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Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons

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Abstract

Gabapentin (GBP) is a cyclic γ-aminobutyric acid (GABA) analog and investigational antiepileptic drug which is effective in the treatment of a variety of human and experimental seizures. GBP's antiepileptic mechanism of action is not known. The present studies tested for effects of GBP on inhibitory (GABA and glycine) and excitatory (*N*-methyl-D-aspartate (NMDA) and non-NMDA) amino acid neurotransmitter receptors, on repetitive firing of sodium (Na^+) action potentials, and on voltage-dependent calcium (Ca^{2+}) channel currents in cultured rodent neurons using intracellular, whole cell, or single channel recording techniques. GBP did not have a significant effect in any experiment when tested at or above concentrations that are therapeutic in humans except for a variable enhancement of NMDA-evoked depolarizations. These results suggest that the antiepileptic activity of GBP is not due to direct effects at receptors for inhibitory or excitatory amino acids or on voltage-dependent Na^+ or Ca^{2+} channels.

Key words: Gabapentin; Investigational antiepileptic drug; Repetitive firing; GABA; Glycine; *N*-Methyl-D-aspartate; Sodium action potentials; Calcium currents; Voltage clamp; Single channel

1. Introduction

Gabapentin (GBP), 1-(aminomethyl) cyclohexane-acetic acid, is a cyclic γ-aminobutyric acid (GABA) analog originally designed to mimic the steric conformation of GABA [22], to have high lipid solubility to penetrate the blood-brain barrier, and to be a centrally active GABA agonist with potential therapeutic value [19]. GBP has been shown to have anticonvulsant activity in a variety of animal seizure models [3] and is effective in the treatment of human partial [5,7,11,23] and generalized tonic-clonic seizures [5,7]. Despite its effectiveness against a variety of seizures, GBP's antiepileptic mechanism of action remains unknown.

There are relatively few reported electrophysiological studies directed at determining GBP's antiepileptic mechanism of action. The present study used a variety of cultured rodent neuronal preparations and electrophysiological techniques including intracellular, whole cell, and single channel recordings to determine any effects of GBP on inhibitory (GABA and glycine) and excitatory (*N*-methyl-D-aspartate (NMDA) and non-NMDA) amino acid neurotransmitter receptors, repetitive firing of sodium (Na^+) action potentials, and on voltage-dependent calcium (Ca^{2+}) channel currents. GBP was tested at or above plasma concentrations which range ~12–88 μM in ongoing clinical stu-

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dies [6]. These studies were chosen to corroborate or confirm the results of other studies suggesting an effect of GBP on excitatory or inhibitory neurotransmitter systems, or on voltage-dependent ion channels. Preliminary results from the present experiments have been reported previously [13,26].

2. Methods

2.1. Cultures

2.1.1. Cortical neurons. Cortices were removed from E 18 rat fetuses, treated with trypsin (type 1, bovine pancreas, Sigma) and mechanically dissociated. Neurons were suspended in MEM supplemented with glucose 30 mM, bicarbonate 26 mM, 10% fetal calf serum and 10% horse serum and were plated on poly-L-lysine-coated culture dishes. 5-Fluoro-2-deoxyuridine (15 μg/ml) and uridine (35 μg/ml) in MEM were added to cultures on the fifth day. Cultures were maintained for 2–5 weeks prior to experiments.

2.1.2. Spinal cord neurons. Spinal cords were dissected from E 12–14 mouse fetuses, minced, mechanically dissociated, and plated. Growth media consisted of MEM supplemented with 5.5 g glucose and 1.5 g $NaHCO_3$ per liter, sera as above and adjusted to 325 mOsm. The cultures were incubated at 35°C in a 90% air/10% CO_2 atmosphere. The $NaCO_3/CO_2$ buffer was maintained at pH 7.4. 5-Fluoro-2-deoxyuridine was added to cultures after 6–8 days. Cultures were maintained for at least 4 weeks prior to experiments.

