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Metabolomic analyses of vigabatrin (VGB)-treated mice: GABA-transaminase inhibition significantly alters amino acid profiles in murine neural and non-neural tissues

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ABSTRACT

The anticonvulsant vigabatrin (VGB; Sabril[R]) irreversibly inhibits GABA transaminase to increase neural GABA, yet its mechanism of retinal toxicity remains unclear. VGB is suggested to alter several amino acids, including homocarnosine, β-alanine, ornithine, glycine, taurine, and 2-aminoadipic acid (AADA), the latter a homologue of glutamic acid. Here, we evaluate the effect of VGB on amino acid concentrations in mice, employing a continuous VGB infusion (subcutaneously implanted osmotic minipumps), dose-escalation paradigm (35–140 mg/kg/d, 12 days), and amino acid quantitation in eye, visual and prefrontal cortex, total brain, liver and plasma. We hypothesized that continuous VGB dosing would reveal numerous hitherto undescribed amino acid disturbances. Consistent amino acid elevations across tissues included GABA, β-alanine, carnosine, ornithine and AADA, as well as neuroactive aspartic and glutamic acids, serine and glycine. Maximal increase of AADA in eye occurred at 35 mg/kg/d (41 ± 2 nmol/g (n = 21, vehicle) to 60 ± 8.5 (n = 8)), and at 70 mg/kg/d for brain (97 ± 6 (n = 21) to 145 ± 6 (n = 6)), visual cortex (128 ± 6 to 215 ± 19) and prefrontal cortex (124 ± 11 to 200 ± 13; mean ± SEM; $p < 0.05$), the first demonstration of tissue AADA accumulation with VGB in mammal. VGB effects on basic amino acids, including guanidino-species, suggested the capacity of VGB to alter urea cycle function and nitrogen disposal. The known toxicity of AADA in retinal glial cells highlights new avenues for assessing VGB retinal toxicity and other off-target effects.

1. Introduction

Anticonvulsants represent diverse pharmaceuticals whose therapeutic goal is the suppression of excessive neuronal firing during seizure (Rogawski and Bazil, 2008). Further, many anticonvulsants possess the potential to decrease the spread of seizure within the brain (Kaminski et al., 2014). Typically, anticonvulsant drugs will block voltage-gated sodium and calcium channels with the ultimate objective of reducing the release of excitatory glutamic acid (Fekete et al., 2009). However, other targets for anticonvulsants can include enhancement of the inhibitory GABAergic system, including $GABA_A$ receptors and GABA transporters (Treven et al., 2015). Nonetheless, the exact mechanism of action for many anticonvulsant agents remains unknown, although their efficacy is appreciated (e.g., topiramate) (Palpacuer et al., 2018).

The mechanism of action of vigabatrin (VGB; 4-aminohex-5-enoic acid; Sabril[R], Fig. 1), directly impacting the GABA metabolic pathway by irreversible inhibition of GABA-transaminase (GABA-T; Rey et al., 1990) (Fig. 1), is unique among antiepileptic drugs. VGB is a first-line

Abbreviations: GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyrate transaminase; β-ala, β-alanine; VGB, vigabatrin; gly, glycine; ala, alanine; ser, serine; cys, cysteine; lys, lysine; cit, citrulline; PEA, phosphoethanolamine; EA, ethanolamine; his, histidine; asp, aspartic acid; glu, glutamic acid; orn, ornithine; arg, arginine; thr, threonine; gln, glutamine; carnosine, carn; AADA, 2-aminoadipic acid; 4-GBA, 4-guanidinobutyric acid; VC, visual cortex; PFC, prefrontal cortex; LNAA, large neutral amino acid

