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Acute effects of gabapentin and pregabalin on rat forebrain cellular GABA, glutamate, and glutamine concentrations

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The effects of antiepileptic drugs, gabapentin, pregabalin and vigabatrin, on brain gamma-aminobutyric acid (GABA), glutamate and glutamine concentrations were studied in Long Evans rats using proton magnetic resonance spectroscopy (MRS) of perchloric acid extracts. Cellular glutamate concentrations significantly decreased by 7% ($P < 0.05$) 2 hours after intraperitoneal injection of 100 mg/kg gabapentin and 4% ($P < 0.05$) with 1000 mg/kg. No differences were observed in cellular GABA and cellular glutamine concentrations in rats treated with gabapentin. Pregabalin, an analogue of gabapentin, significantly decreased cellular glutamate concentrations by 4% ($P < 0.05$), while no effect was observed on cellular GABA or glutamine concentrations in the healthy rat forebrain. Vigabatrin, used as a positive control to increase GABA levels, produced a 50% increase in cellular GABA compared to saline treated rats ($P < 0.003$). Although, gabapentin and pregabalin are anticonvulsants designed to mimic GABA, these drugs do not raise cellular GABA levels acutely but modestly decreased cellular glutamate levels in our healthy rat forebrain model.

Key words: gabapentin; pregabalin; glutamate; GABA; MRS; brain.

INTRODUCTION

The majority of excitatory neurons in the human neocortex release glutamate as their primary neurotransmitter[1]. The glutamate content of brain primarily reflects the glutamate concentration in glutamatergic neurons, whose glutamate content is far greater than non-glutamatergic neurons or glia[2,3]. Enhanced glutamatergic activity is coupled tightly to increased neuron-glia cycling and cerebral energy metabolism[4–7]. Opposing the action of glutamate is GABA, the major inhibitory neurotransmitter in the adult human cortex[1,8]. Because glutamate is its precursor, GABA metabolism is coupled tightly to neuron-glia cycling and cerebral energy metabolism[9–12].

Gabapentin, pregabalin, and vigabatrin are three new antiepileptic drugs (AEDs) originally designed to target the GABAergic system[13,14]. Vigabatrin is a well-characterised vinyl analogue of GABA that irreversibly inhibits GABA-transaminase resulting in a significant increase in GABA levels in both human and rodents[15,16]. The first oral dose of vigabatrin (50 mg/kg) doubles cellular GABA concentrations within 2 hours when used by patients with refractory localisation-related epilepsies[17]. Daily dosing appears to increase cortical glutamine and lower glutamate content[17,18]. Gabapentin and pregabalin are lipophilic analogues of GABA, whose anticonvulsant and analgesic mechanisms of action remain unclear[13,19–21]. The first dose of gabapentin (20 mg/kg) increases cellular GABA within 2 hours when given orally to human subjects[22,23]. Limited studies measuring cortical glutamate and glutamine in epilepsy patients taking gabapentin daily suggest changes would be modest at best[12]. Similar studies to determine the effects of pregabalin on GABA levels in epilepsy patients have yet to be reported.

In animal models, only a few studies have investigated the effects of gabapentin on cortical amino acid neurotransmitter levels. Gabapentin (5–75 mg/kg) fails to increase forebrain GABA concentration[24]. However, in the presence of a GABA catabolism

inhibitor, gabapentin (23 mg/kg) administration results in regional increases in brain GABA synthesis[25]. In addition, acute administration of gabapentin (50 mg/kg) results in an increase in glutamine concentration, whereas, chronic gabapentin administration produces a decrease in glutamate[24]. To date, the role of pregabalin on brain amino acid neurotransmitter concentrations has yet to be examined fully.

Our study investigates the acute effects of gabapentin and pregabalin on rat brain GABA, glutamate and glutamine concentrations to further understand the mechanisms of these antiepileptic drugs. Vigabatrin serves as a positive control because it reliably increases cellular GABA levels in humans and rodents[15,16].

