Protein Kinase A–Dependence of the Supraspinally Mediated Analgesic Effects of Gabapentin on Thermal and Mechanical Hypersensitivity

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Abstract. We have recently shown that gabapentin generates protein kinase A (PKA)-dependent presynaptic inhibition of GABAergic synaptic transmission in locus coeruleus (LC) neurons only under neuropathic states. To verify behaviorally this in vitro electrophysiological finding, the PKA inhibitor H-89 was injected intracerebroventricularly (i.c.v.) before supraspinal application of gabapentin in mice developing thermal and mechanical hypersensitivity after peripheral nerve injury. H-89 dose-dependently attenuated the analgesic effects of i.c.v.-injected gabapentin, suggesting that PKA-dependent removal of GABAergic inhibition of LC neurons is the most plausible synaptic mechanism underlying the supraspinally mediated analgesic effects of gabapentin involving activation of the descending noradrenergic pain-inhibitory system.

Keywords: neuropathic pain, gabapentin, protein kinase A (PKA)

The anti-hypersensitivity actions of gabapentin have been well established, although we are still on the way to full understanding of its underlying mechanisms. We have been focusing on the supraspinal structure as a possible site for its action and have demonstrated that intracerebroventricular (i.c.v.) administration of gabapentin indeed decreases thermal and mechanical hypersensitivity by activating the descending noradrenergic system in a murine chronic pain model involving partial ligation of the sciatic nerve (1 – 3). Our conclusions are supported by Hayashida et al. (4) who have observed recruitment of the descending noradrenergic system by gabapentin in both animal and human postoperative pain conditions. Our recent electrophysiological experiments have shown that gabapentin presynaptically reduces GABAergic inhibitory postsynaptic currents (IPSCs) in locus coeruleus (LC) neurons in slices taken from mice after peripheral nerve injury (5), thereby removing inhibitory influences on LC neurons, leading to activation of the descending noradrenergic system to relieve neuropathic pain. Recent lines of evidence indicate that several protein kinases including PKC and PKA play crucial roles in mediating not only the pathophysiology of chronic pain after nerve injury or inflammation, but also the hyperalgesia-dependent pharmacological actions of gabapentin (6, 7). Interestingly, our in vitro electrophysiological study demonstrated that the PKA inhibitor H-89, but not the PKC inhibitor chelerythrine, abolished the inhibitory action of subsequently superfused gabapentin on IPSCs in LC neurons in slices taken from mice after peripheral nerve injury, suggesting that PKA activity plays an important role in supraspinal gabapentin responsiveness in neuropathic conditions.

The aim of the present study was to behaviorally verify this in vitro electrophysiological finding (5).

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.

We employed a murine neuropathic pain model prepared by partial ligation of the sciatic nerve (Seltzer model) (8). In brief, 4-week-old male ddY-strain mice were anesthetized by intraperitoneal administration of pentobarbital sodium (60 mg/kg). One-third to one-half of the dorsal aspect of the right sciatic nerve was ligated just distal to its branch to the posterior biceps and semitendinosus muscles. Thermal and mechanical hypersensitivity was assessed 7 days after ligation.
Thermal hypersensitivity was assessed using the plantar test (Ugo Basile, Comerio, Italy) following a modification of the method of Hargreaves et al. (9). A mobile radiant heat source, which was located under the glass floor, was focused onto the plantar surface of the right hindpaw, and paw withdrawal latencies (PWLs) were recorded. The intensity of radiant heat was adjusted to give a 7–8 s withdrawal latency in naive mice. A cut-off latency of 15 s was imposed to avoid tissue damage. PWLs were measured in duplicate for the right hindpaw of each animal, and the mean of the two values was used for analysis. Mechanical hypersensitivity was assessed using the von Frey test. A series of calibrated von Frey filaments (Semmes-Weinstein monofilaments; Stoelting, Wood Dale, IL, USA) was used to determine the 50% likelihood of a paw withdrawal response (50% threshold, Chaplan et al.) (10) by the up-down method of Dixon (11). Each hair was applied perpendicularly to the plantar surface of the right hindpaw, with sufficient force to bend the filament, for 3–4 s. In the study presented here, mice that exhibited a PWL of less than 5 s in the plantar test and a 50% threshold of 0.1 g in the von Frey test 7 days after partial ligation of the sciatic nerve were considered to be developing thermal and mechanical hypersensitivity.

Gabapentin (Wako, Osaka), H-89 dihydrochloride (referred to simply as H-89; Sigma, St Louis, MO, USA), chelerythrine chloride (referred to simply as chelerythrine, Sigma), and their vehicle were injected i.c.v. All drugs were dissolved in distilled water and administered in a volume of 5 μl via a disposable 27-gauge needle, which was inserted into the right lateral ventricle (12), with an interval of 15 min. H-89, chelerythrine, or their vehicle was administered 15 min before gabapentin injection. All data were expressed as means ± S.E.M. Two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (two comparisons for three groups on the left and three comparisons for four groups on the right, *P<0.05).