2.1.3. Sensory neurons. Nodose ganglia were taken from 5–10 days old rats, placed in oxygenated Ca^{2+}- and Mg^{2+}-free DPBS, treated with collagenase (type 1A, Sigma) (1 mg/ml), and incubated at 37°C in a 93% air/7% CO_2 atmosphere for 30 min. Following addition of 5% fetal calf serum, the neurons were triturated, centrifuged, resuspended in MEM, plated in collagen-coated dishes, and reincubated prior to use. Dorsal root ganglia (DRG) were taken from 15–25 day old rats (~20/animal), placed in MEM, minced, treated with collagenase (3 mg/ml), and incubated for 50 min before trypsin (1 mg/ml) was added for 10 min. The ganglia were removed, placed in MEM, centrifuged, triturated, recentrifuged, resuspended, plated, reincubated, and 1 ml of MEM was added to each plate after 30 min. Nodose and DRG neurons were used after ~1 h of incubation and were viable for ~12 h.

2.2. Experiments

2.2.1. Inhibitory and excitatory amino acids. Intracellular recordings of spinal cord or cortical neurons were made with glass micropipettes (20–60 MΩ) filled with 3 M KCl (GABA and glycine) or 4 M KAc (glutamate). Membrane potential was recorded with a high-impedance bridge amplifier and recorded on analog tape. The recording solution contained (in mM): NaCl 140, KCl 5, $MgCl_2$ 10, $CaCl_2$ 1, glucose 10, pH 7.3–7.4. GABA (0.5 M, pH 3.2, positive current), glycine (0.5 M, pH 3.0, positive current), or glutamate (0.4 M, pH 10, negative current) was applied by passing 400 ms current pulses every 5 s from a high resistance micropipette placed ~2 μm from the impaled neuron. Retaining current (negative for GABA and glycine, positive for glutamate) was applied to prevent leakage between pulses. Membrane potential was hyperpolarized to −60 to −80 mV. Iontophoretic current was adjusted so that responses were approximately 10 mV in amplitude. GBP was applied by pressure ejection (0.25–1.0 psi, 30 s) from blunt-tipped (10 μm) micropipettes placed 30–100 μm from the neuron. NMDA (10 μM) was applied by pressure ejection (10 s, 1 psi) in the presence or absence of GBP or glycine. Data were expressed as percentage of control amplitude but were not used if the response failed to return to control levels after drug application.

2.2.2. NMDA single channel currents. The external solution for single channel experiments consisted of (in mM): NaCl 142, KCl 1.5, $CaCl_2$ 1, glucose 10, Na^+-HEPES 10 (pH 7.4, ~300–320 mOsm). The internal pipette solution consisted of (in mM): CsCl 153, Cs^+-HEPES 10, EGTA 5 (pH 7.4, ~280–305 mOsm). NMDA (Sigma) and glycine (Sigma) were dissolved in external solution (10–100 μM) and serially diluted to final concentrations. Glycine concentrations were expressed as the amount of glycine added to the external solu-

tion. Drugs were applied to membrane patches by pressure ejection (0.25–1.0 psi) from blunt-tipped (15–25 μm) glass micropipettes positioned ~50 μm from the patch.

Cultures were maintained at room temperature (20–23°C) and were superfused at approximately 1 ml/min during or between recordings. High resistance GΩ seals were obtained with glass micropipettes (5–10 MΩ) for outside-out excised patch recordings. Recordings were performed with a L/M EPC-7 amplifier (LIST-Medical Instruments) and currents were recorded on a video cassette system via a digital audio processor (16 bit, 44 kHz). Currents were simultaneously displayed on a chart recorder using a lowpass (3 db at 1 kHz) 8-pole Bessel filter. Data were played back from the VCR system through a lowpass filter (3 db at 2 kHz) and digitized (20 kHz, 16 bit) for computer analysis. System dead time was 70 μs, so only openings >140 μs (2× system dead time) were considered valid openings.

Single channel data were analyzed by computer using a locally written channel detection program (50% threshold crossing criterion) and locally written analysis programs used to determine opening frequency, and open and closed durations. Percent change in opening frequency was determined in those patches where control solution (NMDA alone) was applied before, during and after application of GBP. Openings from the two higher conductance levels of NMDA receptor single channel currents (40 and 50 pS) were analyzed.