EXPERIMENTAL PROCEDURES

Animal protocol

Male Long Evans rats (Charles River, Wilmington, MA, USA) weighing 190–220 g received an intraperitoneal (i.p.) injection of either gabapentin, pregabalin-active optical isomer, pregabalin-inactive optical isomer (Parke-Davis, Ann Arbor, MI, USA), vigabatrin (500 mg/kg; $n = 8$) or vehicle ($n = 56$; physiological saline, Butler, Columbus, OH, USA). Gabapentin was administered at 100 mg/kg ($n = 10$) and 1000 mg/kg ($n = 16$). Pregabalin has two chiral isomers; only one optical isomer has anticonvulsant properties (pregabalin-active). Pregabalin-active was given at 50 mg/kg ($n = 12$), 250 mg/kg ($n = 12$) and 500 mg/kg ($n = 8$). Pregabalin-inactive was given at 50 mg/kg ($n = 12$) and 250 mg/kg ($n = 12$). At the time of the injections, the experimenters did not know which enantiomer was biologically active. Vigabatrin, a well-characterised GABA-transaminase inhibitor, was used as a positive control to increase cellular GABA[15,16].

Post injection, animals had free access to food and water. Two hours after drug administration, animals received an i.p. injection of 100 mg/kg pentobarbital followed by an i.p. injection of 0.4 ml 2N 3-mercaptopropionic acid (3-MPA; Sigma Chemicals, St. Louis, MO, USA). Four minutes after 3-MPA injection[26,27], animals were decapitated and brains were quickly removed (<90 seconds) rostral to the cerebellum and frozen in liquid nitrogen. Blood was collected after decapitation from rats that received 1000 mg/kg gabapentin rats ($n = 8$) and 500 mg/kg pregabalin ($n = 8$). The 2-hour time period was chosen based on the observation that maximal gabapentin concentrations in brain were achieved within 1 hour and maximal protection from electroshock seizures occurred 2 hours following administration of gabapentin[28,29]. The Yale Animal Care and Use Committee approved the protocol. Rats were purchased through and maintained by Yale Animal Resource Centre.

Extraction method

Frozen brains were extracted in 3.5 ml cold 12% perchloric acid (PCA) stock solution containing 7.7 mM dichloracetic acid (Sigma Chemicals) and centrifuged at 3200 × *g* for 15 minutes at 4 °C. The supernatant was brought to a neutral pH with a solution containing 4.8 M KOH and 0.3 M K_2HPO_4 and centrifuged at 3200 × *g* for 10 minutes at 4 °C. The neutral solution was treated with 0.5 g chelating resin (Sigma Chemicals), filtered, and lyophilised. The dried powder was dissolved in neutral 50 mM deuterated phosphate in D_2O containing 2 mM isopropanol as previously described[30]. Dichloracetic acid was added to the extraction procedure as a concentration standard and chemical shift reference. Blood specimens were extracted as above with 1:1 ratio of blood to PCA solution.

Spectroscopy and data analysis

High resolution proton magnetic resonance spectroscopy (MRS) was performed on a Brucker AM-500 (Billerica, MA, USA) analytical spectrometer at 500 MHz and 22 °C using a 30° pulse width (5 microseconds), 6024 Hz sweep width, 32K digital resolution, and low power presaturation. NUTS 1D (Acorn NMK Inc., Fremont, CA, USA) was used to process and measure proton MR spectra. Fig. 1 shows representative proton MR spectra of extracted from forebrains of rats treated with gabapentin, pregabalin, vigabatrin, and saline. Metabolite concentrations in an individual animal were determined using the creatine signal at 3.0 ppm that was assigned the average absolute value of total creatine (creatine plus phosphocreatine) measured for all animals (12.2 mM). Student *t*-test was used to determine significant differences with Bonferroni correction for multiple comparisons when needed.

