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Gabapentin increases extracellular glutamatergic level in the locus coeruleus via astroglial glutamate transporter-dependent mechanisms

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Abstract

Gabapentin has shown to be effective in animals and humans with acute postoperative and chronic pain. Yet the mechanisms by which gabapentin reduces pain have not been fully addressed. The current study performed *in vivo* microdialysis in the locus coeruleus (LC) in normal and spinal nerve ligated (SNL) rats to examine the effect of gabapentin on extracellular glutamate concentration and its mechanisms of action with focus on presynaptic GABA-B receptors, astroglial glutamate transporter-1 (GLT-1), and interactions with α2δ subunits of voltage-gated Ca^{2+} channels and endogenous noradrenaline. Basal extracellular concentration and tissue content of glutamate in the LC were greater in SNL rats than normal ones. Intravenously administered and LC-perfused gabapentin increased extracellular glutamate concentration in the LC. The net amount of glutamate increased by gabapentin is larger in SNL rats compared with normal ones, although the percentage increases from the baseline did not differ. The gabapentin-related α2δ ligand pregabalin increased extracellular glutamate concentration in the LC, whereas another α2δ ligand, 3-exo-aminobicyclo [2.2.1] heptane-2-exo-carboxylic acid (ABHCA), did not. Selective blockade by the dihydrokainic acid or knock-down of GLT-1 by the small interfering RNA abolished the gabapentin-induced glutamate increase in the LC, whereas blockade of GABA-B receptors by the CGP-35348 and depletion of noradrenalin by the dopamine-β-hydroxylase antibody conjugated to saporin did not. These results suggest that gabapentin induces glutamate release from astrocytes in the LC via GLT-1-dependent mechanisms to stimulate descending inhibition. The present study also demonstrates that this target of gabapentin in astrocytes does not require interaction with α2δ subunits in neurons.

Keywords

Locus coeruleus; glutamate; astrocyte; gabapentin; glutamate transporters; peripheral nerve injury

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1. Introduction

Gabapentin is effective in a wide range of animal pain models and in patients with acute postoperative and chronic pain, and became one of 1st choice treatments for chronic pain. Yet the mechanisms by which gabapentin reduces pain have not been fully addressed. Because spinal plasticity and sensitization play important roles in chronic pain after peripheral nerve injury, most laboratory studies have focused on actions of gabapentin in the spinal cord, where it reduces primary afferent traffic and excitation of spinal nociceptive neurons, via interaction with $\alpha 2\delta$ subunits of voltage-gated Ca^{2+} channels (Hendrich et al., 2008; Li et al., 2006; Luo et al., 2002).

However, it is unlikely that gabapentin relies exclusively on spinal actions for its analgesic efficacy. A recent clinical trial indicates no reliance on the spinal mechanisms for the clinical efficacy of gabapentin by demonstrating that intrathecal gabapentin, in doses from 1 mg/day to 30 mg/day for 3 weeks, failed to show efficacy in patients with chronic pain (Rauck et al., 2013), despite the known efficacy of oral gabapentin in this patient population. We and others have proposed supraspinal actions of gabapentin by demonstrating in rodents after peripheral nerve injury and in patients with chronic pain that systemically administered gabapentin activates descending noradrenergic inhibition to produce analgesia (Hayashida et al., 2007; Hayashida et al., 2008; Tanabe et al., 2005). Gabapentin, systemically administered *in vivo* and locally applied to isolated brainstem slices *in vitro*, activates noradrenergic neurons in the locus coeruleus (LC) (Hayashida et al., 2008). These results suggest that gabapentin acts on the local circuits within the LC to activate descending inhibition.

In rats after peripheral nerve injury, gabapentin-induced analgesia and activation of LC neurons were blocked by the locally applied AMPA glutamate receptor antagonist (Hayashida et al., 2008), suggesting that gabapentin relies on the glutamate-dependent mechanisms in the LC to activate descending inhibition. We and others previously demonstrated that gabapentin reduces presynaptic release of the inhibitory neurotransmitter GABA in the LC via an interaction with $\alpha 2\delta$ subunits (Takasu et al., 2008; Yoshizumi et al., 2012b). GABA is classically known to inhibit glutamate release via presynaptic GABA-B receptor-mediated mechanisms (Harte and O'Connor, 2005; Tanaka et al., 2003). We therefore hypothesized that gabapentin increases extracellular glutamate concentration in the LC by reducing the influence of GABA on glutamatergic terminals.

In the central nervous system, extracellular glutamate concentrations are regulated by two types of astroglial glutamate transporters, predominantly by glutamate transporter-1 (GLT-1) and, to a lesser extent, by glutamate-aspartate transporter (GLAST), and synaptic glutamate concentrations are predominantly regulated by neuronal glutamate transporters (EAAT3) (Medrano et al., 2013; Robinson, 1998; Rothstein et al., 1996). We previously demonstrated in cultured astrocytes that gabapentin enhances Na^{+}-glutamate co-transport through glutamate transporters, induces subsequent Ca^{2+} influx via the reverse mode of Na^{+}/Ca^{2+} exchange, and thereby facilitates Ca^{2+}-dependent glutamate release by glutamate (Yoshizumi et al., 2012a). However, the *in vivo* relevance of this action of gabapentin on astroglial glutamate regulation has not yet been tested in the LC. In addition, noradrenaline

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in the LC may have some roles on gabapentin's action, since it affects glutamate uptake and glutamine production in astrocytes (O'Donnell et al., 2012).

The current study performed *in vivo* microdialysis in the LC in normal and spinal nerve ligated (SNL) rats to examine the effect of gabapentin on extracellular glutamate concentration in the LC and the mechanisms of gabapentin's action with focus on presynaptic GABA-B receptors, GLT-1, and interactions with α2δ subunits and endogenous noradrenaline in the LC. The current study also examined the effect of gabapentin on extracellular glutamate levels in the spinal cord.

