Endogenous GABA Does Not Mediate the Inhibitory Effects of Gabapentin on Spinal Reflexes in Rats

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Abstract. The novel antiepileptic drug gabapentin was designed as a structural analog of \( \gamma \)-aminobutyric acid (GABA). However, its mechanism of action remains unclear. In the present study, we investigated the effect of gabapentin on spinal reflexes in anesthetized rats. The mono- and polysynaptic reflex potentials were recorded from the ipsilateral L5 ventral root after stimulation of the L5 dorsal root. The dorsal root reflex potential, an index of presynaptic inhibition, was recorded from the ipsilateral L4 dorsal root. In non-spinalized (intact) and spinalized rats, intravenously administered gabapentin reduced the mono- and polysynaptic reflex potentials in a dose-dependent manner. These inhibitory effects of gabapentin were not suppressed by the GABA\(_A\) antagonist picrotoxin. Moreover, gabapentin also decreased spinal reflexes in spinalized rats depleted of spinal GABA with semicarbazide, an inhibitor of the GABA-synthesizing enzyme. The dorsal root reflex potentials were not affected by gabapentin. These results suggest that endogenous GABA does not mediate the inhibitory effects of gabapentin on spinal reflexes.

Keywords: spinal reflex, gabapentin, endogenous GABA, dorsal root reflex, pregabalin

Introduction

Gabapentin, 1-(aminomethyl)cyclohexaneacetic acid, is a novel anticonvulsant agent that is effective in various animal seizure models (1, 2) and that has been shown in several placebo-controlled clinical studies to prevent partial seizures and generalized tonic-clonic seizures, both as add-on therapy and monotherapy (3–5). Furthermore, it has been shown that gabapentin exhibits antinociceptive effects on hyperalgesia (6–8) and anxiolytic-like effects (8).

The cellular mechanisms of the pharmacological actions of gabapentin have not been completely determined. Gabapentin was synthesized as a structural analog of \( \gamma \)-aminobutyric acid (GABA) and it penetrates the blood-brain barrier. However, gabapentin does not exhibit affinity for known neurotransmitter binding sites, including the GABA\(_A\) and GABA\(_B\) receptors (9), and does not affect GABA uptake and degradation (10, 11). Instead, gabapentin shows specific binding affinity for \( \alpha_2\delta \) subunits of voltage-dependent calcium channels (12). Moreover, similar specific affinity for \( \alpha_2\delta \) subunits was demonstrated for pregabalin, another GABA derivative with antiepileptic and antinociceptive actions (12). This activity profile may be essential for some aspects of the pharmacological actions of these compounds.

It has been demonstrated that gabapentin elevates the GABA concentration in human (13) and rat (14) brains and increases GABA release from rat striatal slices (15). Similarly, pregabalin increases neuronal GABA content (16). Thus, gabapentin and pregabalin can exert indirect GABAergic actions via an increase in the GABA concentration in the central nervous system, which may underlie their antiepileptic effects. Previous studies have shown that the spinal reflexes are susceptible to a change in GABAergic inhibitory tone. The GABA\(_A\) agonist muscimol and the GABA\(_B\) agonist baclofen
decrease mono- and polysynaptic reflexes (17–19). In addition, the dorsal root reflex, mediated by spinal intrinsic GABAergic interneurons, is increased by benzodiazepines (20). In the present study we examined the effects of gabapentin (and pregabalin in some experiments) on spinal reflexes in an attempt to clarify the involvement of indirect GABAergic inhibition in the spinal cord.

