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Gabapentin and pregabalin ameliorate mechanical hypersensitivity after spinal cord injury in mice

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ABSTRACT

The antiepileptic drugs gabapentin and pregabalin exhibit well-established analgesic effects in patients with several neuropathic conditions. In the present study, we examined their effects on mechanical hypersensitivity in mice subjected to weight-drop spinal cord injury. Hindlimb motor function and mechanical hypersensitivity were evaluated using the Basso–Beattie–Bresnahan (BBB) locomotor rating scale and the von Frey test, respectively, for 4 weeks after spinal cord injury. Despite gradual recovery of hindlimb motor function after spinal cord injury, mice exhibited continuous development of mechanical hypersensitivity. Gabapentin (30 and 100 mg/kg) and pregabalin (10 and 30 mg/kg), administered intraperitoneally on the 28th day after spinal cord injury, reduced mechanical hypersensitivity in a dose-dependent manner. These results suggest that gabapentin and pregabalin could be useful therapeutic tools for patients with neuropathic pain after spinal cord injury.

1. Introduction

The antiepileptic drugs gabapentin (Neurontin) and pregabalin (Lyrica) have been used successfully in the treatment of patients with several neuropathic pain conditions, including diabetic neuropathy and postherpetic neuralgia (Segal and Rordorf, 1996; Rosenstock et al., 2004).

Recent research using gene targeting technology has revealed that the binding of gabapentin and pregabalin to the $\alpha_2\delta$-1 subunit of voltage-dependent Ca^{2+} channels in the brain and spinal cord is essential for their analgesic efficacy (Field et al., 2006). Although additional unidentified mechanisms besides $\alpha_2\delta$ interaction must be taken into consideration (Urban et al., 2005; Lynch et al., 2006), it is most probable that the analgesic action of gabapentin and pregabalin requires plastic changes in the supraspinal structures (Tanabe et al., 2005, Takeuchi et al., 2007; Takasu et al., 2008) and spinal cord, including upregulation of the $\alpha_2\delta$ subunit (Li et al., 2004).

In addition to motor dysfunction, spinal cord injury frequently elicits sensory dysfunction including neuropathic pain such as tactile allodynia and thermal hyperalgesia (Siddall et al., 2003). However, the underlying mechanisms responsible for spinal cord injury-elicited neuropathic pain are poorly understood. Based on the established efficacy of gabapentin and pregabalin in relieving neuropathic pain after peripheral injury, clinical studies evaluating their analgesic efficacy in neuropathic pain developing after spinal cord injury have been increasing (Tzellos et al., 2008). By contrast, there have been only a few preclinical studies (Hao et al., 2000; Hulsebosch et al., 2000). Moreover, there has been a lack of studies assessing the effect of gabapentin and pregabalin on neuropathic pain in a single animal model of spinal cord injury. In the present study, we employed the spinal cord contusion model in mice, and evaluated the analgesic effect of gabapentin and pregabalin on mechanical hypersensitivity developing after spinal cord injury.

2. Materials and methods

All of the experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University, and were carried out according to the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

The surgical procedure was based on that described by Honda et al. (2006). In brief, 4-week-old, female ddY-strain mice were anesthetized by intraperitoneal (i.p.) administration of pentobarbital sodium (50 mg/kg) and given Baytril (10 mg/kg, i.p.) and atropine sulfate (0.01 mg/kg, i.p.). Following a dorsal laminectomy at the T8 vertebral level, a 2.5-g weight was dropped from a height of 5 cm onto the exposed dura, and the incision was then sutured. If necessary, mice received manual bladder expression, and Baytril was administered once a day.

