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Influence of Preemptive Treatment with MK-801, an N-methyl-D-aspartate Receptor Antagonist, on Development of Neuropathic Symptoms Induced by Spinal Nerve Ligation in the Rat

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THERE is previous evidence suggesting that nerve injury-induced activation of N-methyl-D-aspartate (NMDA) receptors might cause intracellular changes, providing a mechanism for chronic pain.¹ To test this hypothesis, we used an experimental model of neuropathy induced by spinal nerve ligation to investigate the preemptive effect of a noncompetitive NMDA receptor antagonist, MK-801, on tactile allodynia and mechanical hyperalgesia.

Materials and Methods

The experiments were performed with adult, male Hannover-Wistar rats (weight, 220–350 g; The Finnish Laboratory Animal Center, Kuopio, Finland). The experimental study protocol was accepted by the Institutional Animal Care Committee of the University of Helsinki.

The unilateral ligation of two spinal nerves (L5 and L6) was performed on the rats during anesthesia. The anesthesia was induced by subcutaneous injection of 5 mg/kg midazolam (Dormicum; Roche, Basel, Switzerland) and 1.0 ml/kg of a combination of fentanyl (0.2 mg/ml) and fluanisone (10 mg/ml; Hypnorm; Janssen Pharmaceuticals, Beerse, Belgium). The surgical tech-

nique is described in detail elsewhere.² In one group of animals (sham-operated rats), the surgical procedure, except nerve ligation, was performed as described above.

For assessment of tactile allodynia, a hind limb withdrawal threshold, evoked by stimulation of the hind paw with calibrated monofilaments with increasing force (Stoelting, Wood Dale, IL; range, 0.1 to 46.6 g), was determined while the rat was standing on a metal grid. The threshold for each rat at each time point was based on three separate measurements.

For assessment of mechanical hyperalgesia, a hind limb withdrawal threshold to noxious mechanical stimulation (paw pressure test) was determined with a Basile Analgesy meter (Ugo Basile, Varese, Italy). A cutoff force of 500 g was used. Two threshold determinations for each hind paw were performed.

There were six experimental groups. In all groups (+)-MK-801 hydrogen maleate (Research Biochemicals Incorporated, Natick, MA) was administered to the animals during anesthesia as follows: (1) MK-801 hydrogen maleate 0.5 mg/kg was administered intravenously in the tail vein 15 min before nerve ligation ($n = 16$); (2) MK-801 0.1 mg/kg was administered 15 min before nerve ligation ($n = 12$); (3) MK-801 0.5 mg/kg was administered 10 min after the end of nerve ligation ($n = 12$); (4) after the development of mechanical allodynia induced by ligation of spinal nerves (> 2 weeks after the nerve ligation), the rats were anesthetized in an identical fashion as for the surgery and received MK-801 at the dose of 0.5 mg/kg ($n = 4$); (5) the rats received saline 15 min before nerve ligation (saline control group; $n = 12$); and (6) a sham operation was performed without any additional drug treatments, except general anesthesia (sham control group; $n = 5$). For groups 1–3, 5, and 6, the behavioral assessment of allodynia and hyperalgesia