Materials and Methods

Surgery

All experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University and Tokyo University of Science, and they were conducted in accordance with the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

Male Wistar rats weighing 200–300 g (NRC Haruna, Gunma) were anesthetized with urethane (1 g/kg, intraperitoneally (i.p.)) and α-chloralose (25 mg/kg, i.p.). Cannulae were inserted into the trachea and the femoral vein for artificial respiration and drug administration, respectively. To produce spinalized rats, the vagus nerves were cut bilaterally in the cervical region to eliminate parasympathomimetic effects on the heart, and the spinal cord was transected at the C1 level under lidocaine anesthesia (4%, 50 μl). A dorsal laminectomy was performed in the lumbo-sacral region of each rat. Both the ventral and dorsal roots below L4 were cut distally at their points of exit from the vertebral column, and the entire exposed surgical area was covered with liquid paraffin that was maintained at 36 ± 0.5°C by radiant heat. Rectal temperature was maintained at 36 ± 0.5°C.

Measurement of the spinal reflex potentials

For the recording of mono- and polysynaptic reflexes, the dorsal and ventral roots of the L5 segment were placed on bipolar Ag-AgCl wire electrodes for stimulation (5 V, 0.2 Hz, 0.05 ms; SEN-7103; Nihon Kohden, Tokyo) and recording, respectively (inset in Fig. 1A). The dorsal root reflex was recorded from the L4 dorsal root with a bipolar Ag-AgCl wire electrode following stimulation of the ipsilateral L5 dorsal root (5 V, 0.2 Hz, 0.05 ms). The reflex potentials were amplified (AVB-10, Nihon Kohden), displayed on an oscilloscope (VC-10, Nihon Kohden) and averaged eight times by an averager (DAT-1100, Nihon Kohden). The amplitudes of mono- and polysynaptic reflex potentials and dorsal root reflex potentials were measured (insets in Figs. 1A and 2A). The latency of the monosynaptic reflex potential was 1.5–2 ms, and the delay of the polysynaptic reflex peak from the monosynaptic reflex peak was 0.8–1 ms. The latency of the dorsal root reflex was 3–4 ms.

Drugs

Gabapentin (1-(aminomethyl)cyclohexanecetic acid) and pregabalin ((S)-(−)-3-(aminomethyl)-5-methyl-hexanoic acid) were kindly supplied by Pfizer (Ann Arbor, MI, USA). Muscimol, picrotoxin, and urethane were obtained from Sigma Aldrich (St. Louis, MO, USA). Semicarbazide hydrochloride and α-chloralose were obtained from Tokyo Kasei (Tokyo). Urethane and α-chloralose were both dissolved in distilled water. All the test compounds were dissolved in 0.9% w/v physiological saline and administered intravenously at 1 ml/kg at least 2 h after the C1 transection. Control rats received vehicle at 1 ml/kg. When gabapentin was examined under blockade of GABA synthesis, semicarbazide hydrochloride (200 mg/kg, i.v.) was pretreated after laminectomy.

Statistical analyses

The amplitudes of the mono- and polysynaptic reflex and dorsal root reflex potentials recorded after drug administration were expressed as percentages of the corresponding predrug (time 0) values. All data were expressed as the means ± S.E.M. Student’s t-test was used to compare the data between two groups, and the t-test with Bonferroni correction following one-way analysis of variance (ANOVA) was used for multiple comparisons of control and treated groups (21). Differences at P<0.05 (two-tailed) were considered to be significant.

Results

Effect of gabapentin on monosynaptic, polysynaptic, and dorsal root reflexes

In non-spinalized (intact) rats, gabapentin reduced the mono- and polysynaptic reflex potentials in a dose-dependent manner. The amplitude of the monosynaptic reflex potential was reduced by gabapentin to 92.9 ± 8.8% (n = 5) and 70.9 ± 9.7% (n = 5) at doses of 10 and 30 mg/kg, i.v., respectively (60 min after administration) (Fig. 1A). The amplitude of the polysynaptic reflex potential was significantly reduced by gabapentin to 47.3 ± 8.2% and 37.2 ± 2.4% at doses of 10 and 30 mg/kg, i.v., respectively (60 min after administration) (Fig. 1B). In spinalized rats, gabapentin also reduced the mono- and polysynaptic reflex potentials in a dose-dependent manner. The amplitude of the monosynaptic reflex potential was reduced by gabapentin to 88.5 ± 11.4% (n = 5) and 65.2 ± 5.2% (n = 5) at doses of
3 and 10 mg/kg, i.v., respectively (60 min after administration) (Fig. 1C). The amplitude of the polysynaptic reflex potential was significantly reduced by gabapentin to 51.7 ± 10.1% and 28.2 ± 7.2% at doses of 3 and 10 mg/kg, i.v., respectively (60 min after administration) (Fig. 1D). In both intact and spinalized rats, the inhibitory effects of gabapentin appeared gradually after administration, and gabapentin reduced the polysynaptic reflex more strongly than the monosynaptic reflex.