2. Materials and Methods

2.1 Animals and surgeries

Male Sprague-Dawley rats (6 week old at arrival) from Harlan Industries (Indianapolis, IN), housed under a 12-h light-dark cycle with free access to food and water, were used. All experiments were approved by Animal Care and Use Committee at Wake Forest University School of Medicine (Winston Salem, NC). L5-L6 spinal nerve ligation (SNL) was performed in 7 week old rats as previously described (Kim and Chung, 1992). Briefly, under anesthesia with 2% isoflurane in oxygen, the right L6 transverse process was removed and the right L5 and L6 spinal nerves were tightly ligated using 5–0 silk suture. Two weeks after SNL, some animals were anesthetized with 2% isoflurane and placed securely in a stereotaxic frame. A sterile steel guide cannula (CXG-8, EICOM CO., Kyoto, Japan) was implanted into the right LC as previously described (Hayashida et al., 2008). The coordinates for placement of the tip of the guide cannula were 9.8 mm posterior and 1.4 mm lateral to the bregma, and 6.5 mm ventral from the surface of the dura mater, according to the rat brain atlas (Paxinos and Watson, 2005). Animals were allowed to recover for at least one week prior to the microdialysis experiments or treatments.

2.2 Depletion of noradrenaline and knock-down of GLT-1 in the LC

For depletion of noradrenaline in the LC, dopamine-β-hydroxylase antibody conjugated to saporin (DβH-saporin, Sigma-Aldrich Co., St. Louis, MO) or its negative control IgG-saporin (Sigma-Aldrich Co.) was dissolved with saline to achieve a final concentration of 0.25 μg/0.5 μl and injected into the LC through the guide cannula at 2 weeks prior to the microdialysis experiment. For knock-down of GLT-1 in the LC, a small interfering RNA (siRNA) mixture for rat GLT-1 (SMARTpool #M-091209-02, Thermo Fisher Scientific Inc., Pittsburgh, PA) or a non-targeting siRNA pool (#D-001206-14, Thermo Fisher Scientific Inc.) was dissolved in double distilled water, diluted with the transfection reagent (i-Fect; Neuromics, Edina, MN) to achieve a final concentration of 8.3 pmol/0.5 μl, and injected through the guide cannula for 5 consecutive days.

2.3 Microdialysis for glutamate

Microdialysis in the spinal cord and LC was performed as previously described (Yoshizumi et al., 2013; Yoshizumi et al., 2012b). On the day of experiment, anesthesia was induced with 2% isoflurane and then maintained with 1.25–1.5% isoflurane during the study. A heating blanket was used to maintain rectal temperature 36.5 ± 0.5°C and the right jugular

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vein was cannulated for saline infusion (2.4 ml/kg/hr) and gabapentin (50 mg/ml in saline, Toronto Research Chemicals Inc., Toronto, Canada) injection. For microdialysis in the spinal dorsal horn, the L3-L6 level of spinal cord was exposed by the T13-L1 laminectomy. A microdialysis probe (OD = 0.22 mm, ID = 0.20 mm, length = 1 mm, CX-I-8-01, EICOM CO.) was inserted into the spinal dorsal horn 1 hr prior to the study and perfused with Ringer's solution (1.0 μL/min). Fractions were collected every 30 min for 2.5 hr starting 1 hr prior to gabapentin perfusion. For microdialysis in the LC, a probe was inserted through the guide cannula 1 hr prior to the experiment and perfused with Ringer's solution with or without the GLT-1 blocker dihydrokainic acid (DHK, Tocris bioscience, Ellisville, MO) or GABA-B receptor blocker CGP-35348 (Sigma-Aldrich Co.) throughout the experiment. After baseline collection for 1 hr, gabapentin, pregabalin (Tocris Bioscience), 3-exo-aminobicyclo[2.2.1] heptane-2-exo-carboxylic acid (ABHCA, Thermo Fisher Scientific INC.), or KCl was perfused for 90 min. After the experiment, the probes, except those for the DBH-saporin and siRNA experiments, were perfused for 10 min with methylene blue to stain the areas surrounding the active dialysate window in the brainstem and then rats were killed by decapitation. The brainstem was removed and post-fixed with 4% buffered formaldehyde overnight. After sectioning, the placement of the probe was verified microscopically. Data were used only from animals with staining in the LC and two animals were excluded from the current study due to the cannula misplacement. Glutamate content in the microdialysates was measured by a high pressure liquid chromatography (HPLC) system with electrochemical detection (HTEC-500, EICOM CO.). The in vitro recovery rate for glutamate with the probe, perfusate, and flow rate in the current condition is 12.0 % according to the manufacture's information.

2.4 Western Blotting for GLT-1

After the microdialysis experiment, siRNA treated animals were killed by decapitation and the brainstem was quickly removed and placed in ice-cold sucrose (0.32 M)-HEPES (10 mM) buffer, pH = 7.4. Brainstem slices (2 mm thickness) containing the LC ipsilateral to the siRNA treatment were obtained using a precision brain slicer (RBM-4000C, ASI Instruments, Inc., Warren, MI), the region of the LC was carefully dissected under the surgical microscope, and homogenized in ice-cold sucrose-HEPES buffer. Western blotting for GLT-1 was performed as we previously reported (Hobo et al., 2011). The LC and adjacent tissue were homogenized, lysed, and centrifuged for 10 min at 4°C at 1000G. Protein content in each supernatant w as measured using a standard Bradford method. Samples (25 μg protein) were placed on the 10–20% gradient gels (Criterion Tris-HCl Gel; Bio-Rad, Hercules, CA), run at 100V for 1 hr, and transferred to polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked with 1% bovine albumin serum in Tris-buffer saline containing 0.1% Tween 20 (TBST), and incubated overnight at 4°C with a guinea pig anti-G LT-1 (1:1000; Millipore, Temecula, CA) or a rabbit α-tubulin (1:1000; Cell Signaling, Danvers, MA). After washing with TBST, the membrane was incubated for 1 hr at room temperature with a corresponding horseradish peroxidase-conjugated secondary antibody (1:5000; anti-guinea pig or 1:1000; anti-rabbit, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), treated for 1 min with the West Pico chemiluminescence (Thermo Fisher Scientific INC.), and exposed to X-ray film (Kodak BioMax film, Sigma-Aldrich Co.). The

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density of each specific band was measured using a computer-assisted imaging analysis system (Sigma Scan Pro 5 software, Systat Software Inc., Chicago, IL).