RESULTS

Cellular glutamate concentrations decreased significantly ($P < 0.005$) in gabapentin-treated rats (Table 1) and this decrease was significant ($P < 0.05$) at both doses of gabapentin. Overall, the pregabalin-active group showed a 4% decrease ($P < 0.05$) of cellular glutamate concentration compared to pregabalin-inactive treated animals. Compared to

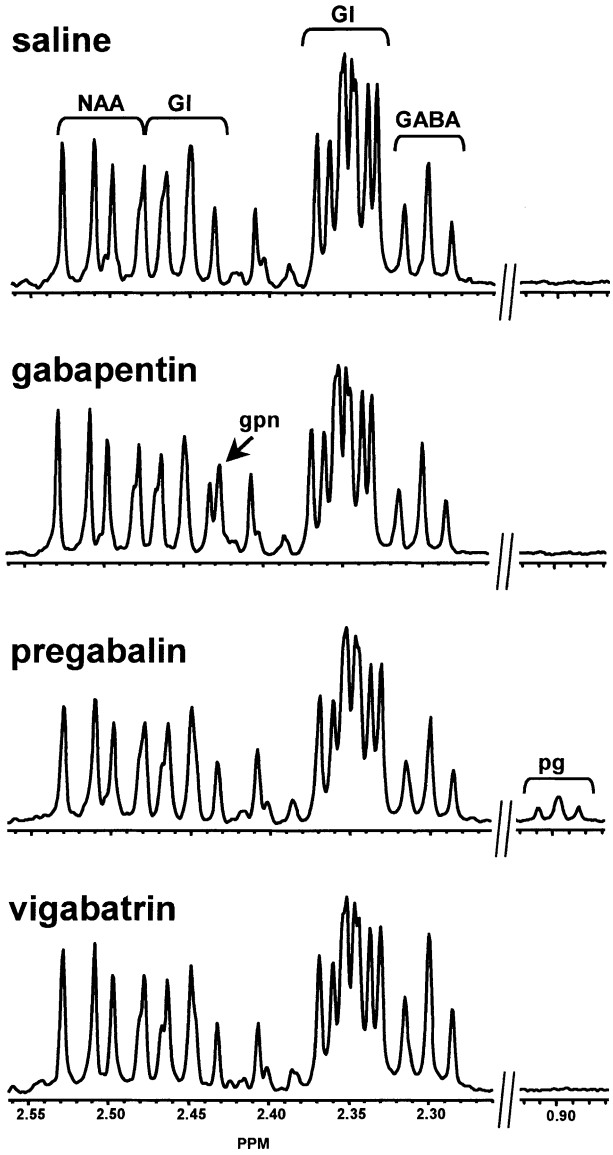

Fig. 1: Representative proton MR spectra of extract from forebrains of rats treated with gabapentin (1000 mg/kg), pregabalin (500 mg/kg), vigabatrin (500 mg/kg) and saline highlight the ability of MRS to measure the forebrain concentrations of antiepileptic drugs. The concentration of gabapentin in brain extracts (0.7 mM; SEM, 0.2) was 70% of the levels found in blood (0.9 mM; SEM, 0.2). In contrast only 30% of the pregabalin levels measured in the blood (0.90 mM; SEM, 0.04) were observed in the brain (0.34 mM; SEM, 0.02). NAA: *N*-acetylaspartate; Gln: glutamine; Glt: glutamate; GABA: gamma-aminobutyric acid; gpn: gabapentin; pgn: pregabalin.

Table 1: Acute effects of gabapentin, pregabalin and vigabatrin on GABA, glutamate and glutamine concentrations in the rat brain.

	GABA	Glutamate	Glutamine
Saline control ($n = 56$)	2.36 ± 0.03	13.1 ± 0.1	3.7 ± 0.1
Vigabatrin ($n = 8$)	3.51 ± 0.18*	12.6 ± 0.4	3.6 ± 0.2
Gabapentin ($n = 26$)	2.42 ± 0.03	12.4 ± 0.2*	3.6 ± 0.1
Pregabalin-active ($n = 32$)	2.41 ± 0.04	12.5 ± 0.2*	3.6 ± 0.1
Pregabalin-inactive ($n = 24$)	2.37 ± 0.03	13.1 ± 0.2	3.7 ± 0.1

Values represent the mean concentrations (mM) with ± SEM.
* $P < 0.05$ versus saline control using the Student *t*-test.