During 4 weeks after injury, mechanical sensitivity was assessed daily by the von Frey test. Mice were placed in individual transparent Perspex cubicles with a wire mesh bottom, and a series of calibrated von Frey filaments (Semmes-Weinstein monofilaments; Stoelting, Wood Dale, IL) was used to determine the 50% likelihood of a paw withdrawal response (50% threshold) by the up-down method of Dixon (1980). Eight von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (von Frey number: 2.36, 2.44, 2.83, 3.22, 3.61, 3.84, 4.08, and 4.17; equivalent

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to 0.02, 0.03, 0.07, 0.16, 0.4, 0.6, 1.0, and 1.4 g force, respectively). Testing was initiated with the 0.16 g hair, and each hair was applied perpendicularly to the plantar surface of the hindpaw, with sufficient force to bend the filament, for 3–4 s. Lifting of the paw indicated a positive response and prompted the use of the next weaker (i.e. lighter) filament. Absence of a paw withdrawal response prompted the use of the next stronger (i.e. heavier) filament. This paradigm was continued until four measurements had been obtained after an initial change in behavior, or until four consecutive positive scores (score of 0.01 g) or five negative scores (score of 1.5 g) had been obtained. The resulting scores were used to calculate the 50% threshold (Chaplan et al., 1994). Testing was made on both the right and left hind paws of each animal, and the mean of the two 50% threshold values was used for analysis. In the study presented here, mice that exhibited a 50% threshold of 0.1 g in the von Frey test 4 weeks after injury were considered to be developing mechanical hypersensitivity, and used to evaluate the effects of gabapentin and pregabalin. We also evaluated hindlimb motor function daily during 4 weeks after injury using the Basso–Beattie–Bresnahan (BBB) locomotor rating scale (Basso et al., 1995). Mice were placed in an open field for 5 min and the observers scored their behavior on a 22-point scale where 0 is complete paralysis and 21 is normal movement. In this study, a total of 52 mice were subjected to spinal cord injury, and the BBI score and mechanical sensitivity were assessable in 46 mice up to 4 weeks after spinal cord injury, except for 6 mice that died during the postoperative period. On the 28th day after spinal cord injury, a total of 42 mice were used to assess the analgesic effect of gabapentin (30 and 100 mg/kg, i.p.) and pregabalin (10 and 30 mg/kg, i.p.) on mechanical hypersensitivity ($n = 7$ in each group). These doses of gabapentin and pregabalin were selected based on our previous studies assessing their analgesic effects on thermal and mechanical hypersensitivity developing after partial sciatic nerve ligation (Tanabe et al., 2005; Takeuchi et al., 2007).

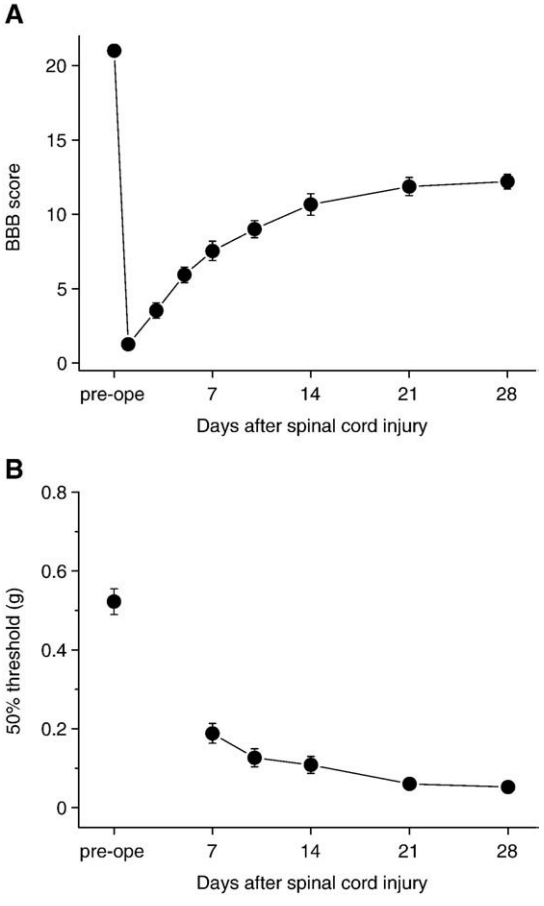

Fig. 1. Evaluation of hindlimb motor function and mechanical sensitivity using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale and the von Frey test, respectively, after spinal cord injury. Each point represents the mean ± S.E.M. of 46 mice. Ordinates: mean BBB scores (A) and 50% thresholds (B). In A, mice were placed in an open field for 5 min and the observers scored their behavior based on a 22-point scale, where 0 represents complete paralysis and 21 represents normal movement. Abscissae: days after spinal cord injury.

