Systemic ketamine inhibits hypersensitivity after surgery via descending inhibitory pathways in rats

[La kétamine intravasculaire inhibe l'hypersensibilité postchirurgicale par des voies inhibitrices descendantes chez les rats]

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Purpose: Systemic ketamine suppresses several types of chronic pain. Although ketamine is used as a general anesthetic agent, the analgesic effect of systemic ketamine for early-stage postoperative pain is not clear. We investigated the efficacy and mechanism of systemic ketamine in a rat model of postoperative pain.

Methods: An incision was made in the plantar aspect of the left hind paw in male Wistar rats. Mechanical hypersensitivity was measured using calibrated von Frey filaments. The anti-hypersensitivity effect of systemic or intrathecal administration of ketamine was determined every hour after making the incision. We examined the effects of intrathecal pretreatment with yohimbine, an α_2-adrenoceptor antagonist, and methysergide, a serotonergic receptor antagonist, on the anti-hypersensitivity effect of ketamine. We also examined the effect of systemic ketamine on the c-fos immunoreactivity in the spinal cord.

Results: Systemic administration of ketamine at doses from 3 to 30 $mg \cdot kg^{-1}$ produced anti-hypersensitivity effects in a dose-dependent manner. Intrathecal administration of ketamine had no effect. There was no significant difference between effects of pre- and post-incisional administration. Intrathecal pretreatment with yohimbine (10 μg) or methysergide (15 μg) completely reversed the anti-hypersensitivity effects of systemic ketamine. Systemic ketamine reduced fos expression in laminae I–II in the dorsal horn of the lumbar spinal cord ipsilateral to the paw incision.

Conclusions: The results suggest that systemic administration of ketamine perioperatively suppresses early-stage postoperative pain via monoaminergic descending inhibitory pathways.

Objectif : *La kétamine intravasculaire supprime certaines douleurs chroniques. Utilisée comme anesthésique général, son effet analgésique pour la douleur postopératoire de stade précoce n'est toutefois pas clair. Nous avons vérifié l'efficacité et le mécanisme de la kétamine intravasculaire chez un modèle rat de douleur postopératoire.*

Méthode : *Une incision a été faite dans la partie plantaire de la patte arrière gauche de rats mâles Wistar. L'hypersensibilité mécanique a été mesurée à l'aide de filaments von Frey calibrés. L'effet d'anti-hypersensibilité de la kétamine vasculaire ou intrathécale a été déterminé toutes les heures après l'incision. Nous avons étudié les effets du prétraitement intrathécal avec yohimbine, un antagoniste des récepteurs α_2-adrénergiques, et méthysergide, un antagoniste des récepteurs sérotoninergiques, sur l'effet anti-hypersensibilité de la kétamine. Nous avons aussi vérifié l'effet de la kétamine intravasculaire sur l'immunoréactivité des c-fos dans la moelle épinière.*

Résultats : *L'administration intravasculaire de kétamine en doses de 3 à 30 $mg \cdot kg^{-1}$ a produit des effets d'anti-hypersensibilité reliés à la dose. La kétamine intrathécale n'a pas eu d'effet. Il n'y avait pas de différence significative entre les effets de l'administration pré-incisionnelle et post-incisionnelle. Le prétraitement intrathécal avec yohimbine (10 μg) ou méthysergide (15 μg) a complètement renversé les effets d'anti-hypersensibilité de la kétamine intravasculaire. La kétamine intravasculaire a réduit l'expression de fos dans la lame I–II de la corne supérieure de la moelle épinière lombaire homolatérale à l'incision de la patte.*

Conclusion : *Les résultats suggèrent que l'administration intravasculaire préopératoire de kétamine supprime la douleur postopératoire de stade précoce par des voies inhibitrices descendantes monoaminergiques.*

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KETAMINE is a widely used general anesthetic with analgesic effects. Ketamine is also administered systemically and produces analgesia in several types of chronic pain, such as phantom limb pain, post-herpetic neuralgia, and orofacial pain.[1–3] Surgery is another common cause of persistent pain and hyperalgesia. Brennan *et al.*[4] developed a postoperative pain model in rats by making an incision in the paw. Hypersensitivity (reduction of withdrawal threshold from stimuli) around the incision site differs from hypersensitivity due to nerve injury or during inflammation, and is pharmacologically unique.[4–6] Although systemic ketamine is effective for postoperative pain in humans,[7,8] the effect of systemic ketamine in this model has not been examined. The first purpose of the current study is to evaluate the efficacy of systemic ketamine for postoperative pain in rats. Anti-hypersensitivity is defined as inhibition of reduction of withdrawal threshold after paw-incision.