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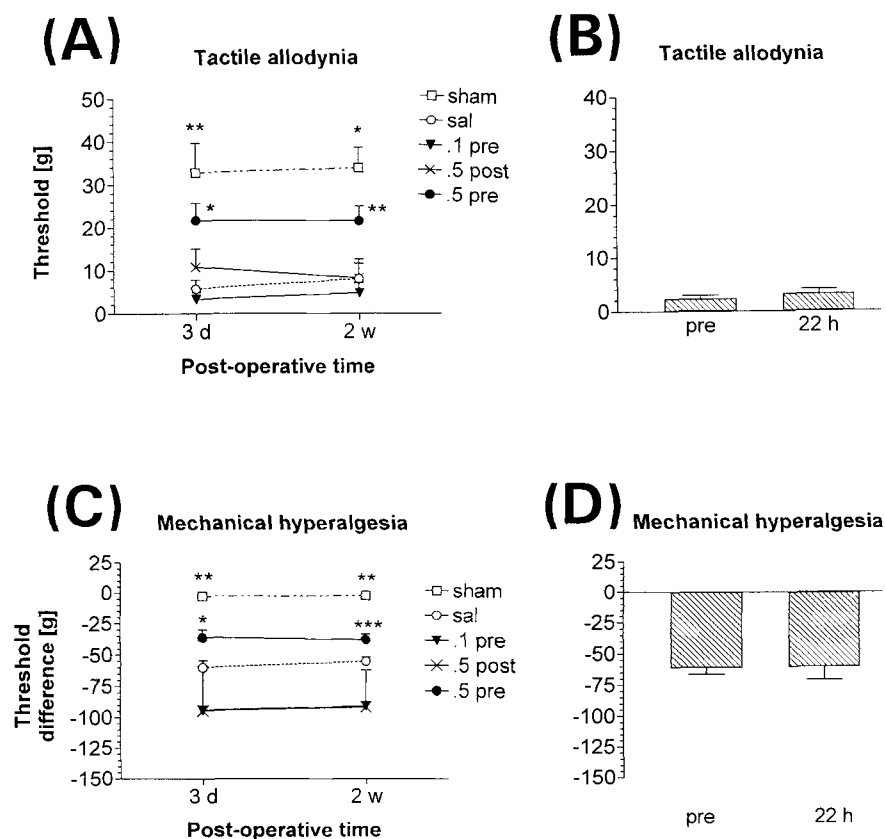


Fig. 1. (A) Effect of preoperative/postoperative treatment with various doses of MK-801 on development of tactile allodynia. (B) Effect of MK-801 0.5 mg/kg on tactile allodynia in fully developed neuropathy (> 2 weeks after the ligation of spinal nerves). (C) Effect of preoperative/postoperative treatment with various doses of MK-801 on development of mechanical hyperalgesia. (D) Effect of MK-801 0.5 mg/kg on mechanical hyperalgesia in fully developed neuropathy. In A and B, y-axis indicates the withdrawal threshold to monofilament stimulation of the operated hind limb. 0.1–0.5 pre/post = dose of MK-801 in mg/kg administered 15 min before (pre) or 10 min after (post) nerve ligation; 2 w = 2 weeks after surgery; 3 d = 3 days after surgery; sal = saline-control group; sham = sham operation. In C and D, y-axis indicates the difference between hind limb withdrawal thresholds (threshold difference < 0 g indicates hyperalgesia of the operated side). pre = before induction of anesthesia and treatment with MK-801; 22 h = 22 h after administration of MK-801 and recovery from anesthesia. The error bars represent SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (reference: the corresponding saline group, Mann-Whitney U test in graphs A and B, Student t test in graphs C and D).

was performed 3 days and 2 weeks after the operation. For group 4, the behavioral assessment of allodynia and hyperalgesia was performed immediately before induction of anesthesia and 22 h after the application of MK-801.

Nonparametric statistics (Mann-Whitney U test) were used to assess treatment-induced differences in mechanical allodynia of the operated limb between the groups. Parametric statistics (t test) were used to assess treatment-induced differences in an index of mechanical hyperalgesia (= threshold difference between the operated limb – the unoperated limb). Two-way analysis of variance (factors: postoperative time and treatment) was used to assess changes in the hind paw withdrawal thresholds to noxious mechanical stimulation of the control limb. $P < 0.05$ (two-tailed) was considered to represent a significant difference.

Results

In saline-treated animals, nerve ligation induced a highly significant decrease of the hind paw withdrawal

threshold to monofilament stimulation of the paw when compared with sham-operated rats. In saline-treated animals, a marked tactile allodynia was observed within the third postoperative day and was maintained at the same level throughout the observation period of 2 weeks. MK-801 at the 0.1-mg/kg dose before surgery or the 0.5-mg/kg dose immediately after surgery did not produce any significant attenuation of the tactile allodynia during the 2-week observation period (fig. 1A). However, the 0.5-mg/kg dose of MK-801 immediately before the surgery significantly attenuated the development of allodynia throughout the 2-week observation period. After the development of allodynia (> 2 weeks after the nerve ligation), the 0.5-mg/kg dose of MK-801 administered in anesthetized animals had no long-term effects, as indicated by monofilament test performed after recovery from anesthesia 22 h after the administration of MK-801 (fig. 1B).