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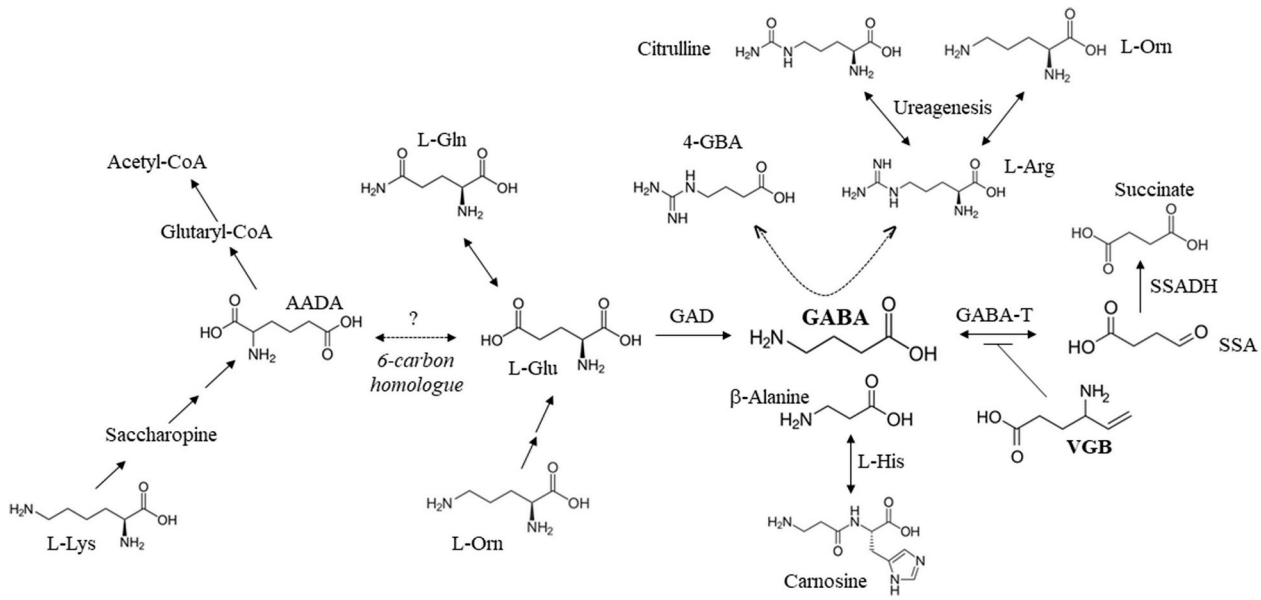

Fig. 1. Metabolic interrelationships of GABA metabolism. The major inhibitory neurotransmitter, GABA is produced from glutamic acid (which can interconvert with glutamine (gln)) in a reaction catalyzed by glutamic acid decarboxylase (GAD). Its catabolism is catalyzed by two sequential steps, the first GABA-transaminase (GABA-T), which produces succinic semialdehyde (SSA) that is subsequently oxidized to succinic acid by succinic semialdehyde dehydrogenase (SSADH). GABA-T is the target of vigabatrin (VGB) which results in its irreversible inactivation. A number of intermediates shown to be dysregulated by chronic VGB administration in mice (current study) are also depicted. Carnosine is the L-histidine dipeptide of β-alanine. The production of the GABA analogue, 4-guanidinobutyrate (4-GBA; not measured in this study but shown to be increased in patients with SSADH deficiency (Jansen et al., 2006)), is postulated to derive from condensation of GABA with arginine (L-arg) catalyzed by arginine amidinotransferase in the pathway of creatine metabolism (Walters et al., 2019). The cycle of ureagenesis encompasses arginine, citrulline (cit) and ornithine (orn). As well, there is non-mammalian evidence that ornithine can produce glutamic acid in a multistep reaction sequence (Brosnan, 2000). The catabolism of lysine (lys) generates 2-aminoadipic acid (AADA) through the pathway of saccharopine metabolism, ultimately generating glutaryl-CoA and eventually acetyl-CoA. AADA is a 6-carbon homologue of glutamic acid, and its exact mechanism of generation during VGB administration, is unknown.

intervention in infantile spasms (Hussain et al., 2018), and it is employed in other convulsive disorders including refractive complex partial seizures, treatment resistant epilepsy, secondary generalized seizures, and others (van der Poest Clement et al., 2018). Whereas VGB has been in use in Europe and Canada for decades, it was not approved in the United States until ~2009, primarily related to reports of visual field defects (Riikonen et al., 2015). The mechanism by which VGB selectively affects the visual system has not been clearly defined (Shields and Pellock, 2011; Tuğcu et al., 2017; Peng et al., 2017; Yang et al., 2012), while other studies suggest that VGB does not display an increased association with visual field abnormalities (Schwarz et al., 2016). More recently, VGB has been associated with white matter spongiosis and restricted globus pallidus diffusion (Pearl et al., 2018; Trindade et al., 2018).