2.5 Measurements for glutamate and noradrenaline contents

The brainstem tissues containing LC were obtained from normal or SNL rats at 2 weeks after surgery for glutamate assay and from DBH-saporin or IgG-saporin treated rats for noradrenaline assay. After treatment with 0.1 M perchloric acid, the tissues were homogenized on ice and centrifuged, and supernatants were collected. Noradrenaline and glutamate contents in the supernatants were individually measured by HPLC systems as we previously described (Hayashida et al., 2008; Yoshizumi et al., 2013).

2.6 Statistical Analyses

Data are presented as mean ± S.E.M. No data were excluded from analysis. Microdialysis data were analyzed by one-way or two-way repeated-measures analysis of variance (ANOVA) followed by Tukey *post hoc* test. Other data were analyzed by one-way ANOVA followed by Dunn's *post hoc* test. $P<0.05$ was considered significant.

3 Results

3.1 Gabapentin increases extracellular glutamate in the LC

Basal glutamate concentrations in microdialysates were significantly greater from the LC ipsilateral to SNL than from the LC in animals without surgery (Table 1). Glutamate content in the brainstem tissue containing LC ipsilateral to SNL (9.8 ± 0.6 nmol/mg tissue weight, n=14) was also significantly greater than from LC in normal animals (7.3 ± 0.8 nmol/mg tissue weight, n=13, $p<0.05$).

In normal and SNL rats, intravenous injection of gabapentin (50 mg/kg), a previously shown effective dose to activate LC neurons(Hayashida et al., 2008), significantly increased glutamate concentrations in microdialysates from the LC compared with saline, with a peak effect of gabapentin 60 min after injection (Fig. 1A). Although the percentage increase from the baseline after gabapentin did not differ in normal and SNL rats (P=0.36), the quantitative glutamate concentrations in the dialysates collected from 0 to 60 min after gabapentin injection were significantly greater in SNL rats (42 ± 9.1 pmol/60 μl, n=10) than in those without surgery (17 ± 3.8 pmol/60 μl, n=11, $P<0.05$). Similar to its systemic effect, local perfusion of gabapentin into the LC concentration-dependently increased glutamate concentrations in microdialysates compared with vehicle in normal and SNL rats (Fig. 1B). Based on this result, we selected 10 mM gabapentin for the following experiments in normal rats.

3.2 Roles of α2δ subunits, GABA-B receptors, glial glutamate transporters, and noradrenaline on gabapentin-induced glutamate increase in the LC

A local perfusion of the gabapentin-related α2δ ligand pregabalin (10 mM) increased glutamate concentrations in microdialysate from the LC compared with vehicle and this effect did not differ from gabapentin's effect (Fig. 2, P=0.18). On the other hand, ABHCA

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(10 mM), which has a similar α2δ binding affinity to gabapentin and pregabalin (Lynch et al., 2006), showed no effect.

A local perfusion of the GLT-1 selective blocker DHK (1 mM) significantly increased basal glutamate concentrations in microdialysates from the LC(Table 1), consistent with the previous study that DHK increased the spontaneous firing rate of LC neurons via an AMPA receptor-mediated mechanism (Medrano et al., 2013). DHK abolished the gabapentin-induced glutamate increase (Fig. 3A). The GABA-B receptor antagonist CGP-35348 (1 mM) affected neither basal glutamate level nor gabapentin's effect, but augmented KCl-induced glutamate increase in the LC (Fig. 3A and 3B).

Fig. 4A depicts representative immunoblotting images of GLT-1 in the LC treated with the GLT-1 selective siRNA (8.3 pmol/rat) or non-targeting siRNA (8.3 pmol/rat) for 5 consecutive days. Quantitatively, GLT-1 selective siRNA treatment significantly reduced expression of GLT-1 in the LC (ratio to α-tubulin: 0.27 ± 0.05) compared to the non-targeting siRNA treatment (ratio to α-tubulin: 0.56 ± 0.03. $p<0.01$). Similar to the effect of DHK, GLT-1 selective siRNA treatment significantly increased basal glutamate concentrations in microdialysates and abolished the gabapentin-induced glutamate increase in the LC, whereas non-targeting siRNA treatment affected neither basal glutamate nor gabapentin's effect (Table 1 and Fig. 4B).

A local injection of DβH-saporin (0.25 ug/rat) significantly reduced noradrenaline content in the brainstem tissue containing the LC (0.40 ± 0.04 ng/mg tissue weight, n=8) compared to the same dose (0.25 ug/rat) of IgG-saporin (5.46 ± 0.44 ng/mg tissue weight, n=9, $P<0.05$). DβH-saporin treatment significantly increased basal glutamate concentrations in microdialysate from the LC compared to IgG-saporin and buffer treatments (Table 1). Although the percentage increases from the baseline by gabapentin did not differ in DβH-saporin and IgG-saporin treated rats (Fig. 5., P=0.49), the quantitative glutamate concentrations in the microdialysates collected from 0 to 60 min during gabapentin perfusion were significantly greater in DβH-saporin treated rats (70 ± 9.9 pmol/60 μl) than IgG-saporin treated ones (22 ± 3.1 pmol/60 μl, $P<0.05$).