2.2.3. Repetitive firing and spontaneous synaptically driven Na^+ action potentials. Intracellular recordings of spinal cord neurons were made in a manner similar to those in the inhibitory and excitatory amino acid experiments. Only cultures in which all neurons exhibited sustained repetitive firing and spontaneous action potentials (3–5 neurons examined per culture) in control medium were used for experiments. To elicit repetitive firing, 450 ms depolarizing current pulses of varying amplitudes were used. If a neuron fired action potentials throughout any of the 450 ms pulses, firing was judged as sustained. A neuron was used when the resting membrane potential was −40 mV or more negative and had overshooting action potentials. All experiments were conducted at 35–37°C. GBP was dissolved in recording solution and was applied to the bath. Neurons were tested at membrane potentials of approximately −50 mV for the ability to fire sustained action potentials. Data were expressed as the percentage of neurons showing sustained firing. Because recordings were done with a low concentration of Mg^{2+} in the recording solution, synaptically driven action potentials were recorded when neurons were held at resting potential (−50 to −60 mV). Effects of GBP on spontaneous action potentials were qualitatively assessed and cells were judged to have either normal or reduced levels of spontaneous action potentials.

2.2.4. Voltage-dependent Ca^{2+} channel currents. Recordings of Ca^{2+} currents were made with glass micropipettes (1.5–2.5 MΩ) filled with a solution of (in mM): CsCl 140, CsOH 30, HEPES 10, EGTA 10, ATP 5 and GTP 0.1, pH 7.2–7.3, ~300 mOsm. Neurons were bathed in a solution of (in mM): choline Cl 67, TEA 100, glucose 5.6, KCl 5.3, $CaCl_2$ 5.0, $MgCl_2$ 0.8, HEPES 10, pH 7.35, ~320 mOsm. Following sealing of the micropipette to the neuron and rupture of the membrane patch, the neuron was hyperpolarized to a holding potential (V_h) = −90 mV. The program pCLAMP (Axon Instruments) was used to generate voltage step commands and to digitize (5.6 kHz) and store current traces. All experiments were conducted at room temperature. GBP was prepared on the day of experiments as described above and put in external solution. The GBP solution was drawn up into a blunt tipped (15–20 μm) micropipette and lowered to ~50 μm from the neuronal soma and applied by passive diffusion or pressure ejection (1 psi) shortly before and during voltage step commands.

T currents were isolated by evoking currents from a V_h = −90 mV at clamp potentials (V_c) at or positive to −55 mV (typically, V_c = −65 to −20 mV). N/L currents were evoked from a V_h = −80 or −90 mV at a V_c at or positive to −20 mV. T and N/L currents were measured at the point of peak inward current and at 100 ms, and were leak subtracted for analysis. In order to distinguish between the N and L components qualitatively, currents were evoked at V_c = −10 mV

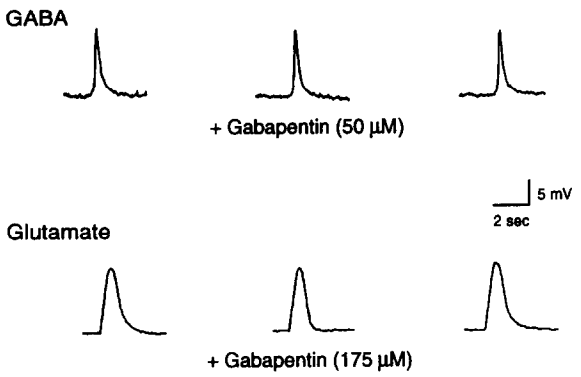

Fig. 1. GBP had no effect on iontophoretic GABA and glutamate responses in cultured spinal cord neurons. Traces represent intracellular recordings of membrane depolarizations that were evoked by the application of GABA (upper) and glutamate (lower). Application of GBP did not change the amplitude of membrane depolarizations.

from V_h = −80 mV and −40 mV. Current evoked from V_h = −40 mV was used as an estimate of L current and the additional current evoked from V_h = −80 mV was used as an estimate of N current. Ethosuximide (1 mM) was used to block T current; nifedipine (10 μM) was used to block L current; ω-conotoxin GVIA (10 μM) was used to block N current. Each was applied by passive diffusion from blunt tipped (35–50 μM) micropipettes positioned ~50 μm from the neuronal soma prior to voltage step commands.