As for any therapeutic, VGB might manifest both on- and off-target toxicity. An example of the former includes VGB's capacity to elevate GABA, homocarnosine, β-alanine, and potentially 4-guanidinobutyrate (Fig. 1; Grove et al., 1981; Schechter, 1989; Jansen et al., 2006, 2008), the latter a derivate of arginine and GABA. All of these intermediates are predicted to arise via GABA-T inhibition, and their role, if any, in the ocular toxicity of VGB remains unknown (Hosking and Hilton, 2002; Vogel et al., 2017a, 2017b). As well, VGB use may associate with a number of amino acid alterations, including ornithine, glycine, taurine and the glutamate homologue, 2-aminoadipic acid (AADA) (Yu and Eldred, 2005; Sorri et al., 2010; Tao et al., 2016; Vallat et al., 1996). Whether these amino acid alterations are on- or off-target is also unresolved, but might be addressed through comparison of GABA-transaminase siRNA to VGB application.

The catabolic pathway of GABA to succinic acid (Fig. 1), thereby priming the Krebs cycle, occupies a central role in central nervous system metabolism. Accordingly, here we have undertaken a metabolomics approach focused on evaluating amino acid levels in mice treated with increasing levels of VGB via continuous administration. Constant dosing obviates the time-dependent parameter associated with VGB's irreversible inhibition of GABA-T ($t_{1/2}$ VGB 5–8 h), and ensures that our amino acid profiles represent "steady-state" conditions. Our central hypothesis posited that chronic VGB intervention would result in multiple amino acid alterations in eye and visual cortex (VC), potentially providing novel clues to VGB's selective ocular toxicity. As control regions, we also assessed comprehensive amino acids in prefrontal cortex (PFC), brain (post-VC and PFC excision), liver, and plasma. Refinement of the on- and off-target effects of VGB on the visual system, most likely in a cell line of ocular origin, may help to delineate pharmacotherapeutic approaches to mitigate this toxicity and significantly extend the utility of this important anticonvulsant agent.

2. Materials and methods

Preparation and purification of VGB, as well as subcutaneous infusion of VGB to mice, has been recently described (Walters et al., 2019). In brief, C57BL/6 J mice (Jackson Laboratories, Bar Harbor, ME) were bred in-house to obtain required cohort size (n = 6–8 per dose group, male only), and were 8–10 weeks of age and 20.8–26.1 g in weight. Final animal numbers for study analyses included vehicle (n = 21, representing a pool of animals across all studies), 35 mg/kg/d (n = 8), 70 mg/kg/d (n = 6) and 140 mg/kg/d (n = 8). Osmotic minipumps, model 2002 (Alzet, Cupertino, CA) were prepared 18–24 h prior to surgery. The minipumps deliver drug at a constant flow rate of 0.5 μL/h for up to 14 days. To achieve the desired delivery concentrations of 35, 70 and 140 mg/kg/d VGB (based upon an average mouse weight of 25 g), pumps were filled with vehicle (PBS) or 73, 146 and 291 mg/mL VGB solution in PBS, respectively. All procedures were approved by the Washington State University Institutional Animal Care and use Committee (Protocols ASAF 4232–42 and 6134), and consistent with the National Institutes of Health guide for the care and use of laboratory animals.

Animals were randomly assigned to vehicle or drug cohorts. Loading of pumps, surgical implantation, and duration of use (12 days)