Preemptive analgesia, pain relief strategies performed before tissue injury, can modify the development and maintenance of postoperative pain in patients.[9,10] The efficacy of preemptive analgesia clinically[11] and in postoperative pain models is controversial.[5] The second purpose of the present study is to compare the effects of pre- and post-incisional ketamine administration in this model.

Although the antinociceptive effects of systemic ketamine are considered to be mediated by spinal cord and supraspinal sites,[12] the precise mechanism is not fully understood. Activation of the N-methyl-D-aspartate (NMDA) receptors in the spinal cord is important for the induction and maintenance of sustained pain.[13,14] Ketamine inhibits NMDA receptors in a noncompetitive manner,[15] and intrathecal or systemic administration of ketamine reduces nerve injury-induced hyperalgesia or inflammatory pain in rats.[13,14,16–18] Ketamine also has antinociceptive effects for acute pain induced by the tail-flick test,[19,20] in which NMDA receptor antagonists are not effective.[21] Several behavioural and electrophysiologic studies suggest that ketamine produces antinociceptive effects through activation of monoaminergic descending inhibitory pathways.[19,20,22–24] The mechanism of the antinociceptive effects of ketamine is suggested to depend upon the presence peripheral inflammation.[22] The third purpose of the current study is to clarify the mechanism of the antinociceptive effects of systemic ketamine. Site of action and involvement of noradrenergic and serotonergic systems were examined in the postoperative pain model.

Immunocytochemical localization of Fos, which is the protein product of the immediate-early proto-oncogene c-fos, is widely used to identify populations of neurons in the spinal cord that are activated by peripheral noxious stimuli.[25,26] We examined the effect of systemically administered ketamine on Fos immunoreactivity induced by paw incision.

Materials and methods

This investigation was approved by the Animal Care and Use Committee of Gunma University Graduate School of Medicine (Maebashi, Japan). Male Wistar rats weighing 250 to 300 g were used in all experiments. All surgical procedures were performed under general anesthesia (isoflurane 2–3% in oxygen). At the end of the protocol, all animals were sacrificed with an overdose of pentobarbital.

Foot incision

The surgery was based upon the procedure described by Brennan *et al.*[4] A longitudinal 1-cm incision was made through the skin and fascia, starting at 0.5 cm from the edge of the heel and extending toward the toes of the left hind paw. The plantaris muscle was elevated using forceps and incised longitudinally. The wound was closed with two mattress sutures of 5–0 nylon. Gentamycin solution was applied to the wound site. After surgery, the rats recovered in their cages.

Intrathecal catheter placement

Intrathecal catheters were placed in some rats for intrathecal administration of drugs. After sterile preparation of the posterior neck, a polyethylene catheter (PE-10) was inserted through an opening in the atlanto-occipital membrane to the lumbar spinal cord (8.0 cm) according to the method described by Yaksh and Rudy.[27] After recovery, the animal was examined for any apparent motor or sensory deficits; if any were present, the animal was excluded from the study. Experiments were performed for five to seven days after intrathecal catheter placement.

Behavioural testing

Rats were placed individually in a cage with a wire mesh bottom that allowed full access to the paws. Withdrawal thresholds to punctate mechanical stimulation were determined using the method described by Brennan *et al.*[4] with calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA). Each von Frey filament was applied vertically to an area adjacent to the wound for six seconds while the filament was gently bent, starting with 0.2 g and continuing until a withdrawal response occurred or 28.8 g (cut-off value) was reached. This procedure was repeated three times with a three- to five-minute test-free period between with-

drawal responses. The lowest force obtained in the three tests producing a response was defined as the withdrawal threshold. The cut-off value, 28.8 g, was recorded even if there was no withdrawal response to this force. The withdrawal threshold was measured before the incision was made (baseline threshold), and then at one-hour intervals for four or six hours thereafter. The general behaviour of the rats was carefully observed after drug injection. Motor function was evaluated by examining the righting reflex, stepping reflex, posture, and ambulation. Sedation was assessed in terms of spontaneous movement such as grooming and chewing, as well as evoked movement (a startle reflex evoked by tapping on the cage). The investigator responsible for the behavioural studies was blind to the drug treatment of each animal.