3. Results

3.1. GBP did not affect membrane potential

GBP (18–500 μM) did not affect membrane potential upon direct application to spinal cord neurons (N = 20). No depolarization or hyperpolarization was noted upon application, unlike the action of agonists that interact with $GABA_A$ or glutamate receptors which produce depolarization or hyperpolarization, depending upon recording conditions. These results suggest that GBP is not acting as an agonist at inhibitory or excitatory amino acid receptors in spinal cord neurons.

3.2. GBP did not affect inhibitory amino acid responses

Iontophoretic application of GABA or glycine, with 3 M KCl in the recording electrode, resulted in a depolarization of membrane potential. Iontophoretic ejection current was adjusted to produce depolarization of approximately 10 mV. GBP (18–500 μM) applied by pressure ejection did not reduce the amplitude of GABA- or glycine-evoked depolarizations (Fig. 1, Table 1). Diazepam (100 nM) increased the amplitude of iontophoretic GABA responses by 81% (N = 5) in a similar preparation.

3.3. GBP had variable effects on excitatory amino acid responses

Iontophoretic application of glutamate in the presence of Mg^{2+} (10 mM) activates both α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate subtypes of postsynaptic glu-

TABLE 1

Effect of GBP on inhibitory and excitatory neurotransmitter responses in cultured fetal cortical (ctx) and spinal cord (sc) neurons

	Prep.	Agonist application	GBP (μM)	% Control (SEM, N)
GABA	sc	ionto	18	100 ± 1.5 (N = 5)
			50	103 ± 2.3 (N = 5)
Glutamate	sc	ionto	175	98 ± 1.5 (N = 5)
Glycine	ctx	ionto	500	99 ± 3.1 (N = 5)
NMDA	ctx	local 10 μM (+ 1 μM glycine)	100	159 ± 18.0 (N = 18)*
				106 ± 6.4 (N = 6)

*$P < 0.05$, two-tailed t-test.

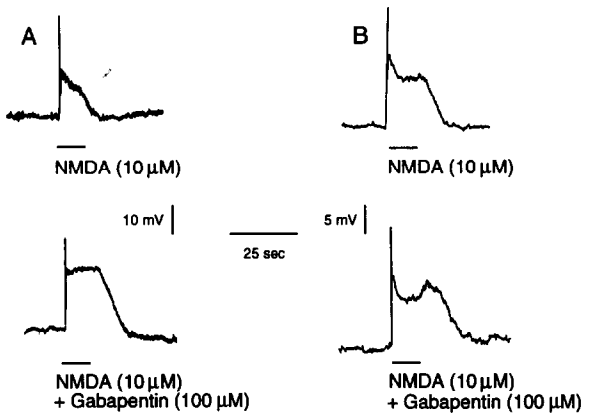

Fig. 2. GBP had some effect on the amplitude of NMDA-evoked depolarizations in the absence of added glycine in cultured cortical neurons. Traces represent intracellular recordings of membrane depolarizations evoked by the local application of NMDA (upper) or in combination with GBP (lower). The amplitude of the NMDA-evoked depolarization at the end of the application was measured. The left panel (A) is an example of a neuron where the addition of GBP resulted in an increase in the amplitude of the sustained portion of the depolarization. No effect was seen in the neuron represented in B tested under the same conditions.

tamate receptors. GBP (175 μM) applied by pressure ejection had no effect on the amplitude of iontophoretic glutamate responses (Fig. 1, Table 1). Pentobarbital (200 μM) reduced the amplitude of glutamate responses by 16% (N = 5).

However, GBP (100 μM) had a variable effect on the responses of cortical neurons when applied with NMDA (Table 1, Fig. 2). In the absence of added glycine, GBP enhanced the amplitude of the sustained portion of NMDA-evoked responses in seven of 18 neurons with an average increase of 59% (Fig. 2A). Eleven of 18 cells showed no enhancement (<20% change from control) of NMDA responses by 100 μM GBP (Fig. 2B). Glycine was effective in enhancing NMDA-evoked depolarizations in all neurons tested with the addition of glycine (1 μM) enhancing the amplitude of the sustained portion of NMDA-evoked depolarizations by 212% (N = 4).