The hind limb withdrawal thresholds induced by noxious mechanical stimulation of the paw contralateral to the surgery were not significantly different between treatments ($F_{4,96} = 0.78$, two-way analysis of variance).

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The withdrawal threshold evoked by noxious mechanical stimulation of the paw contralateral to the surgery also did not change with postoperative time ($F_{1,96} = 0.57$, two-way analysis of variance).

In saline-treated animals, nerve ligation induced a highly significant mechanical hyperalgesia in the operated side when compared with sham-operated animals. This hyperalgesic effect was observed throughout the 2-week observation period (fig. 1C). The 0.1-mg/kg dose of MK-801 administered before surgery or the 0.5-mg/kg dose administered immediately after surgery had no significant effect on the development of mechanical hyperalgesia. However, the 0.5-mg/kg dose of MK-801 administered before nerve ligation significantly attenuated the development of mechanical hyperalgesia throughout the 2-week observation period. After the development of mechanical hyperalgesia (> 2 weeks after nerve ligation), the 0.5-mg/kg dose of MK-801 administered to anesthetized animals had no long-term effects, as indicated by paw pressure test performed after recovery from anesthesia 22 h after the administration of MK-801 (fig. 1D).

Discussion

In accordance with previous results obtained with other experimental models of pathophysiologic pain or other submodalities of painful test stimulation,³⁻⁸ the results of this study indicate that an NMDA receptor antagonist administered preoperatively significantly attenuated the development of mechanical allodynia and hyperalgesia induced by unilateral ligation of two spinal nerves. This antiallodynic and antihyperalgesic effect induced by a single dose of MK-801 was dose-related, long-lasting, and critically dependent on the timing of drug administration. The administration of a single dose of MK-801 15 min before nerve ligation produced a significant antiallodynic and antihyperalgesic effect for at least 2 weeks, whereas the administration of the same drug dose 10 min after the nerve ligation or after the development of neuropathic symptoms had no significant long-term effect on tactile allodynia or mechanical hyperalgesia. This finding indicates that the antiallodynic and antihyperalgesic effect of MK-801 was truly preemptive. This finding also indicates that the preemptive effect of MK-801 cannot be explained because of interaction with the compounds used for induction of anesthesia, because the level of anesthesia was the same during administrations of MK-801 in all experimental conditions.

A plausible explanation for the present findings is that the nerve ligation induced an afferent barrage that triggered mechanisms, possibly at the spinal cord level, that maintained the allodynia and hyperalgesia. According to the present results, NMDA receptors are critically involved in the triggering of mechanical allodynia and hyperalgesia. Previous studies indicate that among the potential trigger mechanisms that NMDA receptor antagonists suppress in the spinal cord are C-fiber-induced sensitization of central pain-relay neurons (e.g., "wind up" or long-term potentiation),^{9,10} activation of various intracellular second-messenger systems,¹ induction of immediate-early genes,¹¹ and transsynaptic apoptosis of the spinal dorsal horn neurons.¹²

Because MK-801 was always administered in anesthetized animals and testing could be performed adequately only after recovery from anesthesia, the short-lasting (up to 6 h) antinociceptive effects of MK-801 shown in previous investigations^{7,13-17} could not be studied in the present experiment. Moreover, the doses of MK-801 used (0.1–0.5 mg/kg) were high and likely to produce strong motor impairment when administered in awake animals.¹⁸ However, at time points used for testing (> 22 h from administration of MK-801), no motor impairment was observed. This is also shown by the finding that the withdrawal responses of the unoperated limb were not influenced by any of the experimental conditions.

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