Drugs and injections

Ketamine hydrochloride, yohimbine hydrochloride, an α_2-adrenoceptor antagonist, and methysergide hydrochloride, a non-selective serotonergic receptor antagonist, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Systemic drug administration was subcutaneously injected in a single injection volume of 0.5 mL. Rats were injected systemically with ketamine either immediately after (3, 10, and 30 $mg \cdot kg^{-1}$) or ten minutes before making the incision (10 and 30 $mg \cdot kg^{-1}$). In the preliminary experiment, *sc* injection of ketamine produced the effect within ten minutes. Intrathecal drug administration was performed using a microinjection syringe (Hamilton, Reno, NV, USA) in a volume of 10 μL, followed by 10 μL saline to flush the catheter. Rats were injected with ketamine (10, 30, and 100 μg) intrathecally immediately after making the incision. To verify that the effect of systemic ketamine on post-incisional hypersensitivity was produced by descending monoaminergic inhibitory pathways, 10 μg of yohimbine or 15 μg of methysergide were administered intrathecally ten minutes before making the incision, followed by *sc* administration of 30 $mg \cdot kg^{-1}$ of ketamine immediately after making the incision. The doses of antagonists were selected according to a previous report.[22] All drugs were dissolved in saline.

c-fos protein-like immunoreactivity

Rats received either 30 $mg \cdot kg^{-1}$ of ketamine ($n = 5$) or saline ($n = 6$) subcutaneously immediately after paw incision, and the expression of Fos protein was examined two hours after making the incision. The rats were deeply anesthetized with pentobarbital sodium (100–150 $mg \cdot kg^{-1}$, intraperitoneally) and transcardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4). The spinal cord was dissected out, postfixed in the same fixative overnight, and cryoprotected by immersion in 0.1 M phosphate buffer containing 20% sucrose. Three days later, the spinal cord segments containing L4–L5 were frozen with powdered dry ice, and then cut transversely into 30 μm sections using a cryostat. The sections were immunostained using the avidin-biotin complex with nickel ammonium sulfate intensification. The primary antibody for c-fos protein (Ab-5, Oncogene Research Products, MA, USA) was diluted 1:10000 with 0.1 M phosphate buffer saline (PBS) containing 5% normal rabbit serum. In this solution, the sections were incubated for 72 hr at 4°C. After rinsing with 0.1 M PBS, they were incubated in biotinylated rabbit anti-sheep IgG (Vector Laboratories, Burlingame, CA, USA) diluted 1:400 in PBS containing 5% normal rabbit serum for 24 hr at 4°C. After rinsing with PBS, the sections were incubated in 30% methanol and 1% H_2O_2 in 0.1 M PBS for 15 min to inactivate endogenous peroxidase. The sections were incubated with avidin-biotin complex reagent (Vector Laboratories) in PBS for two hours at 4°C. The sections were then reacted in 0.05% diaminobenzidine and 0.01% H_2O_2 in 0.1 M Tris-