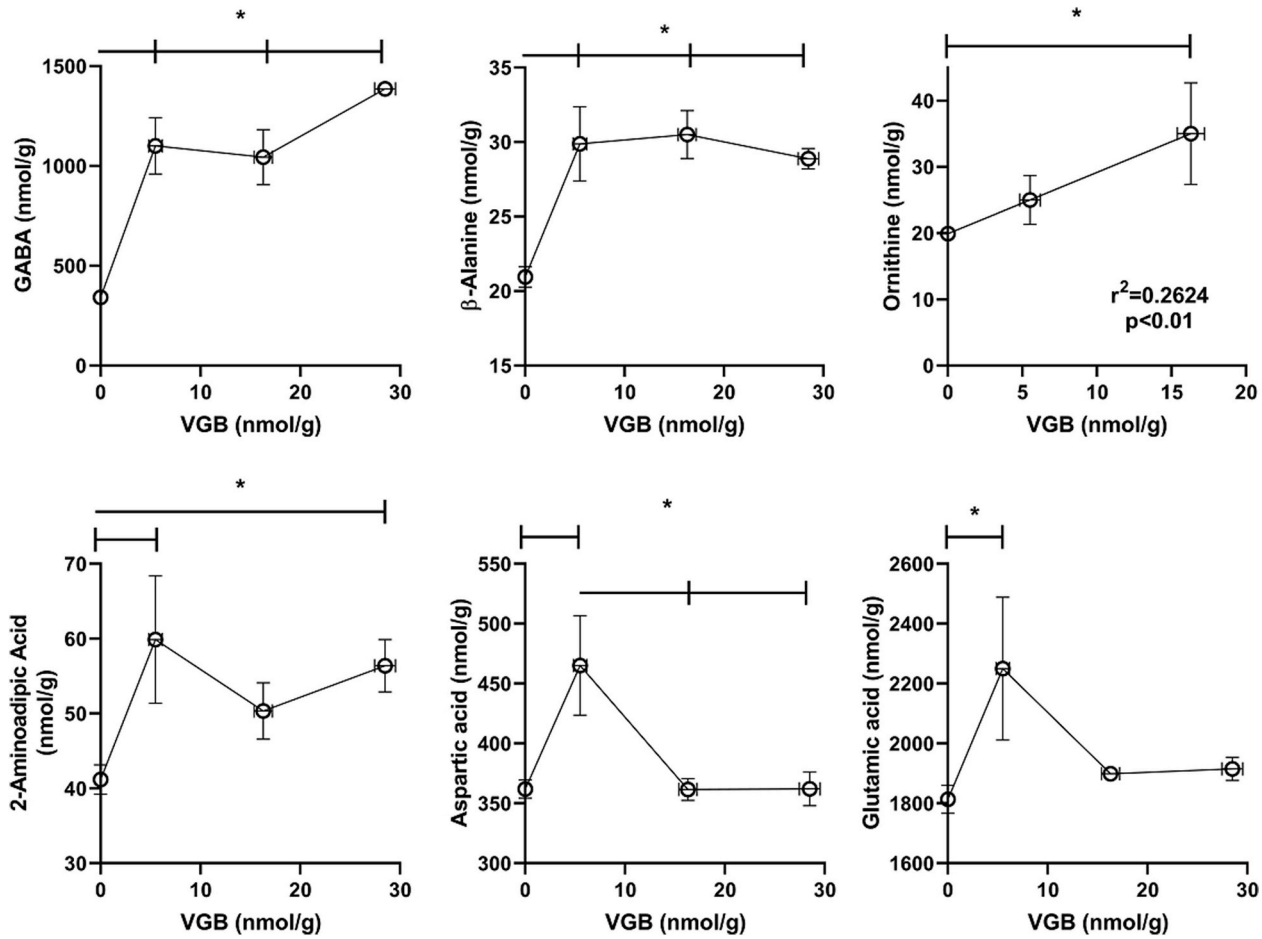

Fig. 2. Selected amino acids in eye as a function of vigabatrin dose. Amino acids included GABA, β-alanine, ornithine, 2-aminoadipic acid, aspartic and glutamic acids. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set at $p < 0.05$). For ornithine, linear regression analysis revealed a statistically significant slope ($r^2 = 0.2624$; $p < 0.01$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8). Data was not available for ornithine with high-dose VGB due to interference.

Table 1
Amino acid content in eye as a function of increasing vigabatrin administered subcutaneously.

Description	Metabolite	Vehicle	35 mg/kg/d	70 mg/kg/d	140 mg/kg/d
Drug	*Vigabatrin*	–	6 (1)	16 (1)	29 (1)
Drug-Related	Carnosine	9 (1)	nd	nd	15 (2)
Neutral Amino Acids	Glycine	575 (16)	728 (76)[a]	576 (16)	618 (19)
	Alanine	530 (12)	671 (77)[a]	576 (16)	541 (17)
	Serine	220 (7)	281 (30)[a]	250 (16)	249 (12)
	Cysteine	1.3 (1)	nd	nd	23 (1)[a]
Basic Amino Acids	Lysine	306 (12)	398 (55)[a]	300 (9)	299 (6)
	Citrulline	9 (1)	nd	nd	13 (2)[a]
Amines	Phosphoethanolamine	547 (18)	735 (92)[a]	576 (16)	591 (12)
	Ethanolamine	46 (2)	61 (7)[a]	43 (2)	44 (3)[b]