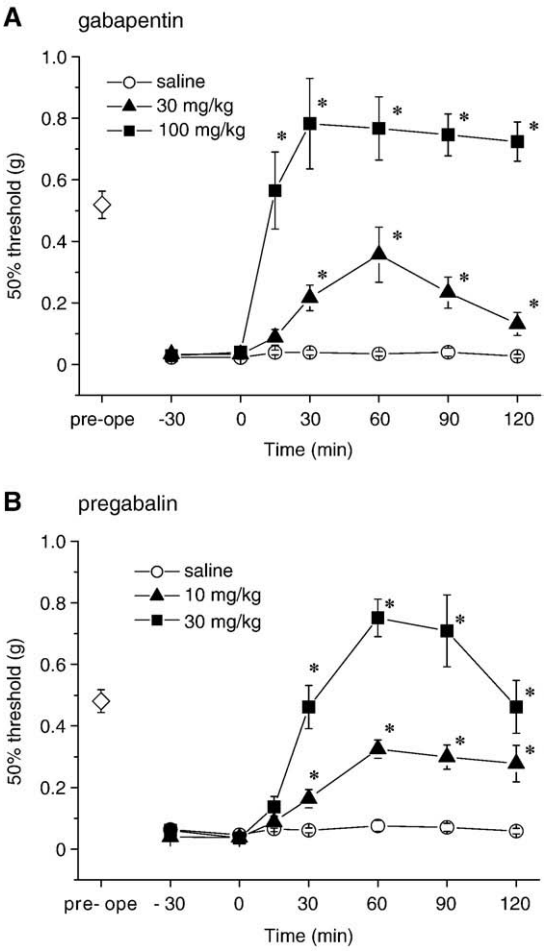

Fig. 2. Analgesic effects of gabapentin and pregabalin on mechanical hypersensitivity developing after spinal cord injury. Mechanical hypersensitivity was assessed using the von Frey test. Gabapentin (30 and 100 mg/kg, in A) and pregabalin (10 and 30 mg/kg, in B) were administered intraperitoneally at time zero. Each point represents the mean ± S.E.M. of 7 separate experiments. Ordinate: mean 50% withdrawal thresholds. Abscissae: 28 days before (pre-ope) and time in minutes after gabapentin (A) or pregabalin (B) application. Clear diamond indicates the mean of pooled 50% thresholds obtained before spinal cord injury in the three groups of mice. The asterisks indicate data points for which a significant difference between the control (clear circles) and drug-treated (solid triangles and squares) animals was observed, as determined by two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (2 comparisons in 3 groups, $^*P < 0.05$).

Gabapentin and pregabalin were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). They were dissolved in 0.9% saline and administered i.p. All data are expressed as means ± S.E.M. The effects of drugs on the nociceptive threshold in the von Frey test were evaluated with respect to time; the time of administration of drugs was defined as time zero. Two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal–Wallis test (Glantz, 1992) was employed. Differences at $P < 0.05$ were considered significant.

3. Results

Fig. 1A illustrates the time course of the BBB score after spinal cord injury. One day after spinal cord injury, the average BBB score was 1.3 ± 0.3, and therefore animals were able to move one or two of their

hindlimb joints slightly. Mice then exhibited gradual recovery of their hindlimb motor function with an average BBB score of 7.5 ± 0.7 and 12.2 ± 0.5 at 1 and 4 weeks after spinal cord injury, respectively ($n = 46$). Therefore, mice were able to move all three hindlimb joints at least 1 week after spinal cord injury, and exert consistent weight-supported steps and occasional coordinated forelimb–hindlimb movements at 4 weeks after spinal cord injury.