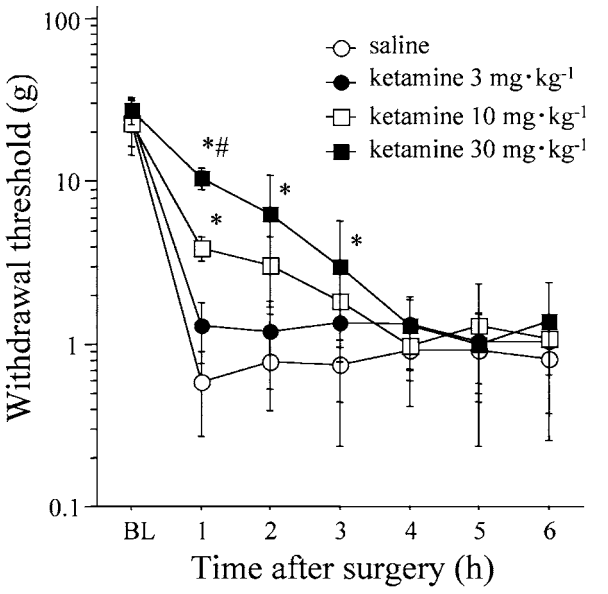

FIGURE 1 Dose-dependent reduction in punctate mechanical hypersensitivity produced by systemic ketamine administered subcutaneously immediately after incision. Each point represents the mean ± SD ($n = 8$ in each group). The withdrawal thresholds are on a log scale. BL = baseline withdrawal threshold measured before paw incision surgery. *$P < 0.05$ *vs* saline-treated group. #$P < 0.05$ *vs* 10 $mg \cdot kg^{-1}$ of ketamine-treated group.

HCl-buffered saline (pH = 7.4) containing 0.2% nickel ammonium sulfate for five minutes. The tissue sections were mounted onto gelatin-coated slides, air dried, dehydrated in a graded series of ethanol, cleared in xylene, and coverslipped.

Five to ten sections of the spinal cord segments (L4–L5) were randomly selected from each rat. The dorsal horn of each section was divided into three regions (laminae I/II, III/IV, and V/VI) as described previously,[28] and the number of c-fos protein positive neurons in each area was counted. The average number of c-fos protein-positive neurons in the five to ten sections was defined as the number of c-fos-positive neurons.

Statistical analysis

Data are presented as the mean ± SD. The data of withdrawal threshold were analyzed with nonparametric statistics. The Kruskal-Wallis test and Mann-Whitney test were used. Multiple comparisons after the Kruskal-Wallis test were performed by using the Dunn test. One-way analysis of variance was used to assess differences in number of c-fos immunoreactive neurons, followed by post hoc testing using the Dunn test for multiple comparison. A P value of less than 0.05 was considered statistically significant.

Results

Effects of ketamine on general behaviour and incision-induced mechanical hypersensitivity

Paw incision reduced the paw withdrawal threshold one hour after surgery compared to pre-surgical values (22.9 g before, 0.6 g after, $P < 0.05$, $n = 8$, Figure 1). Systemic administration of ketamine produced anti-hypersensitivity effects in a dose-dependent manner ($P < 0.05$, Dunn test). The maximum dose of ketamine increased the withdrawal threshold for three hours after surgery compared to saline ($P < 0.05$, Dunn test, Figure 1). There were no differences in the withdrawal thresholds between the group that received 10 or 30 mg·kg^{-1} of systemic ketamine ten minutes before and the group that received the same doses of systemic ketamine immediately after the incision was made (Figure 2). The rats were not sedated and motor function assessed by the righting/stepping reflex, posture, and ambulation was normally preserved at 60 min after systemic administration of ketamine. Intrathecal administration of ketamine at doses from 10 to 100 μg did not produce an anti-hypersensitivity effect (Figure 3). Systemic injection of 10 or 30 mg·kg^{-1} of ketamine produced several characteristic behavioural effects, in addition to analgesia. After administration, the rats exhibited head weaving and circling behaviour that

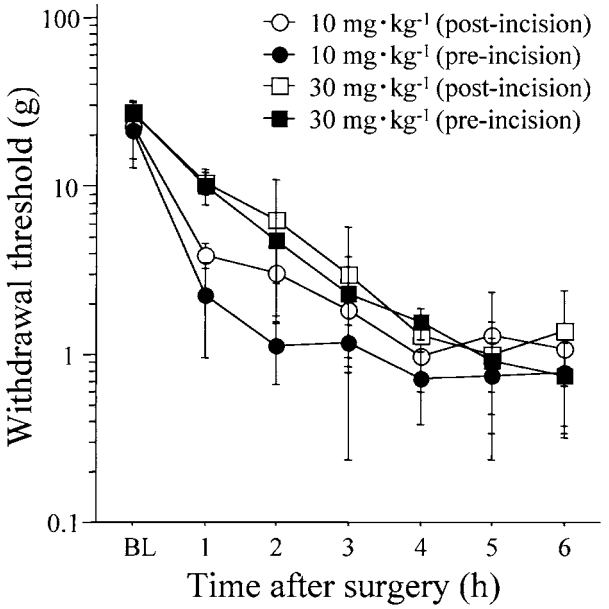

FIGURE 2 Comparison of pre- *vs* post-incisional administration of systemic ketamine on the withdrawal threshold after paw surgery. Each point represents the mean ± SD ($n = 8$ in each group). The withdrawal thresholds are on a log scale. BL = baseline withdrawal threshold measured before paw incision surgery.

