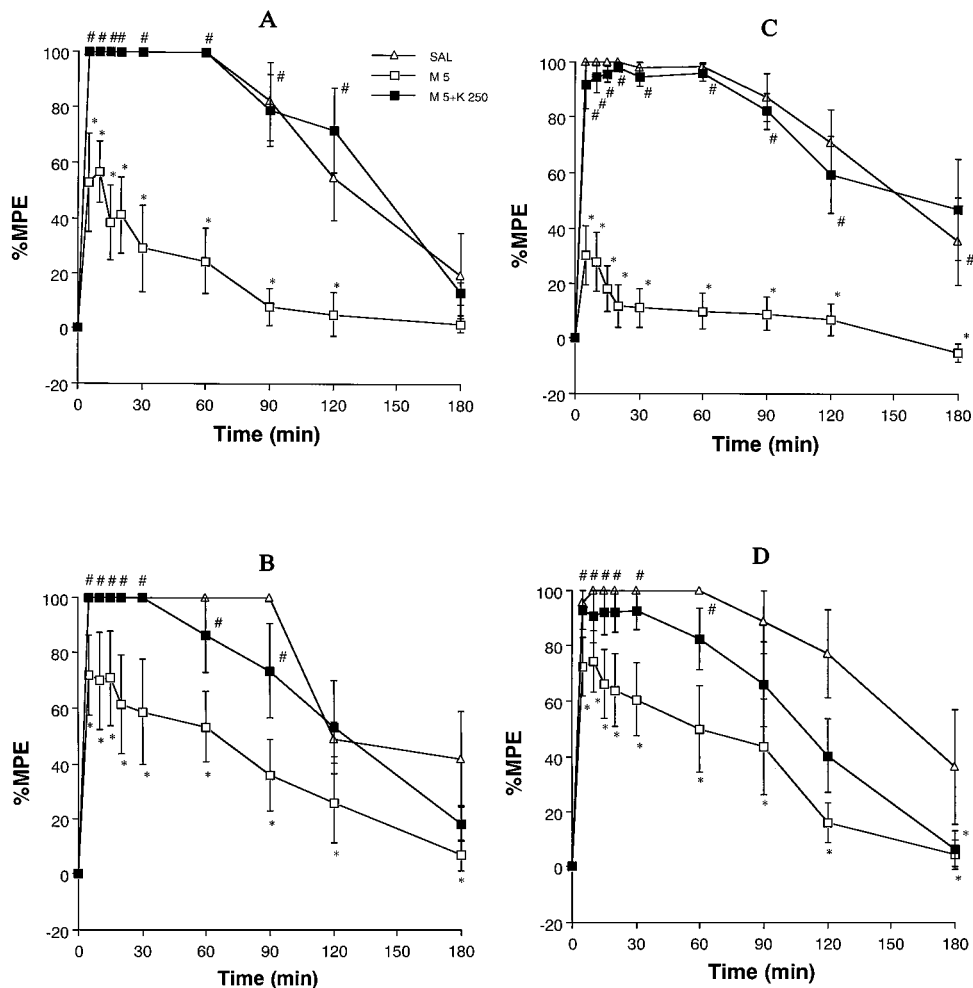
**Figure 2.** Changes in percent of maximal possible effect (%MPE) in the tail flick (TF) test (A and C) and colorectal distension (CD) test (B and D) after the intrathecal administration of morphine 5  $\mu\text{g}/10 \mu\text{L}$  on Day 5 (A and B) and Day 7 (C and D) in morphine 1  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1), morphine plus ketamine 1 + 250  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M1 + K250), and saline (SAL). \* $P < 0.05$  versus SAL. + $P < 0.05$  versus M1.

dose-response curve to assess the magnitude of tolerance (1-4,13). The challenge test, with a single dose of intrathecal morphine, provides information regarding the changes of antinociceptive effects in both intensity and duration. Another reason for choosing the time-response curve is to minimize the number of animals used because of ethical considerations. Despite the different assessments, these observations from two studies clearly indicate that ketamine attenuates the development of morphine tolerance to antinociceptive effects, including their duration as well as magnitude, at the spinal level.