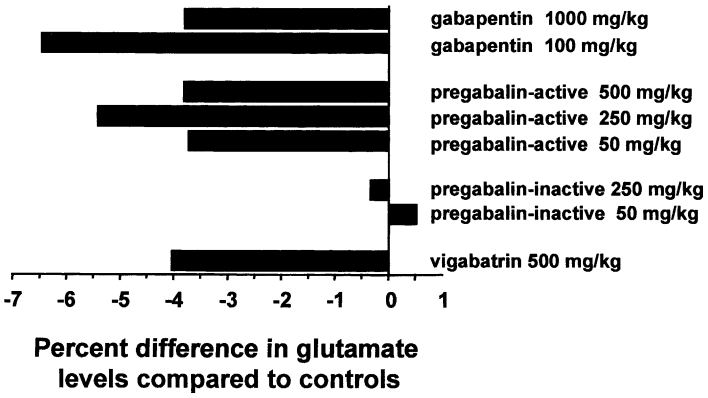

Fig. 2: Comparison of acute antiepileptic drug (AED) treatment to percent difference of glutamate concentrations when compared to controls.

saline treated controls, the pregabalin-active group showed a significant decrease ($P < 0.05$, corrected for a three-way comparison) in cellular glutamate levels (Table 1). The level of significance at individual dosages for rats treated with 50 and 250 mg/kg pregabalin-active showed a similar decrease in cellular glutamate levels when compared to saline controls (Fig. 2). None of the AEDs examined had any significant effect on cellular glutamine levels measured when compared to saline treated rats (Table 1).

Gabapentin and pregabalin, at low and high doses, failed to increase cellular GABA concentration in rat forebrain after acute drug treatment, whereas, the control AED, vigabatrin showed a 45% increase in GABA (Table 1).

DISCUSSION

Gabapentin administration results in a significant though modest decrease in forebrain cellular glutamate levels in our model, without significant changes in cellular GABA or glutamine content. A ten-fold increase in dose did not magnify the effect. Gabapentin crosses the blood–brain barrier by a transporter-mediated mechanism that appears to be saturated at a dose of 100 mg/kg[31]. Forebrain gabapentin content (0.7 mM; SEM, 0.2) achieves 70% of the concentration in the blood, measured 2 hours after intraperitoneal injection of 1000 mg/kg; a dose that should saturate gabapentin transport. At this concentration, gabapentin would be expected to inhibit the system-L branched-chain-amino acid (BCAA) transporter (K_i, 0.03–0.18 mM) competitively and enhance GABA release through reversal of the GABA-uptake transporters (0.05–0.1 mM)[32–34].

Gabapentin modulates activities of enzymes and transporters that affect glutamate metabolism decreasing glutamate and glutamine content in several model systems[35–38]. Glutamatergic neurotransmission is highly dependent on the net synthesis of glutamate by neurons[3,6]. Neurotransmitter release of glutamate results in the obligate loss of tricarboxylic acid (TCA) cycle intermediates because neurons lack sufficient enzymatic capacity to resynthesise glutamate lost through neurotransmission from glucose without depleting mitochondrial stores of TCA cycle intermediates[7,12,39]. A large fraction of the glutamate released by neurons is taken up by surrounding astrocytes and converted to glutamine, which is released by system-L and system-N transporters[40]. Transported into neurons by system-A and system-L, glutamine is deaminated by phosphate activated glutaminase, which serves as the main neuronal enzyme for the net synthesis of glutamate[7,12,41]. The complete pathway is called the glutamine–glutamate cycle (Fig. 3). Anaesthesia with chloralose or pentobarbital-coma slows the glutamine–glutamate cycle significantly and decreases cortical glutamate content by 16–28%[42].