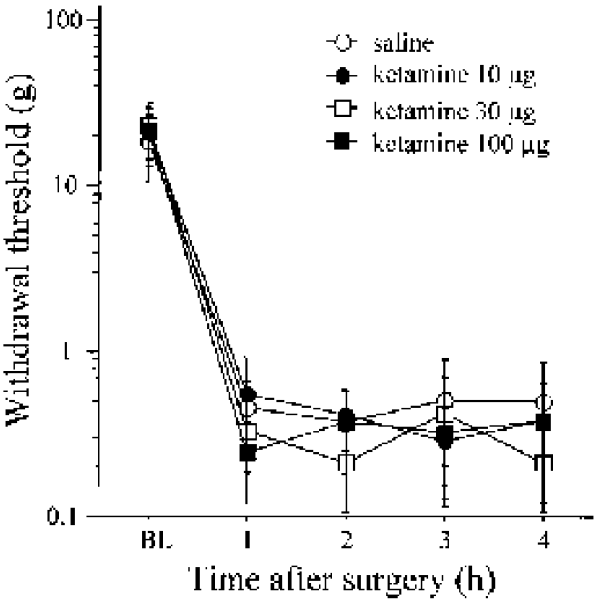

FIGURE 3 The effect of intrathecal administration of ketamine on withdrawal threshold after paw incision. Each point represents the mean ± SD ($n = 6$ in each group). The withdrawal thresholds are on a log scale. BL = baseline withdrawal threshold measured before paw incision surgery.

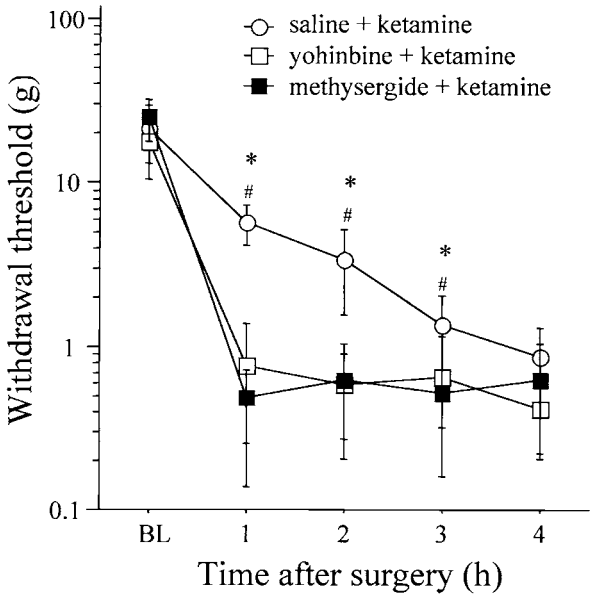

FIGURE 4 Effects of pretreatment with intrathecal administration of yohimbine or methysergide on antihypersensitivity effects of 30 mg·kg^{-1} of systemic ketamine. Each point represents the mean ± SD (n = 6 in each group). The withdrawal thresholds are on a log scale. BL = baseline withdrawal threshold measured before paw incision surgery. *P < 0.05 *vs* yohimbine-pretreated group. #P < 0.05 *vs* methysergide-pretreated group.