The increased duration of morphine antinociception, as shown in Figure 1, may indicate the enhancement of morphine antinociception as well as the attenuation of tolerance, because ketamine enhanced morphine antinociception in the early period when the tolerance did not completely develop. Wong et al. (9) reported that intrathecally coadministered NMDA receptor antagonists enhanced the morphine antinociception effect. This observation supports the enhancement

of morphine antinociception by co-infused ketamine in our results. In contrast, Shiomoyama et al. (6) demonstrated that intrathecally administered ketamine did not enhance morphine antinociception, although it attenuated the tolerance to morphine. They administered ketamine 10 minutes before daily morphine injection, whereas Wong et al. (9) and we coadministered NMDA receptor antagonists with morphine. The different methods of administering drugs may contribute to this different effect of ketamine on morphine antinociception.

Coadministered ketamine attenuated the morphine tolerance to visceral antinociception as well as somatic antinociception. Previous studies have focused only on somatic antinociception (1-4,6,7,9). The visceral component of pain is important in pain control, especially in cancer pain (8). However, there has been little information concerning the characteristics of development of tolerance to opioid analgesia at the spinal level. Our results demonstrated that intrathecal infusion of morphine produced a decreased antinociceptive effect on



**Figure 3.** Changes in percent of maximal possible effect (%MPE) in the tail flick (TF) test (A and C) and colorectal distension (CD) test (B and D) after the intrathecal administration of morphine 5  $\mu\text{g}/10 \mu\text{L}$  on Day 5 (A and B) and Day 7 (C and D) in morphine, 5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5), morphine plus ketamine 5 + 250  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$  (M5 + K250), and saline (SAL). \* $P < 0.05$  versus saline SAL. # $P < 0.05$  versus M5.

visceral pain compared with somatic pain (Fig. 1). The morphine challenge test indicated decreased development of tolerance to visceral antinociception, as shown in Figure 3. Although the mechanism is not clear, the anatomical features in visceral pain pathways, including the different terminations of the primary afferent fibers in the dorsal horn (14), low density of sensory innervation and extensive divergence of input at the spinal level (15) may contribute to the present results. It is difficult to compare the analgesic effects on both pain models.

Visceral pain, especially in cancer patients, is a poor responder (16), but the present results suggest the possibility of controlling visceral cancer pain by using the coadministration of ketamine with morphine. To obtain satisfactory analgesia for visceral pain, the required dose of opioids is gradually increased because of tolerance, but the increased dose may lead to more complications. Our results demonstrate that ketamine attenuated morphine tolerance and enhanced visceral antinociception and

support that coadministered ketamine and morphine has an advantage in controlling long-lasting visceral pain.

Another aspect of the interaction between NMDA antagonists and morphine, the interaction with side effects, should be discussed. Trujillo et al. (17) reported that NMDA receptor antagonist MK-801 led to an increase in morphine-associated catalepsy and lethality. However, in this study, no animals showed catalepsy or died as a result of the side effects of morphine and/or ketamine. The different results between the two studies may come from the different drugs administered, different modes of administration, and different doses of morphine. It is likely that the incidence of side effects is increased, because Trujillo et al. (17) used a much larger dose of morphine (1.0–100 mg/kg) than we did (0.17 and 0.87 mg/kg). Although our results may suggest the safety of that combination, further studies in different settings, including different animals, are necessary before clinical applications can be made.

The side effects of opioids, such as respiratory depression, nausea, vomiting, pruritus, or urinary retention, or the side effects of ketamine, such as psychotomimetic activity, often restrict the increase of the opioid dose, because the incidence of side effects increases dose-dependently (18). The coadministration of ketamine with opioids may contribute to a decrease in the incidence of side effects of opioids and ketamine by decreasing the required dose of each drug. In fact, epidurally coadministered ketamine with morphine reduced some symptoms, including drowsiness, nausea, vomiting, and pruritus, in postoperative patients undergoing major joint replacement (10).

Neuronal toxicity of ketamine should be considered in the clinical use of epidural ketamine (19). The toxicity is mainly caused by its preservatives (19,20). There is evidence that a large concentration of ketamine causes focal degeneration with myelin loss of spinal cord in the rat (21). However, the concentration used in clinical practice may not be responsible for neuronal toxicity. Although there have been no reports regarding apparent neuronal deficits after the use of epidural ketamine in humans, we strongly recommend use of preservative-free ketamine of a small concentration.

We conclude that co-infused ketamine attenuated the development of tolerance to somatic and visceral antinociceptive effects of morphine and enhanced those effects during intrathecal infusion. These results suggest that the coadministration of ketamine may have an advantage in the long-term use of opioids for controlling visceral as well as somatic pain.

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