3.3 Gabapentin reduced extracellular glutamate in the spinal cord

In contrast to its effect in the LC, gabapentin (10 mM), at the same concentration used in the LC, significantly reduced glutamate concentrations in microdialysates from the spinal cord within 30 min compared to vehicle and this degree of inhibition was maintained for at least 90 min (Fig 6).

4 Discussion

Despite its name and structural similarity to GABA, gabapentin does not bind to GABA receptors but acts on α2δ subunits of Ca^{2+} channels to reduce neuronal excitation, directly via reduction of Ca^{2+} influx or indirectly via inhibition of trafficking of those channels to the cell membrane (Hendrich et al., 2008; Li et al., 2006; Luo et al., 2002). A focus on the spinal cord as a key site of pain processing and plasticity after nerve injury has led to a theory that gabapentin has a primary site of action in the spinal cord. However, a clinical

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trial, originally designed to test this theory, shows no efficacy of intrathecal gabapentin in patients with chronic pain (Rauck et al., 2013). We and others proposed the supra-spinal actions of gabapentin are important to its analgesic mechanisms, by demonstrating that noradrenergic neurons in the LC are excited by gabapentin, coincident with noradrenaline release in the spinal cord and behavioral antihypersensitivity in rodents after peripheral nerve injury (Hayashida et al., 2008; Tanabe et al., 2005), and that oral gabapentin, in a dose that produces postoperative analgesia, increases noradrenaline concentration in cerebrospinal fluid in patients with chronic pain (Hayashida et al., 2007). The current study extends these observations and demonstrates the novel *in vivo* mechanism of gabapentin's action on astrocytes to increase glutamate tone in the LC but not in the spinal cord, and that this effect of gabapentin is augmented by peripheral nerve injury.

Peripheral nerve injury has been shown to induce functional and histological changes in the LC. Sciatic nerve injury time-dependently increases bursting activity of LC neurons in rats (Alba-Delgado et al., 2013), consistent with the current observation that SNL increased contents and basal extracellular concentrations of glutamate in the LC. Although another study showed that SNL did not alter spontaneous discharge rate of LC neurons (Viisanen and Pertovaara, 2007), the current study demonstrates that SNL increases basal glutamate tone in the LC, consistent with previous observations that SNL increases neuronal activity in the LC, measured by the expression of phosphorylated cyclic adenosine monophosphate response element binding protein (pCREB), and also increases basal noradrenaline release in the spinal dorsal horn (Hayashida et al., 2008). This difference may be due to the differing measures of neuronal activity (mean firing rate *vs.* pCREB expression and noradrenaline release). Although the current study did not examine the mechanisms by which SNL alters basal glutamate level in the LC, the effect of gabapentin to increase glutamate is preserved after SNL, consistent with the previous result that gabapentin induces a similar degree of pCREB up-regulation in LC neurons and spinal noradrenaline release in normal and SNL rats (Hayashida et al., 2008). Since SNL reduces expression of astroglial glutamate transporters in the spinal cord (Hobo et al., 2011) and since blockade or knock-down of GLT-1 increases extracellular glutamate concentrations in the LC, one could argue that SNL might reduce the expression of astroglial glutamate transporters to increase extracellular glutamate concentration in the LC. Against this argument is the current observation that gabapentin lost its action on glutamate regulation in GLT-1 siRNA treated rats but not in SNL rats. This result suggests that the knock-down of GLT-1 does not mimic the effect of SNL on glutamate regulation in the LC.

In addition to its direct inhibition on LC neurons via postsynaptic GABA-A receptors, GABA is known to inhibit glutamate release via presynaptic GABA-B receptors (Harte and O'Connor, 2005; Tanaka et al., 2003), while. Since gabapentin reduces presynaptic release of GABA in the LC via interactions with α2δ subunits (Takasu et al., 2008; Yoshizumi et al., 2012b), we hypothesized that gabapentin increases glutamate release from glutamatergic terminals in the LC by reducing the influence of GABA. However, in contract to this hypothesis, blockade of GABA-B receptors affected neither basal glutamate level nor gabapentin-induced glutamate increase in the LC. This result suggests that tonic influence of GABA on glutamatergic terminals via presynaptic GABA-B receptors is either minor or

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absent in the LC and that gabapentin's effect on the glutamate level is independent from GABA-mediated mechanisms.

We previously demonstrated that gabapentin increases co-transport of Na^+ ions and glutamate via glutamate transporters, enhances the glutamate-induced intracellular Ca^{2+} response via the reverse mode of Na^+-Ca^{2+} exchange, and by this mechanism facilitates glutamate release in cultured astrocytes (Yoshizumi et al., 2012a). Although GLT-1 may express in glutamatergic terminals in the LC (Medrano et al., 2013), the current study shows the *in vivo* relevance of this gabapentin's action in astrocytes by demonstrating the role of GLT-1 in the gabapentin-induced glutamate increase in the LC. Since there is no evidence for the presence of α2δ subunits in astrocytes, α2δ interactions may not contribute to the effect of gabapentin on astrocytes. This is further supported by the current observation that the gabapentin-related α2δ ligand pregabalin increased extracellular glutamate concentration in the LC, while ABHCA did not, despite its high binding affinity to α2δ subunits (Lynch et al., 2006). However, the current study tested only a single concentration of ABHCA and further studies are required to examine the effect of α2δ ligands in astrocytes.