GBP did not enhance NMDA-evoked depolarizations in the presence of glycine. With glycine (1 μM), GBP at 100 μM (106% of control, N = 6) or at 1 mM (104% of control, N = 10) was not effective in enhancing NMDA-evoked depolarizations when applied by pressure ejection. Application of 1 mM GBP by superfusion was also ineffective in affecting NMDA-evoked depolarizations (106 $\pm$ 3.1% control, N = 5). At higher concentrations of NMDA (25 μM) and glycine (10 μM), GBP at 10 μM (107 $\pm$ 9.8% control, N = 5) and 100 μM (104 $\pm$ 7.5% control) had no effect on the amplitude of the sustained portion of the NMDA-evoked depolarizations.

GBP did not reduce the amplitude of NMDA-evoked depolarizations under any of the conditions tested. However, NMDA (10 μM) responses could be completely blocked by the competitive NMDA antagonist 3-(($\pm$)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (100 μM, N = 3) and the amplitude of the sustained portion of NMDA-evoked depolarizations was reduced to 9 $\pm$ 4.5% of control by the glycine site partial agonist HA-966 (500 μM, N = 6).

Single channel recordings of NMDA receptor currents from excised outside-out patches were made to further investigate the possibility of an interaction between GBP and NMDA receptors. In the absence of added glycine NMDA (5 μM) evoked single channel openings at a frequency of 2.2/s. Addition of 1 mM GBP did not increase the frequency of openings (1.4/s) in these patches but the frequency of NMDA receptor channel openings was increased 5-fold (10.4/s) by the addition of 2.5 μM glycine (N = 4 patches). In the presence of 2.5 μM glycine, GBP (1 mM) did not change average open duration (2.6 ms control, 2.5 ms GBP), opening frequency (10.7/s control, 9.9/s GBP), burst duration (6.7 ms control, 7.5 ms GPB), the number of openings/burst (1.7 control, 1.9 GBP) or the burst frequency (6.0/s control, 5.3/s GBP) (N = 6 patches, 2894–9159 openings, 1682–4824 bursts, Fig. 3). GBP did not alter the amplitude of NMDA receptor single channel openings in the presence or absence of glycine (Fig. 3). These data indicate that GBP does not act like an agonist or partial agonist at the glycine site of the NMDA receptor, having no effect on the frequency of channel openings, and that GBP also had no effect on the kinetics of NMDA receptor single channels.

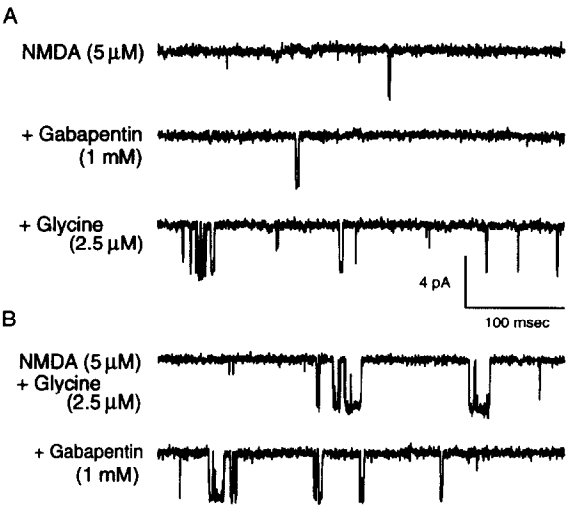

Fig. 3. GBP did not act like an agonist or antagonist at the glycine site of the NMDA receptor in single channel recordings from outside-out excised patches. In the absence of added glycine (A), NMDA (5 μM) evoked very few openings of NMDA receptor single channels. Addition of GBP (1 mM) had little effect on opening frequency while addition of glycine (2.5 μM) greatly increased the frequency of channel opening. In the presence of NMDA and glycine (B), addition of GBP (1 mM) had no effect on the kinetics of single channel openings.

3.4. GBP did not block sustained repetitive firing of Na^+ action potentials or synaptically driven action potentials

Control neurons (N = 17) responded to 450 ms depolarizations with trains of action potentials that were sustained throughout the depolarization (Fig. 4, left panel). Addition of GBP (18–175 μM) to the bathing solution for 10–30 min had no effect on the sustained repetitive firing of action potentials (Table 2, Fig. 4, right panel). In experiments performed in low Mg^{2+} (1 mM), the effect of GBP on spontaneous synaptically driven action potentials was evaluated. Bath application of GBP (18–175 μM) had no effect on spontaneous action potentials (Table 2).