The glutamine–glutamate cycle is coupled tightly with ammonia detoxification[43,44]. Neuronal glutamate dehydrogenase (GDH) can detoxify ammonia by combining it with alpha-ketoglutarate (α-KG), a critical TCA cycle intermediate, to form glutamate. The branched-chain-amino-acid cycle ferries the ammonia from neurons to glia in a non-toxic form and regenerates α-KG, avoiding depletion of neuronal TCA cycle intermediates[36,38,45]. Interfering with the uptake of leucine has a major impact on ammonia detoxification and would be expected to lower cellular glutamate concentrations (Fig. 3).

Gabapentin can modulate ammonia handling, and thus, affect the glutamine–glutamate cycle in several ways[21,32,35,38,46]. It is a competitive inhibitor of system-L (leucine) transporters, thereby decreasing the branched-chain-amino-acid content of astrocytes and potentially glial glutamate needed for efficient

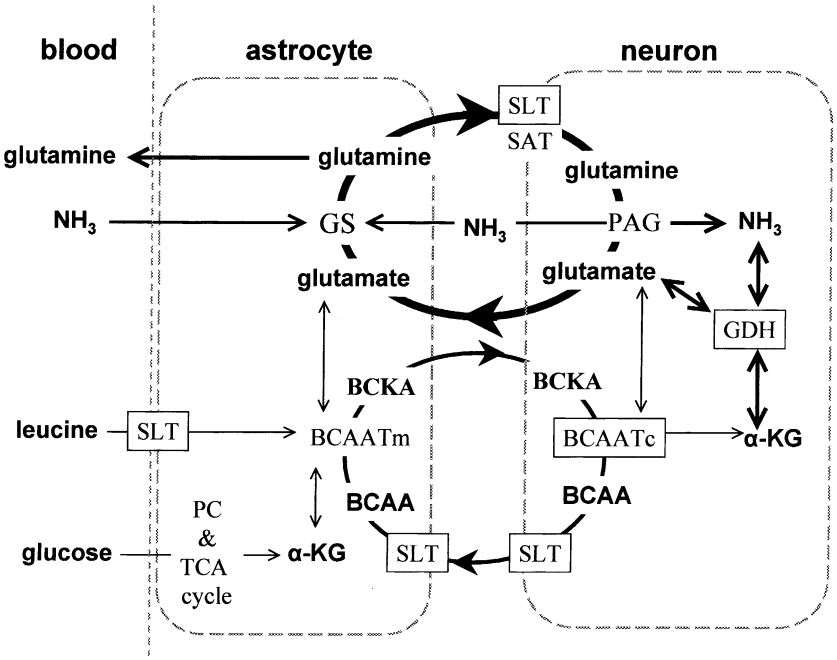

Fig. 3: Schematic of the glutamine–glutamate cycle and branched-chain-amino-acid cycle. In order to detoxify ammonia (NH_3) entering the brain from the blood, glia synthesise glutamate *de novo* from glucose using pyruvate carboxylase (PC), the TCA cycle, and the mitochondrial form of branched-chain-amino-acid transaminase (BCAA-T). Ammonia entering from the blood or released by cerebral metabolism is detoxified by glutamine synthetase (GS). Astrocytes release glutamine into the blood or extracellular space using primarily system-N, but also system-L (SLT), transporters. Neurons take up glutamine by the system-A (SAT) transporter and SLT systems to replace glutamate lost through glutamatergic neurotransmission (glutamine–glutamate cycle), GABA synthesis, or oxidation. The mitochondrial enzyme, phosphate activated glutaminase (PAG), converts glutamine to glutamate and ammonia. Ammonia strongly inhibits PAG, thereby decreasing neuronal glutamate synthesis. Ammonia released by neuronal metabolism can diffuse into the glia to be detoxified by GS. Alternatively, neuronal GDH can detoxify ammonia by combining it with α-KG to form additional glutamate at the expense of depleting neuronal TCA cycle intermediates. The branched-chain-amino-acid cycle ferries the ammonia from neurons to glia in a non-toxic form and regenerates α-KG, avoiding depletion of neuronal TCA cycle intermediates. Gabapentin slows the branched-chain-amino-acid cycle by inhibiting both the neuronal form of BCAA-T and SLT, responsible for the neuronal release of leucine and valine and their glial uptake. Gabapentin appears to activate GDH, both to detoxify and release ammonia, which would make the equilibrium more dependent on the availability of α-KG. Low levels of α-KG would lead to increasing neuronal ammonia levels and inhibition of PAG, thereby decreasing neuronal glutamate concentrations.