The LC is the principal site for noradrenaline production in the brain. Noradrenaline has been shown to reduce presynaptic glutamate release via α-2 adrenoceptors and increase glutamate uptake in astrocytes via α-1 adrenoceptors as important mechanisms for regulation of extracellular glutamate level in the central nervous system (O'Donnell et al., 2012), consistent with the current observation that depletion of noradrenaline increased extracellular glutamate level in the LC. However, depletion of noradrenaline did not affect the gabapentin-induced glutamate increase in the LC, suggesting that noradrenaline is not essential for gabapentin's action. In contrast to its effect in the LC, a local perfusion of gabapentin reduces, rather than increases, extracellular glutamate level in the spinal cord, consistent with its inhibitory action on the primary sensory terminals at the spinal level via α2δ interactions (Hendrich et al., 2008; Li et al., 2006; Luo et al., 2002). This result suggests that the gabapentin-induced glutamate increase is not a global phenomenon in the central nervous system. We are currently studying which factors or structures are required for this unique gabapentin's action in the LC.

In summary, the present study demonstrates a novel site of gabapentin's action in astrocytes aside from α2δ subunits in neurons by showing that gabapentin increases extracellular glutamate by astroglial glutamate transporter-mediated mechanisms but not by α2δ interactions. The present study also demonstrates that peripheral nerve injury increases tonic glutamatergic tone in the LC to augment this gabapentin's effect. These results suggest that gabapentin increases glutamatergic activity in the LC as one mechanism by which it activates noradrenergic descending inhibition to reduce pain.

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Highlights

- Spinal nerve injury increases tonic glutamatergic tone in the locus coeruleus.
- Gabapentin increases extracellular glutamate level in the locus coeruleus.
- This effect of gabapentin is mediated by astroglial glutamate transporters.

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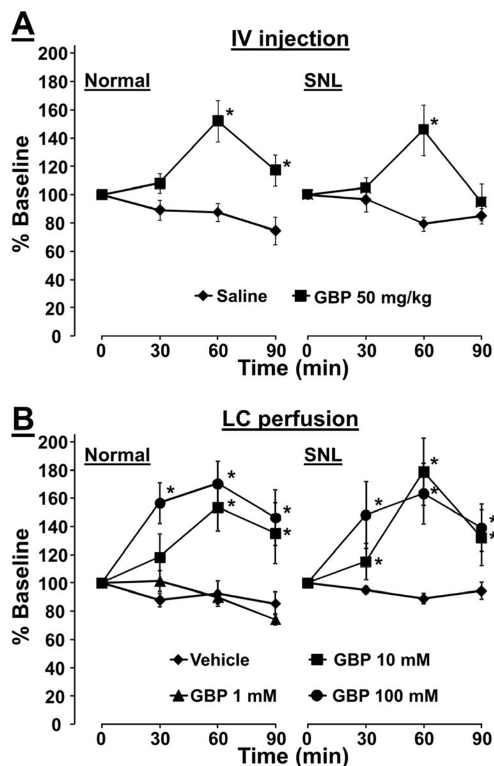

Fig. 1.

Effects of systemically and locally administered gabapentin (GBP) on extracellular glutamate concentrations in the LC in normal and SNL rats. (A) Saline (Normal: n=10, SNL: n=8) or GBP (50 mg/kg, Normal: n=11, SNL: n=12) was intravenously injected. (B) Vehicle (Normal: n=11, SNL: n=7), GBP 1 mM (Normal: n=11), GBP 10 mM (Normal: n=8, SNL: n=12), or GBP 100 mM (Normal: n=12, SNL: n=8) was perfused into the LC for 90 min through the microdialysis probe. Changes in glutamate concentrations in microdialysates from the LC are presented over time as percentage of baseline. *P<0.05 vs. saline or vehicle.

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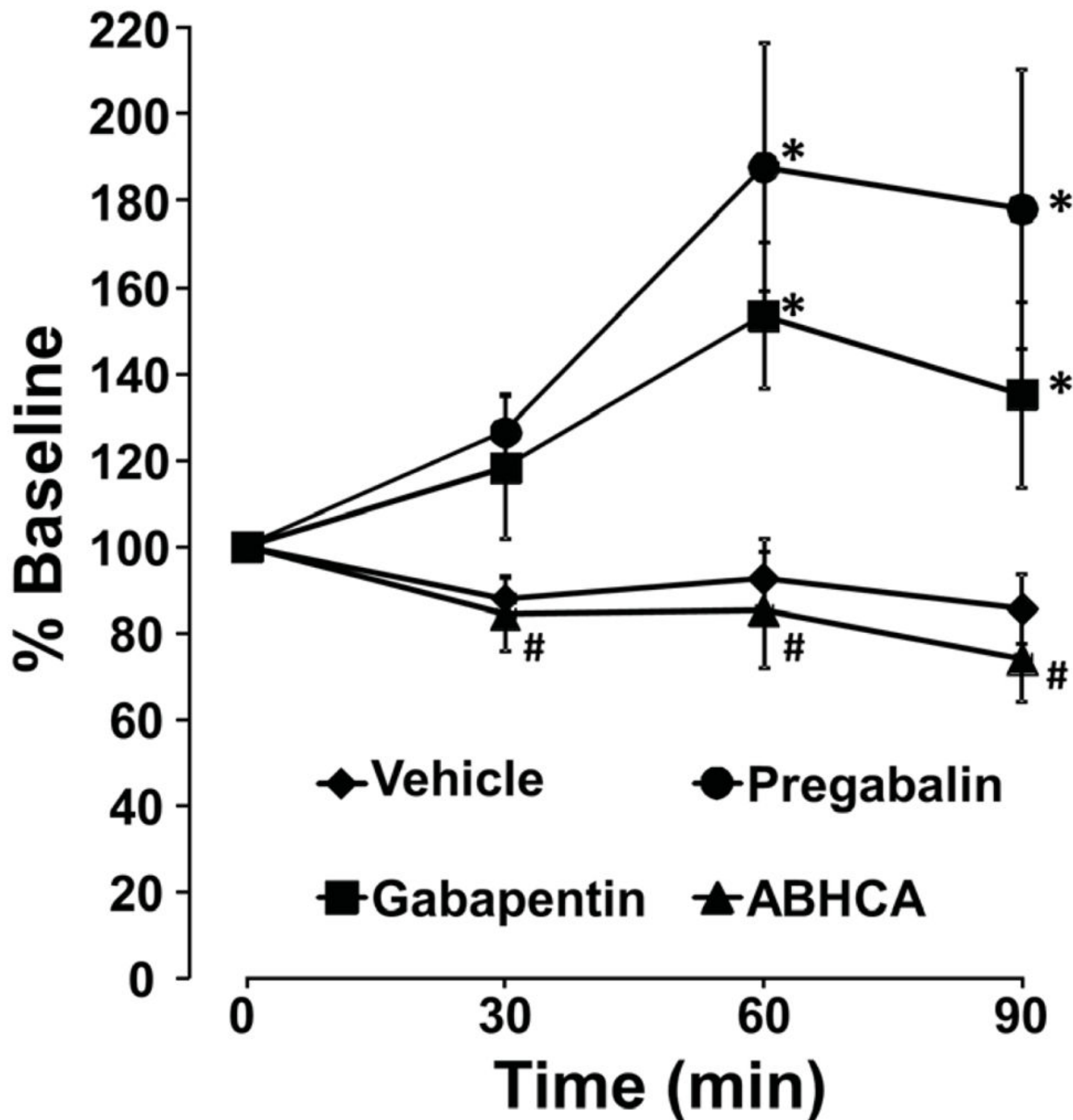