![Diagram](https://via.placeholder.com/150)

Fig. 1. Blockade of supraspinal PKA activity markedly reduces the analgesic effects of i.c.v.-injected gabapentin on thermal and mechanical hypersensitivity. Thermal and mechanical hypersensitivity was assessed by the plantar and von Frey tests, respectively. H-89 (1 and 3 μg in the plantar test and 0.3, 1, and 3 μg in the von Frey test) or vehicle was administered i.c.v. 15 min before the administration of gabapentin (100 μg, i.c.v., administered at time zero). Each point represents the mean ± S.E.M. of 5–7 separate mice. Ordinates: mean PWLs (plantar test: left) and 50% thresholds (von Frey test: right). Abscissae: 7 days before (pre-ope) and time in minutes after gabapentin administration. The clear diamond in each graph represents the mean of pooled PWLs (left) or 50% thresholds (right) obtained before ligation in the three (plantar test) or four (von Frey test) groups of mice. The asterisks indicate data points for which a significant difference between the gabapentin-only (clear circles) and H-89–treated (solid triangles and squares) groups was observed, as determined by two-tailed nonparametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (two comparisons for three groups on the left and three comparisons for four groups on the right, *P<0.05).
PKA-Dependent Analgesic Effects of Gabapentin

Chelerythrine were then examined. Chelerythrine at up to 3 μg exhibited almost negligible effects on the supraspinally mediated analgesic effects of gabapentin (100 μg, i.c.v.) on thermal and mechanical hypersensitivity (Fig. 2). When the chelerythrine dose was increased to 10 μg, it generated partial but significant reversal of the analgesic effects of gabapentin on mechanical, but not thermal, hypersensitivity. However, chelerythrine alone at 10 μg significantly elevated the paw withdrawal threshold in response to a thermal stimulus (plantar test), and therefore higher doses were not tested further.

The present behavioral findings clearly demonstrate that the supraspinally mediated analgesic effects of gabapentin on thermal and mechanical hypersensitivity, which are mediated by recruitment of the descending noradrenergic pain-inhibitory system (1–3), depend largely on supraspinal PKA activity. This finding is compatible with our recent in vitro electrophysiological study showing that gabapentin produced injury-specific inhibition of GABAergic IPSCs via presynaptic mechanisms in LC neurons in mouse brainstem slices (5). This injury-specific removal of GABAergic inhibition in LC neurons was PKA-dependent since gabapentin applied after blockade of PKA with H-89, but not PKC with chelerythrine, was unable to generate IPSC inhibition even in slices prepared from neuropathic mice (5).

Although an increase of PKC and/or PKA activity has been shown to be essential for some of the pharmacological properties of gabapentin (6, 7), we observed no reduction of IPSCs in response to gabapentin applied after PKC or PKA activation in slices prepared from mice given a sham operation, suggesting that the IPSC-inhibitory action of gabapentin in LC neurons requires basal PKA activity under neuropathic conditions that may be crucial to maintain translocation of gabapentin-binding sites on GABAAergic terminals in the LC (5). Thus, under blockade of supraspinal basal PKA activity after i.c.v. injection of H-89 in mice developing neuropathic pain, i.c.v.-applied gabapentin hardly removes the GABAergic inhibition of LC neurons to drive the descending noradrenergic pain-inhibitory system. Since H-89 potently attenuated mechanical hypersensitivity more than thermal hypersensitivity, we do not rule out another PKA-independent contribution to the supraspinal analgesic action of gabapentin on thermal hypersensitivity.

Chelerythrine at the highest dose used here (10 μg, i.c.v.) reduced the analgesic effect of gabapentin on mechanical hypersensitivity, suggesting that mechanisms other than presynaptic removal of GABAergic inhibition of LC neurons may partly contribute to the supraspinally mediated analgesic effects of gabapentin. However, we consider that this dose of chelerythrine

Fig. 2. Little contribution of supraspinal PKC activity to the analgesic effects of i.c.v.-injected gabapentin on thermal and mechanical hypersensitivity. Thermal and mechanical hypersensitivity was assessed by the plantar and von Frey tests, respectively. Chelerythrine (che: 1, 3, and 10 μg) or vehicle was administered i.c.v. 15 min before the administration of gabapentin (100 μg, i.c.v., administered at time zero). Each point represents the mean ± S.E.M. of 5–6 separate mice. Ordinates: mean PWLs (plantar test: left) and 50% thresholds (von Frey test: right). Abscissae: 7 days before (pre-ope) and time in minutes after gabapentin administration. The clear diamond in each graph represents the mean of pooled PWLs (left) or 50% thresholds (right) obtained before ligation in the four groups of mice. The asterisks indicate data points for which a significant difference between the gabapentin-only (clear circles) and chelerythrine-treated (solid triangles and squares) groups was observed, as determined by two-tailed nonparametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (three comparisons for four groups on the right, *P<0.05).
(10 μg) should be enough to block PKC when applied locally in rodents (14, 15). Further studies will be needed to clarify the supraspinal PKC-dependent cellular mechanisms employed by gabapentin.

In conclusion, the present study has provided behavioral evidence that gabapentin produces PKA-dependent supraspinally mediated analgesic effects on thermal and mechanical hypersensitivity. This finding strongly supports the notion that PKA-dependent removal of GABAergic inhibition of LC neurons is the most plausible synaptic mechanism underlying the supraspinally mediated analgesic effects of gabapentin involving activation of the descending noradrenergic pain-inhibitory system.

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