Because of severe lower-limb paralysis, particularly during the first week after spinal cord injury, we were unable to carry out proper assessment of mechanical sensitivity for almost 1 week after injury. Mice exhibited a reduction of the 50% threshold in the von Frey test, which was already obvious at 1 week after spinal cord injury (0.19 ± 0.03 g vs. 0.52 ± 0.03 before injury). They were considered to be developing mechanical hypersensitivity 3 weeks after spinal cord injury (0.06 ± 0.01 g), which further developed 4 weeks after spinal cord injury to 0.05 ± 0.01 g (Fig. 1B, $n = 46$). The analgesic effects of gabapentin and pregabalin were assessed in 42 mice exhibiting a 50% threshold of less than 0.1 g, (Fig. 2) and this mechanical hypersensitivity was significantly reversed by systemically administering gabapentin and pregabalin in a dose-dependent manner.

4. Discussion

Despite recent clinical studies suggesting the effectiveness of gabapentin (To et al., 2002; Levendoglu et al., 2004) and pregabalin (Siddall et al., 2006) in relieving neuropathic pain associated with spinal cord injury, only a few studies have evaluated gabapentin in animal models of spinal cord injury (Hao et al., 2000; Hulsebosch et al., 2000). The present study, employing the spinal cord contusion model, clearly demonstrates that gabapentin and pregabalin reverse the mechanical hypersensitivity that develops after spinal cord injury, and the results are considered to warrant further clinical research in this field.

Despite gradual recovery of hindlimb motor function, mice exhibited continuous development of mechanical hypersensitivity after spinal cord injury. In particular, loss of the descending serotonergic inputs caudal to the injury site contributes to motor dysfunction and mechanical hypersensitivity below the level of spinal cord injury (Saruhashi et al., 1996), and restoration of serotonergic fibers and terminals at the lumbosacral spinal cord leads to reduced sensory hypersensitivity and enhanced locomotor recovery (Saruhashi et al., 1996; Oatway et al., 2005; Erschbamer et al., 2007). We speculate that incomplete spinal cord injury induced by weight-drop results in sparing of serotonergic nerve fibers in the ventral spinal cord, which may partly explain the gradual recovery of locomotor function despite continuous development of mechanical hypersensitivity.

As we have recently demonstrated, both spinal and supraspinal actions contribute to the analgesic effects of systemically administered gabapentin and pregabalin (Tanabe et al., 2005; Takeuchi et al., 2007). These supraspinally mediated analgesic effects involve the descending noradrenergic pain inhibitory system presumably via disinhibition of the noradrenergic locus coeruleus neurons projecting to the spinal dorsal horn (Takasu et al., 2008). However, we consider that this supraspinal contribution to the analgesic effect of systemic administration of gabapentin and pregabalin is limited in the spinal cord contusion model used in the present study, since the noradrenaline content of the spinal cord was reduced to nearly half the level in the sham-treated group (Ono et al., 2008). Therefore, it is likely that the analgesic effects of gabapentin and pregabalin on mechanical hypersensitivity after spinal cord injury are largely mediated via their action on the spinal cord. Moreover, in neuropathic states developing after spinal cord injury, there must be some plastic changes including upregulation of the $\alpha_2\delta$-1 subunit of the voltage-sensitive Ca^{2+} channel, the only known specific binding site of gabapentin and pregabalin (Gee et al., 1996), which most likely mediates their analgesic actions (Field et al., 2006) in the spinal dorsal horn, as demonstrated after peripheral nerve injury (Li et al., 2004).

Both gabapentin and pregabalin at a higher dose (100 mg/kg and 30 mg/kg, respectively), generated an analgesic effect beyond nociceptive withdrawal levels obtained before spinal cord injury. Given that gabapentin and pregabalin produce injury-specific analgesic effects and do not exert any effects against acute nociception (Field et al., 1997; Tanabe et al., 2005; Takeuchi et al., 2007), the analgesic effects beyond nociceptive withdrawal levels obtained before spinal cord injury may reflect plastic changes in the spinal cord that have occurred after spinal cord injury.

In conclusion, the present study has demonstrated the effectiveness of gabapentin and pregabalin in reducing the mechanical hypersensitivity that develops after spinal cord injury, strongly supporting their clinical application for the treatment of patients with chronic pain after spinal cord injury. Although it seems most likely that the $\alpha_2\delta$-1 subunit at the spinal level is the primary target of gabapentin and pregabalin, their precise analgesic mechanisms in the neuropathic conditions after spinal cord injury remain to be clarified in further investigations.

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