Legend: nmol/g tissue (mean ± (SEM)).
Abbreviations: nd, not detected. As an irreversible inhibitor of GABA-transaminase (GABA-T), vigabatrin is predicted to elevate both GABA and β-alanine (the latter a substrate for GABA-T). Carnosine is the L-histidine dipeptide of β-alanine. Animal numbers (n) were: n = 21 for vehicle; n = 8 for 35 mg/kg/d; n = 6 for 70 mg/kg/d; and n = 8 for 140 mg/kg/d.
[a] $p < 0.05$ compared to vehicle.
[b] $p < 0.05$ between 35 and 140 dosing.

has been described (Walters et al., 2019). Tissues isolated for analysis included intact eye, liver, prefrontal cortex (PFC, including both gray and white matter), visual cortex (VC), the remainder of the intact brain, and plasma. Retina was not isolated from intact eye for these studies. For isolation of PFC and VC, regions were identified and dissected according to the method of Spijker (2011), employing the stereotactic coordinates outlined by Paxinos and Franklin (2012). Blood was collected by cardiac puncture using a heparanized syringe, and plasma obtained following low-speed centrifugation. Following sacrifice, tissues were rapidly removed onto an ice-cold glass plate, washed with ice-cold PBS, weighed and flash-frozen in liquid nitrogen. Tissues were stored at − 80 °C until analysis. For quantitative amino acid analysis, tissues were homogenized on ice in 0.1 M perchloric acid, followed by sonication and centrifugation to remove cellular debris. Amino acids