Fig. 2.

Effects of $\alpha_2\delta$ subunit ligands on extracellular glutamate concentrations in the LC. Vehicle (n=11), gabapentin (10 mM, n=8), pregabalin (10 mM, n=11), or ABHCA (10 mM, n=8) was perfused into the LC for 90 min through the microdialysis probe in normal rats. Changes in glutamate concentrations in microdialysates from the LC are presented over time as percentage of baseline. *P<0.05 vs. vehicle. #P<0.05 vs. gabapentin.

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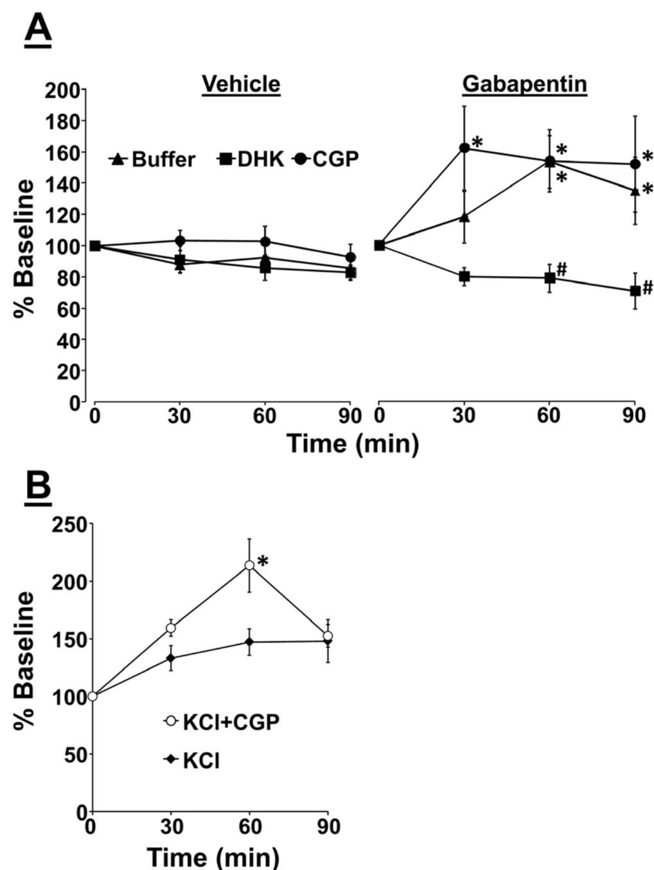

Fig. 3.

Effects of GLT-1 and GABA-B receptor blockers on gabapentin-induced increase in extracellular glutamate in the LC. (A) Vehicle or gabapentin (10 mM) was perfused into the LC for 90 min through the microdialysis probe in normal rats in the presence of buffer (vehicle: n=11, gabapentin: n=11), dihydrokainic acid (DHK, 1 mM, vehicle: n=7, gabapentin: n=7), or CGP 35345 (CGP, 1 mM, vehicle: n=9, gabapentin: n=9). Changes in glutamate concentrations in microdialysates from the LC are presented over time as percentage of baseline. *P<0.05 vs. vehicle. #P<0.05 vs. gabapentin.. (B) KCl (100 mM) was perfused into the LC for 90 min through the microdialysis probe in normal rats in the absence (n=6) or presence of CGP (n=6). *P<0.05 vs. KCl alone.