TABLE 2

Effect of GBP on sustained repetitive firing and spontaneous synaptically driven action potentials in cultured fetal spinal cord neurons

GBP (μM)	% of neurons with sustained repetitive firing (N)	% of neurons with normal spontaneous activity (N)
0 (control)	100 (17)	100 (29)
18	100 (7)	82 (11)
50	100 (10)	73 (15)
175	88 (8)	90 (10)

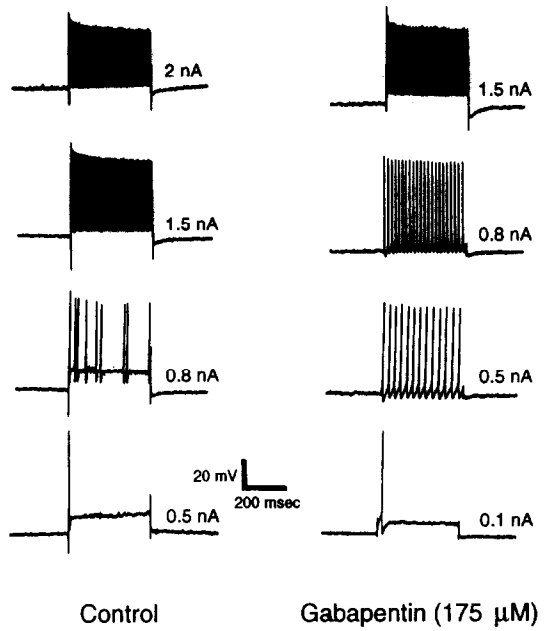

Fig. 4. GBP had no effect on sustained repetitive firing of Na^+ action potentials in cultured spinal cord neurons. In control (left panel), membrane depolarization was increased by increasing the magnitude of injected current (current magnitudes indicated at the right of each trace (nA)) resulting in an increase in the number and frequency of action potentials. In a recording of a different neuron, application of GBP (175 μM) made no difference in the ability of the neuron to fire a sustained train of action potentials. Resting membrane potential was −50 mV (control) and −55 mV (GBP).

3.5. GBP did not affect Ca^{2+} channel currents

GBP (1 mM) was tested for effects on Ca^{2+} channel currents in nodose and dorsal root ganglion neurons (N = 18). Low threshold (T) and high threshold (N/L) currents were examined individually. GBP had no effect on peak current, current at 100 ms, or on apparent current activation or inactivation (Fig. 5). Individual Ca^{2+} channel currents were reduced by the application of specific blockers. Ethosuximide (1 mM) reduced peak T current (16%, N = 3). Nifedipine (10 μM) reduced L current (30% of evoked current from V_h = −40 mV at a V_c = −10 mV, N = 3). ω-Conotoxin (10 μM) reduced N current (45% of evoked current from V_h = −90 mV at V_c = −10 mV, N = 3) (data not shown).

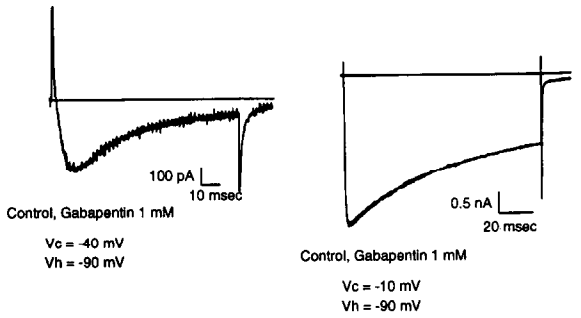

Fig. 5. GBP had no effect on low (T) (left) or high (N/L) (right) threshold Ca^{2+} channel currents in dorsal root ganglion neurons. GBP (1 mM) did not affect peak current, current at 100 ms, current activation or inactivation. V_c, clamp potential; V_h, holding potential.