Stimulation of the L5 dorsal root produces the dorsal root reflex potential in the L4 dorsal root. Gabapentin (10 mg/kg, i.v.) did not affect the dorsal root reflex (Fig. 2).

**Influence of GABA_A-receptor antagonist on gabapentin-induced effects in spinalized rats**

In the following studies, we used a higher dose of gabapentin (30 mg/kg), since this dose of gabapentin exhibited a more stable reduction of the monosynaptic reflex than 10 mg/kg.

In spinalized rats, the GABA_A-receptor antagonist picrotoxin at a dose of 3 mg/kg alone had no effect on the monosynaptic reflex potential (n = 6, Fig. 3A). This dose of picrotoxin sufficed to block GABA_A receptor-mediated responses, as shown in Fig. 3B. Muscimol (3 mg/kg) gradually decreased the monosynaptic reflex potential to 53.5 ± 7.0% at 60 min after administration,
which was partially reversed by picrotoxin injected 30 min after muscimol (inhibition to 74.52 ± 6.7% at 30 min after picrotoxin, n = 7). However, picrotoxin failed to reverse the gabapentin-induced decrease of monosynaptic reflex potential (inhibition to 58.5 ± 10.8% and 63.7 ± 14.5% at 60 min after gabapentin in the control and picrotoxin-treated groups, respectively, n = 6; Fig. 3C).

Influence of semicarbazide on gabapentin-induced effects in spinalized rats

GABA is synthesized from glutamate by glutamic acid decarboxylase (GAD) at GABAergic terminals. Systemic administration of semicarbazide hydrochloride (200 mg/kg, i.v.), an inhibitor of GAD, was shown to deplete GABA to about 15% in the cat spinal cord in 3 h (22). In the present study, the same dose of semicarbazide hydrochloride resulted in a gradual increase in the monosynaptic reflex to 142.4 ± 3.6% in 3 h, and this enhancement was maintained for at least 1 h (n = 4, Fig. 4A), consistent with a marked decrease in GABA in the spinal cord (22). Nevertheless, gabapentin still decreased the spinal reflex (Fig. 4B).

Effect of pregabalin on the mono- and polysynaptic reflexes

In spinalized rats, pregabalin reduced the mono- and polysynaptic reflex potentials in a dose-dependent

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**Fig. 2.** Gabapentin does not change the dorsal root reflex potential in spinalized rats. Each point represents the mean ± S.E.M. of 5 animals. Abscissae: time in minutes after the injection of gabapentin. Ordinate: amplitude of spinal reflexes expressed as a percentage of the corresponding values at time 0. The significance of the differences between the test (closed circle: 10 mg/kg, i.v.) and control (open circle) values was determined by the two-tailed Student’s t-test; *P<0.05 vs control. Inset shows a schematic representation of the experimental situation and a sample trace of the dorsal root reflex potential.