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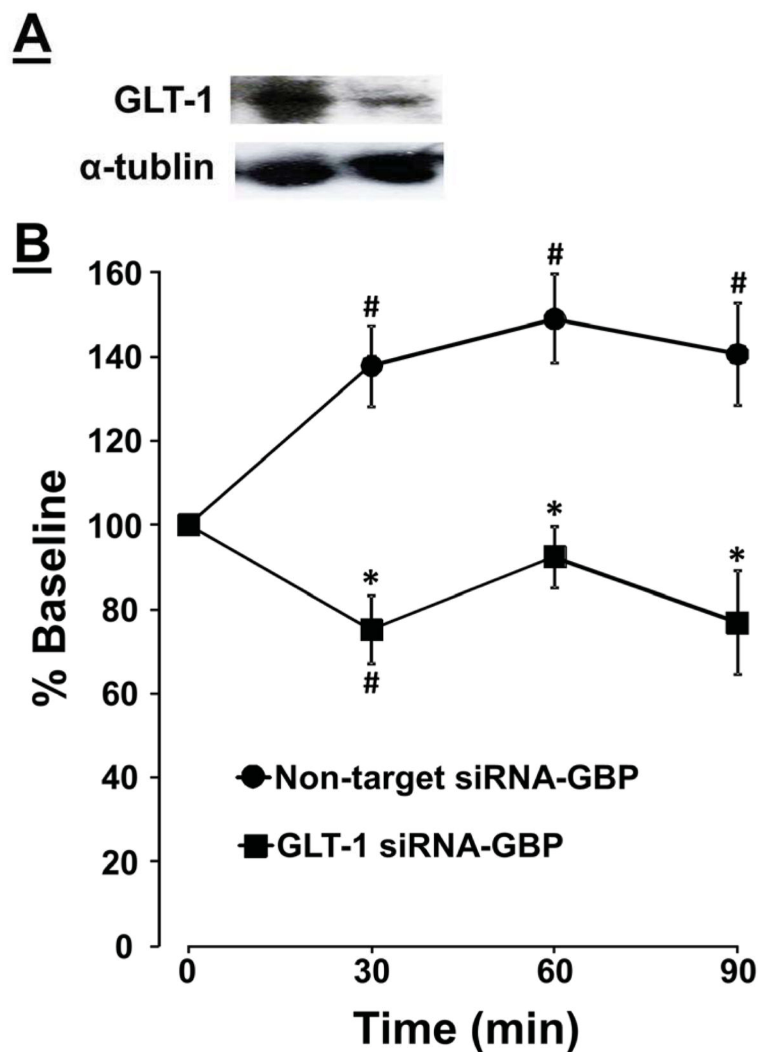

Fig. 4.

Knockdown of GLT-1 abolished gabapentin-induced increase in extracellular glutamate in the LC. Normal rats were treated with intra-LC injections of non-targeting (8.3 pmol/rat, n=11) or GLT-1 selective siRNA (8.3 pmol/rat, n=11) for 5 consecutive days prior to the experiment. (A) Representative western blotting images of GLT-1 in the LC from normal rats treated with non-targeting (left images) or GLT-1 selective siRNA (right images). (B) Changes in glutamate concentrations in microdialysates from the LC during perfusion of gabapentin (GBP, 10 mM) are presented over time as percentage of baseline. *$P<0.05$ vs. non-targeting siRNA. #$P<0.05$ vs. baseline.

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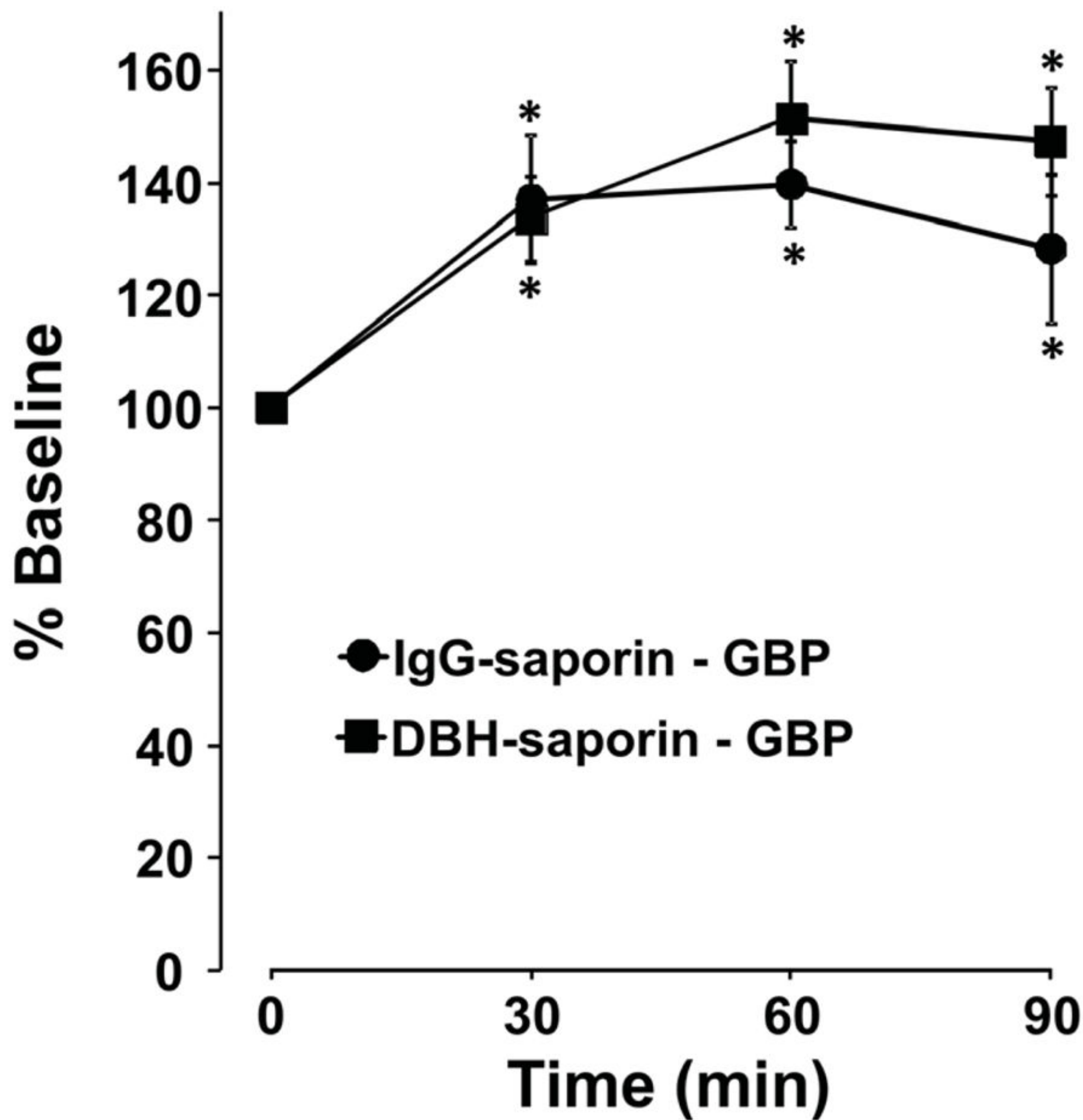

Fig. 5.