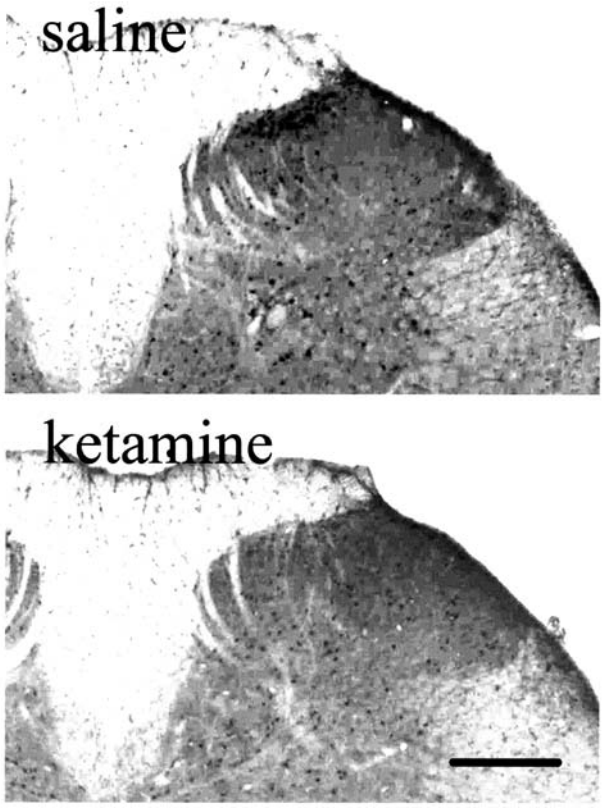

FIGURE 5 Photomicrographs of the ipsilateral dorsal horn of the lumbar spinal cord illustrating c-fos expression in rats treated with saline or 30 mg·kg^{-1} of ketamine immediately after surgery. Scale bar = 100 μm.

persisted for 30 to 50 min. Systemic ketamine at 3 mg·kg^{-1} or intrathecal ketamine at any dose did not produce abnormal behaviour.

Effects of intrathecal pretreatment of yohimbine and methysergide on the anti-hypersensitive action of systemic ketamine

Intrathecal pretreatment with yohimbine or methysergide inhibited the anti-hypersensitivity effects of 30 mg·kg^{-1} of systemic ketamine (P < 0.05, Dunn test, Figure 4). Pretreatment did not attenuate adverse effects such as abnormal behaviour associated with administration of 30 mg·kg^{-1} of systemic ketamine. Intrathecal administration of each antagonist alone did not alter the withdrawal threshold (data not shown).

Effects of systemic ketamine on c-fos expression

c-fos protein immunoreactivity two hours after paw incision was detected mainly in the superficial laminae (laminae I/II) of the lumbar (L4–L5 segments) spinal cord ipsilateral to the side of the paw incision (Figure 5). There were no differences in the number of c-fos immunoreactive neurons between the ketamine-treated group and saline-treated group on the side of the spinal cord contralateral to the paw incision (data not shown). When 30 mg·kg^{-1} of ketamine were administered immediately after paw incision, the number of c-fos immunoreactive neurons in laminae I–II was smaller than that in the saline-treated group (P < 0.05). The number of c-fos immunoreactive neurons in laminae III–IV and V–VI did not differ (Figures 5 and 6).

Discussion

In the present study, systemic but not intrathecal administration of ketamine produced anti-hypersensitivity effects at the early stage in a rat model of postoperative pain. Pretreatment with intrathecal yohimbine and methysergide reversed the effects of systemic ketamine in this model. These results suggest that ketamine activates monoaminergic descending inhibitory pathways at the supraspinal sites to produce

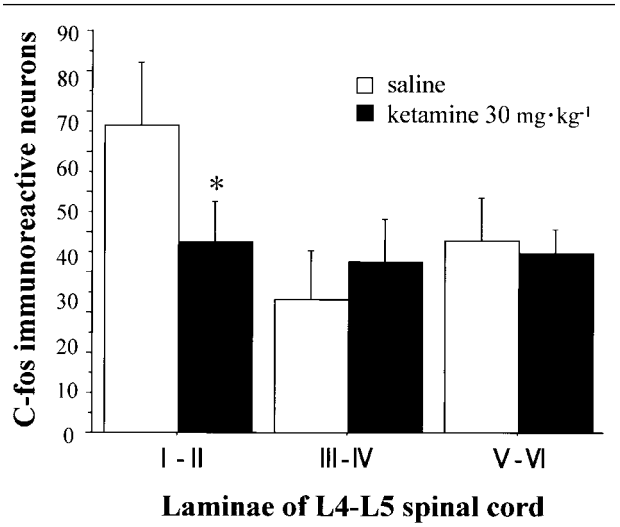

FIGURE 6 Effect of systemic ketamine on the incision-evoked c-fos protein expression in the spinal cord. Each bar represents the mean ± SD, ketamine-treated group ($n = 5$), saline-treated group ($n = 6$). *$P < 0.05$ *vs* saline-treated group.