4. Discussion

GBP was designed to have a steric conformation similar to GABA and to have high lipid solubility to penetrate the blood-brain barrier for potential therapeutic value as a GABA agonist. GBP has shown potent anticonvulsant activity in a variety of animal seizure models and is effective in treating human partial and generalized tonic-clonic seizures. Several types of experimental studies have been performed to determine the nature of GBP's antiepileptic effects. These studies include possible interaction with ligand- and voltage-gated ions channels. Inhibitory and excitatory amino acid receptor systems have been examined extensively without conclusive results. Fewer studies have been directed at voltage-gated ion channels yielding negative or inconclusive results.

4.1. Inhibitory amino acid systems

Early work suggested that GBP may act on GABA systems since it protected mice from tonic extension in chemical convulsion models directed at inhibition of GABA synthesis (3-mercaptopropionic acid. isonicotinic acid, semicarbazide) or inhibition at the $GABA_A$ receptor complex (bicuculline, picrotoxin) [2,4]. However, subsequent work has not clearly demonstrated a specific effect of GBP on GABAergic systems. The inhibition of monoamine release by GBP in electrically stimulated rabbit caudate nucleus [18] and rat cortex [21] was not modified by GABA, baclofen, or bicuculline suggesting that GBP's action was not at $GABA_A$ or $GABA_B$ receptors. Binding experiments in rat brain and spinal cord have shown that GBP has no significant affinity to the $GABA_A$ or $GABA_B$ binding sites measured by ^{3}H-muscimol and ^{3}H-baclofen displacement, respectively. GBP did not significantly inhibit the binding of ^{3}H-diazepam, had only a weak inhibitory effect on the GABA degrading enzyme GABA-aminotransferase, did not elevate GABA content in nerve terminals, and did not affect the GABA uptake system [3]. However, GBP has been shown to increase GABA turnover in several regions of rat brain [15] and recent work has shown that GBP binds to a novel high-affinity site in the central nervous system [12,25] and is potently displaced by the anticonvulsant 3-isobutyl GABA [27]. Additionally, GBP has been shown to be a substrate for a saturable L-amino acid transport system in rat gut tissues [24].

In electrophysiological studies, GBP did not affect depolarizations elicited by iontophoretic application of GABA on cultured mouse spinal cord neurons in the present study or previously [26] although a use-dependent reduction of GABA responses has been reported [29]. Additionally, GBP appeared to act by GABA receptor-independent mechanisms in studies with rat hippocampal slices [10] and the feline trigeminal nucleus [14]. GBP has been shown to decrease the inhibition evoked by paired-pulse orthodromic stimulation of pyramidal neurons in the hippocampal slice preparation [8,26] in a manner similar to that of phenytoin. This stimulation paradigm is used to evaluate the effect of activating inhibitory neurons via GABAergic mechanisms, however, the specific effect of GBP is not known.

GBP protected mice from convulsions caused by strychnine, a glycine receptor antagonist, but was unable to displace ^{3}H-strychnine in binding studies at the highest concentrations tested [3]. The present studies showed no effect of GBP on the response of spinal cord neurons to iontophoretically applied glycine.

4.2. Excitatory amino acid systems

GBP has been tested in animal seizure models where seizures are induced by administration of excitatory amino acids. GBP prolonged the onset

latency of clonic convulsions and tonic extension and death in mice following intraperitoneal (i.p.) injections of NMDA, but not kainic acid or quinolinic acid. GBP did not have a clear effect on convulsions when these compounds or glutamate were injected into the lateral ventricle of rats [1]. I.p. injections in mice of GBP or the NMDA receptor competitive antagonist CPP antagonized tonic seizures. The effect of GBP, but not CPP, was dose-dependently antagonized by the administration of serine, a glycine receptor agonist, suggesting an involvement of the strychnine-insensitive glycine site of the NMDA receptor in the anticonvulsant activity of GBP [17].