Depletion of noradrenergic neurons did not alter gabapentin-induced increase in extracellular glutamate in the LC. Normal rats were treated with an intra-LC injection of IgG-saporin (0.25 μg/rat, n=9) or DBH-saporin (0.25 μg/rat, n=8) at 2 weeks prior to the experiment. Changes in glutamate concentrations in microdialysates from the LC during perfusion of gabapentin (GBP, 10 mM) are presented over time as percentage of baseline. *P<0.05 vs. baseline.

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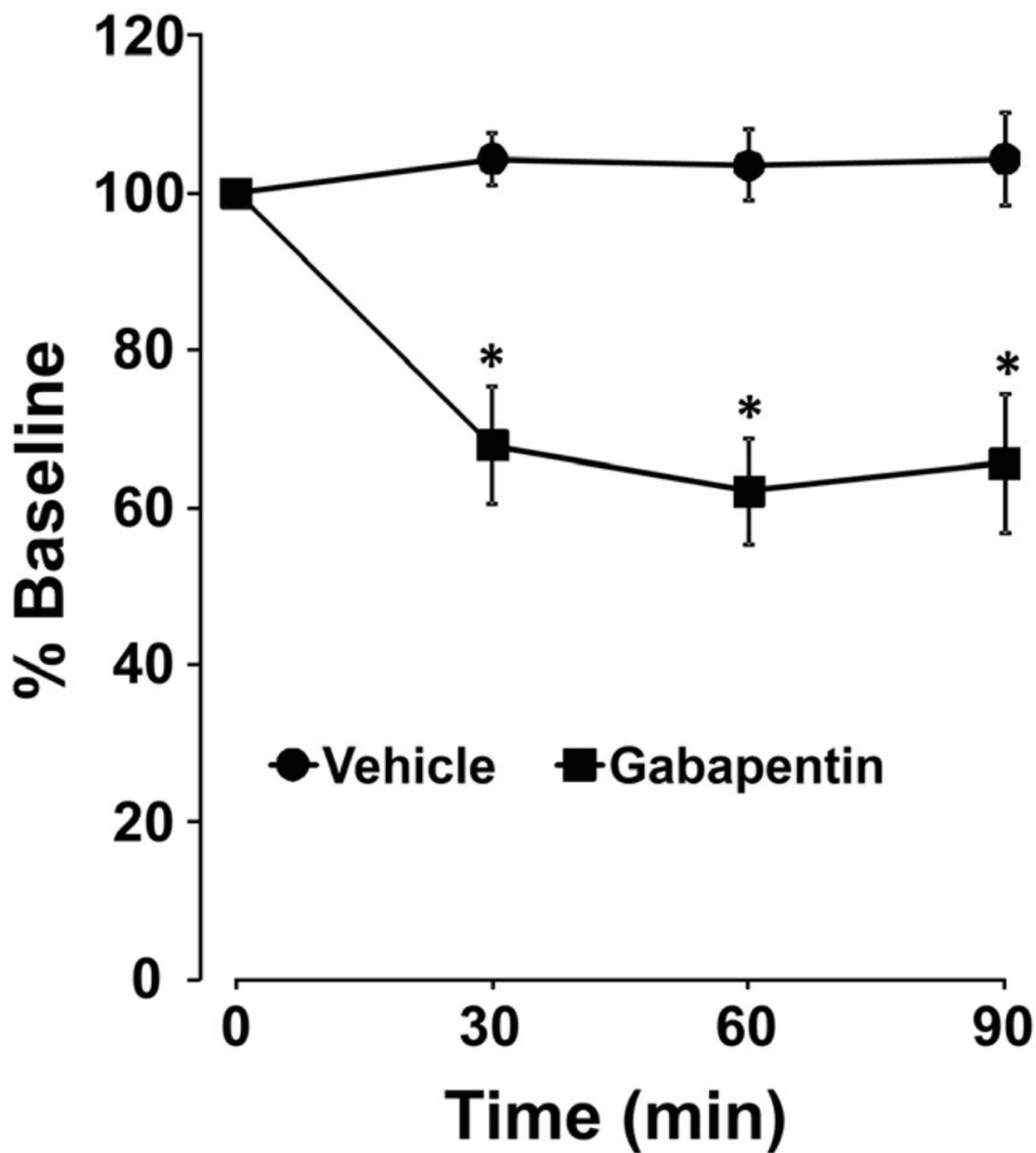

Fig. 6.

Effect of locally administered gabapentin on extracellular glutamate concentrations in the spinal cord. Vehicle or gabapentin (10 mM) was perfused into the spinal dorsal horn for 90 min through the microdialysis probe in normal rats (n=9 in each group). Changes in glutamate concentrations in microdialysates from the spinal cord are presented over time as percentage of baseline. *P<0.05 vs. vehicle.

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Table 1

Basal glutamate concentrations in microdialysates from the LC

Treatment	Glutamate (pmol/30μL)
Buffer (n=88)	11.2 ± 0.7
CGP-35348 (n=24)	10.1 ± 1.0
DHK (n=14)	19.3 ± 2.8[*]
GLT-1 siRNA (n=11)	25.6 ± 3.4[*,#]
Non-target siRNA (n=11)	13.4 ± 0.9
DβH-saporin (n=8)	25.4 ± 4.1[*,$]
IgG-saporin (n=8)	8.2 ± 1.2
SNL Buffer (n=45)	49.1 ± 7.9[*]

[*] $p<0.05$ vs. buffer,

[#] $p<0.05$ vs. non-target siRNA,

[$] $p<0.05$ vs. IgG-sap