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**Fig. 3.** The GABA_A receptor antagonist picrotoxin does not counteract the inhibition of the monosynaptic reflex potential by gabapentin in spinalized rats. A: picrotoxin (open circle: control, closed circle: 3 mg/kg, i.v.) alone did not alter the monosynaptic reflex. B: the inhibitory effect of muscimol (3 mg/kg, i.v.) was partially reversed by picrotoxin (open triangle: muscimol, closed triangle: +picrotoxin). C: the inhibitory effect of gabapentin (30 mg/kg, i.v.) was not affected by picrotoxin (open triangle down: gabapentin, closed triangle down: +picrotoxin). Picrotoxin was administered 30 min after muscimol or gabapentin. Each point represents the mean ± S.E.M. of 5–7 animals. Abscissae: time in minutes. Ordinate: amplitude of spinal reflexes expressed as a percentage of the corresponding values at time 0. The significance of the differences between the test and control values was determined by the two-tailed Student’s t-test; *P<0.05 vs control.
The amplitude of the monosynaptic reflex potential was reduced by pregabalin to 89.6 ± 5.2% and 76.6 ± 5.1% at doses of 3 and 10 mg/kg, i.v., respectively (60 min after administration) (n = 6, Fig. 5A). The amplitude of the polysynaptic reflex potential was significantly reduced by pregabalin to 50.8 ± 11.6% and 24.4 ± 3.5% at doses of 3 and 10 mg/kg, i.v., respectively (60 min after administration) (n = 5, Fig. 5B). The inhibitory effects of pregabalin appeared gradually after intravenous administration, and pregabalin reduced the polysynaptic reflex more strongly than the monosynaptic reflex.

Discussion

The spinal reflex employed in the present study consists of the mono- and polysynaptic reflexes. Glutamatergic monosynaptic excitation of motoneurons (23) is evoked by excitation of group Ia primary afferent fibers originating from muscle spindles located in
skeletal muscles. The polysynaptic reflex measured in this study is evoked by disynaptic excitation of motoneurons via one interneuron. There is GABAergic synaptic inhibition on these spinal reflex transmissions (24).

We have shown that gabapentin decreased the monoand polysynaptic reflexes in intact and spinalized rats (Fig. 1: A – D). While studies have been focused on the well-known effect on chronic pain at the spinal cord (25 – 27), we now for the first time revealed that gabapentin can reduce motor output from the spinal cord at the spinal level.

Although it is controversial whether gabapentin directly activates GABA receptors, indirect GABAergic inhibition using endogenous GABA (13 – 15) might mediate some pharmacological actions elicited by gabapentin including depression of the spinal reflex in this study. The observations reported here, however, exclude this possibility. First, blockade of GABA synthesis by the GAD inhibitor semicarbazide did not affect the action of gabapentin (Fig. 4B). Secondly, the dorsal root reflex, which is mediated by spinal intrinsic GABAergic interneurons (24), was not affected by gabapentin (Fig. 2). Finally, depression of the monosynaptic reflex by gabapentin was not reversed by the GABA receptor antagonist picrotoxin (Fig. 3C). Furthermore, the lack of change in the dorsal root reflex reveals that GABA receptors do not mediate the actions of gabapentin, since the selective GABA receptor agonist baclofen strongly decreases the dorsal root reflex (17). In addition, the preferential effects of baclofen on monosynaptic reflex rather than polysynaptic reflex transmission support this conclusion (17, 19). However, presently, there exist several lines of evidence that argue for (28, 29) and against (30) the mediation of GABA receptors in the action of gabapentin. Moreover, gabapentin may reveal a specific agonism of the GABA receptor subtypes (29). Further studies will be required to clarify the involvement of GABA receptors in the inhibition of spinal reflexes by gabapentin.

The inhibitory effects of pregabalin on spinal reflexes were similar to those of gabapentin; both drugs produced stronger effects on the polysynaptic reflex than on the monosynaptic reflex, and the onset of effects were slow (Figs. 1 and 5). Moreover, there is evidence that pregabalin increases neuronal GABA content (16) and that it selectively binds to the α2δ subunit of calcium channels with high affinity in the nanomolar range (12). Together with the present results, these observations suggest that gabapentin and pregabalin may inhibit spinal reflex transmission via binding to the α2δ subunit. Further studies are required to clarify the detailed mechanisms of action of gabapentin on spinal reflex transmission.

In conclusion, we showed that gabapentin decreased the spinal reflexes in intact and spinalized rats by a process not mediated by endogenous GABA.

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References

Gabapentin and Spinal Reflexes