ammonia detoxification[44]. Similarly, gabapentin would disrupt the branched-chain-amino-acid cycle between neurons and glia by inhibiting transporter mediated leucine release from neurons and astrocytic uptake (Fig. 3). Gabapentin appears to affect glutamine transport, promoting the release of glutamine from astrocytes[35]. In neurons, it could potentially slow glutamine uptake.

At the brain concentrations (0.7 mM with 1000 mg/kg) achieved in our model, gabapentin would be expected to inhibit the neuronal form of branched-chain-amino-acid transaminase (BCAA-T; K_i 0.8 mM for leucine, 0.7 mM for ketoisovalerate), further disrupting the branched-chain-amino-acid cycle[35]. Gabapentin significantly impairs glutamate and glutamine synthesis in a perfused rat retina model at concentrations of 0.2–1.0 mM, thereby decreasing glutamate content[36–38]. The largest impact was on the *de novo* synthesis of glutamate from glucose through the anaplerotic (pyruvate carboxylase) pathway.

Gabapentin has no significant effect on glutamine synthetase[47] or glutaminase[48]. We observe a minimal decrease in glutamine (0.1 mM, ~3%) that failed to reach the 0.05 level of statistical significance. At higher concentrations, gabapentin inhibits GABA degradation by GABA-transaminase (K_i, 17–20 mM) and stimulates glutamic acid decarboxylase (at 1–3 mM)[25, 48–50]. A minor increase in cellular GABA (0.06 mM, ~3%) is observed, but failed to reach significance.

Pregabalin is the isobutyl analogue of gabapentin designed to circumvent in part the limitations in absorption and transport that appear to limit the bioavailability of gabapentin[13, 19, 50, 51]. Administration of pregabalin-active, the enantiomer with antiepileptic properties, results in a significant though very modest decrease (0.6 mM, ~4%) in forebrain cellular glutamate levels. The pregabalin enantiomer lacking antiepileptic activity has no apparent effect. At the forebrain concentrations (0.34 mM with 500 mg/kg)

achieved in our study, pregabalin-active would be expected to affect potassium-evoked glutamate release, which is decreased by 11–26% in hippocampal and neocortical slices with the application of 0.1 mM pregabalin-active[52]. Pregabalin-active is reported to be a weaker inhibitor of BCAA-T (K_i, 0.84) than gabapentin and would not be expected to have significant effects at concentrations achieved in our animals[50]. Acute administration of pregabalin-active (2.5 mM) enhances GAD activity by 43% in a concentration dependent manner that could lead to the increase production of GABA resulting in the anticonvulsant effect observed in animal models[51]. As with gabapentin, pregabalin-active appears to be associated with a minimal (0.1 mM, ~4%) decrease in glutamine. There is no change in glutamine with pregabalin-inactive. In our model, pregabalin does not increase cellular GABA content significantly (pregabalin-active increases GABA by 2% and pregabalin-inactive has no effect). Recently, Whitworth and Quick report that pregabalin, like gabapentin, promotes GABA release through reversal of the GABA-transporter (GAT1) at concentrations (25–100 μM) grossly exceeded in our animals[34]. Overall, the effects of pregabalin-active appear similar to those of gabapentin, whereas values of animals treated with pregabalin-inactive are identical to saline controls.

CONCLUSION

Gabapentin and the isoform of pregabalin with antiepileptic effects significantly decreased cellular glutamate by 0.6–0.9 mM (4–7%) in healthy rat forebrain. Whether the decrease in glutamate content is proconvulsant or anticonvulsant appears to depends on conditions of the model studied[53]. Although the decrease was modest, gabapentin and pregabalin could reverse in part the increase in cortical glutamate associated with the epileptic state[12,54,55]. The human and rodent GABA metabolism differ in their response to antiepileptic drugs[23,56].

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