In unpublished studies, GBP reportedly antagonized NMDA-, but not kainate-induced depolarizations in thalamic and hippocampal slice preparations, and antagonized NMDA-induced currents in the presence of glycine in cultured striatal neurons, an effect that was reversible by the addition of serine or increased glycine [6]. The present studies did not show a significant effect of GBP on neuronal responses to iontophoretic application of glutamate and did not block membrane depolarizations and single channel currents evoked by NMDA with or without coapplication of glycine. These results, in part, are similar to the findings of others where GBP had no effect on spinal cord neuron depolarizations elicited by iontophoretically applied glutamate [26] or pressure-ejected NMDA [28]. Additionally, in extracellular recordings from rat hippocampal slice preparations, GBP had no effect on long-term potentiation making it unlike antagonists of the NMDA receptor [26]. GBP (100 μM) also did not reduce the neuronal damage that occurs following brief applications of 500 μM glutamate to rat cortical cell cultures, a paradigm where competitive, noncompetitive and glycine-site NMDA antagonists effectively block glutamate-induced cell damage (D. Rock and G. Campbell, unpublished observations). These results suggest that the anticonvulsant action of GBP is not due to an effect on excitatory amino acid receptors.

4.3. Voltage-gated channels

Sustained repetitive firing (SRF) of action potentials in neurons can be inhibited by antiepileptic drugs that block the voltage-dependent Na^+ channel [16]. GBP had no effect on SRF in the present studies or previously [26] using mouse spinal cord neurons. In preliminary experiments using the same neuronal preparation, high concentrations of GBP (100 μM) reduced SRF and following overnight exposure of the cultures to GBP, SRF was reduced by GBP (1 μM) [29]. The significance of these findings is not fully established at this time. Recent voltage clamp experiments on Chinese hamster ovary cells with stably expressed α subunits of voltage-dependent Na^+ channels suggest that GBP does not interact directly with Na^+ channels (C. Taylor, personal communication).

Voltage-dependent Ca^{2+} channel currents are involved in numerous physiologic functions of the neuron. L currents are involved in the presynaptic release of neurotransmitters, N currents may be primary regulators of the inhibition of release of neurotransmitters or neuromodulators, and T currents are probably responsible for the low threshold Ca^{2+} spike that underlies some neuronal oscillatory bursting behavior. T currents may also be involved in the generation of thalamocortical rhythms such as sleep spindles, and possibly, the abnormal electroencephalographic 3 Hz spike and wave rhythm seen in human absence seizures. Although GBP is most effective in the treatment of human partial and generalized tonic-clonic seizures, the effect of GBP on absence seizures has been studied both in animal models of absence seizures and as add-on therapy in drug-resistant epileptic patients. In animal studies using pentylenetetrazol-induced clonic seizures, GBP protected mice from clonic convulsions in both the s.c. Metrazol test (ED_{50} 147 mg/kg) and the i.v. threshold test (120% control, 30–50 mg/kg) [3]. However, in a rat genetic model of absence epilepsy, GBP increased electroencephalographic spike and wave bursts in a dose-dependent manner at 25 and 100 mg/kg [9]. In studies of mouse spinal cord neurons, GBP blocked responses to Bay K 8644, an agonist at the dihydropyridine binding site of the L channel [28]. In human studies, GBP reduced $>50\%$ of absence seizures in half of the patients in one study [5], and in another study, GBP reduced absence seizures and generalized spike and wave complexes in patients undergoing 24 h EEG monitoring [20].

In the present studies, GBP did not affect any component of the Ca^{2+} channel current subtype studied (T, L, or N). Since GBP did not affect residual current following use of both nifedipine and ω-conotoxin, there was no evidence to suggest that GBP might be capable of blocking P-type Ca^{2+} channel current. These results suggest that GBP's basic mechanism of action was not on voltage-dependent Ca^{2+} channels.

In summary, the results of the present studies do not demonstrate a major effect of GBP on any of the several different experimental systems tested, even when tested at concentrations well above plasma concentrations which commonly range $\sim$12–88 μM in clinical studies [6]. Specifically, the studies do not show significant effects of GBP on neuronal responses to applied GABA, glycine, or glutamate. At high concentrations, GBP shows a variable enhancement of NMDA responses, however, GBP produces no reductions in NMDA responses under the conditions tested. GBP does not show effects on sustained repetitive firing of Na^+ channel action potentials or on voltage-dependent Ca^{2+} channel currents. Each of the experimental systems tested specific voltage- or ligand-gated responses that previous animal and/or human studies suggested as potentially important in the mechanism of action of GBP. Our results fail to corroborate the results of previous investigations other than those which were similar in design and yielded negative results. The mechanism of action of GBP on neurons involved in the generation of seizure activity remains to be determined.

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