anti-hypersensitivity effects in this model. In normal rats, ketamine produces antinociceptive effects through activation of the monoaminergic descending inhibitory pathways for acute thermal pain.[22] The precise mechanisms underlying the interaction of ketamine with the monoaminergic descending inhibitory pathways remain unclear. Although the mechanism for the analgesic action of ketamine is similar to that of morphine, it may be different.[19] Further, the periaqueductal grey region of the rat brain, which contains mu but no other opioid receptors, is not involved in ketamine-mediated analgesia.[20] Kappa receptors are suggested to have a role in ketamine-mediated analgesia. Activation of a supraspinal site containing ketamine-sensitive opioid receptors on interneurons other than mu receptors might be the mechanism of analgesia of systemic administration of ketamine.

There were no anti-hypersensitivity effects of ketamine after intrathecal administration in the present study. A previous study also demonstrated that intrathecal ketamine does not have antinociceptive effects in acute thermal pain in rats.[22] Other studies, however, demonstrate that intrathecal administration of ketamine reduces nerve injury-induced hyperalgesia[14,16] or inflammatory pain in rats.[13,18] Although ketamine has NMDA receptor antagonistic activity,[15] intrathecal administration of NMDA receptor antagonists does not modify the pain behaviours in this rat model of postoperative pain.[6] Systemic ketamine produces antinociceptive effects via the monoaminergic descending inhibitory pathways in normal rats, but these pathways are not involved in the antihyperalgesic effects of systemic ketamine in rats with peripheral inflammation.[22] Taken together, these observations indicate that the nature of incision-induced hypersensitivity in the postoperative pain model better approximates that of acute nociceptive pain than neuropathic pain or chemical inflammatory pain. Ketamine might also mediate antihyperalgesic effects via a peripheral mechanism.[29,30] Peripheral mechanisms for the anti-hypersensitivity effects of systemic ketamine in the present study, however, cannot be excluded.

Systemic administration of ketamine produced adverse effects such as head weaving and circling behaviour. Although it persisted for 30 to 50 min, these abnormal behaviours might not affect the withdrawal threshold, because rats returned to normal one hour after the administration, and righting and stepping reflexes are normally preserved. Intrathecal pretreatment with yohimbine and methysergide inhibited the anti-hypersensitivity effects of ketamine, but not the ketamine-induced abnormal behaviour. This finding suggests that the monoaminergic descending inhibitory pathways are not involved in the abnormal behaviour following ketamine administration.

In the present study, there was no difference between pre- and post-incisional administration of systemic ketamine on mechanical hypersensitivity after paw incision. Brennan *et al.*[5] also reported that there was no difference in hyperalgesia induced by pre- and post-incision intrathecal morphine or bupivacaine treatment. Although several investigators examined preemptive analgesia with systemically administered ketamine in postoperative patients, most reported that systemic ketamine had no preemptive analgesic effect.[31–33] These observations are consistent with our results. Immunocytochemical study revealed c-fos protein expression mainly in laminae I–II of the spinal cord two hours after incision of the plantar surface. The systemic administration of 30 $mg \cdot kg^{-1}$ of ketamine immediately after surgery reduced c-fos protein expression in laminae I-II, but had no effect on the c-fos protein expression in laminae III–VI. These data strongly suggest that systemic administration of ketamine inhibits nociceptive input to the superficial laminae and produces an analgesic effect in the postoperative pain model. Huang and Simpson[34] demonstrated that c-fos gene expression was induced in the dorsal horn by skin incision and that c-fos immunoreactivity was attenuated by systemic ketamine (37.5 $mg \cdot kg^{-1}$). Our observation is consistent with this report.

In summary, systemic but not intrathecal administration of ketamine produced anti-hypersensitivity effects in a rat model of postoperative pain. c-fos-positive neurons were decreased in the superficial laminae of the spinal cord in the ketamine-treated rats. The anti-hypersensitivity effects of systemic ketamine were inhibited by intrathecal pretreatment of either yohimbine or methysergide, indicating that activation of monoaminergic descending inhibitory pathways is, at least in part, involved in this action. Our results suggest that systemic ketamine inhibits early stage postoperative pain by a different mechanism than that of neuropathic pain or inflammatory pain.

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