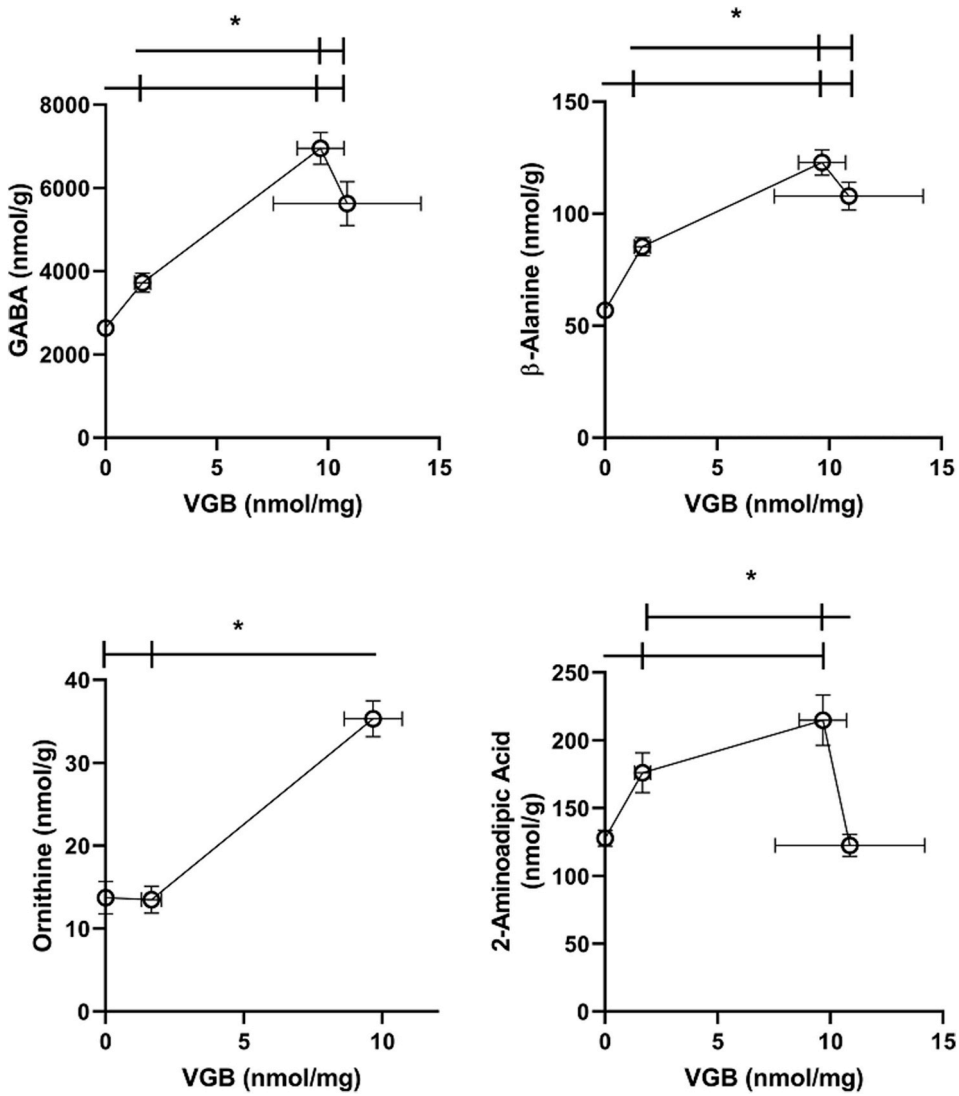

Fig. 3. Selected amino acids in visual cortex as a function of vigabatrin dose. Amino acids shown included GABA, β-alanine, ornithine and 2-aminoadipic acid. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set at $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

were quantified by UPLC (Waters UPLC® MassTrak™ Amino Acid Analysis (AAA) Solution) as described (Vogel et al. 2017c). VGB was quantified by liquid-chromatography tandem mass spectrometry employing $^{2}H_4$-gabapentin as described (Walters et al., 2019). GraphPad Prism (version 8.0; San Diego, CA) was used for data and statistical analyses, the latter using one-way ANOVA with post-hoc analysis and significance set at $p < 0.05$.

3. Results

3.1. Eye

VGB eye levels increased with dose in an approximately linear fashion. Amino acids whose tissue concentration in eye was impacted by VGB are displayed in Fig. 2 and Table 1. For GABA and β-ala, increasing VGB levels essentially revealed saturation, while ornithine (orn) and 2-aminoadipic acid (AADA) showed roughly linear increases with VGB dose (orn data not available for the high VGB dose due to interference). Excitatory amino acids, including aspartic and glutamic acids (asp, glu) only revealed significant differences at the lowest VGB dose. This low-dose effect was also observed for other dysregulated amino acids, including glycine (gly), alanine (ala), serine (ser), lysine (lys), phosphoethanolamine (PEA) and ethanolamine (EA). Conversely, both cysteine (cys) and citrulline (cit) only demonstrated increased content with the highest dose of VGB (Table 1). All other amino acids (total of 27 analytes quantified) were unaffected by VGB administration (data not shown).

3.2. Visual cortex

VGB visual cortex levels increased with dose, plateauing between 70 and 140 mg/kg/d. Amino acids whose tissue concentration in visual cortex (VC) was impacted by VGB are displayed in Fig. 3 and Table 2. As for eye, the content of GABA and β-ala revealed saturation with VGB dose, and a waning effect with high-dose VGB. Similar effects were observed for AADA, but the high-dose effect was even more pronounced (Fig. 3) and returned AADA levels to that seen with vehicle. Levels of carnosine (carn), the dipeptide of β-ala and L-histidine (his), revealed increased levels in VC with higher dose VGB. For orn (Fig. 3), as well as threonine (thr), gly and ser, maximal levels were observed only at intermediate (70 mg/kg/d) dosing (Table 2). Conversely, the effect on

Table 2
Amino acid content in visual cortex as a function of increasing vigabatrin administered subcutaneously.

Description	Metabolite	Vehicle	35 mg/kg/d	70 mg/kg/d	140 mg/kg/d
Drug	*Vigabatrin*	–	1.7 (0.4)	9.7 (1.0)	10.9 (3.3)
Drug-Related	Carnosine	55 (3)	65 (3)	76 (5)[a]	78 (4)[a]
Neutral Amino Acids	Glycine	1331 (42)	1443 (68)[b]	1825 (69)[a]	1337 (65)[c]
	Alanine	1075 (93)	911 (42)	1876 (54)[d]	874 (34)
	Serine	1111 (31)	1059 (42)	1026 (25)	954 (44)[a]
	Threonine	475 (34)	478 (39)	619 (45)[c]	373 (43)
Basic Amino Acids	Arginine	152 (7)	126 (6)[e]	157 (8)	169 (14)

Legend: nmol/g tissue (mean ± (SEM)).
[a] $p < 0.05$ compared to vehicle.
[b] $p < 0.05$ between 35 and 140 dosing.
[c] $p < 0.05$ between 70 and 140 dosing.
[d] $p < 0.05$ compared to all other dosing (including vehicle).
[e] $p < 0.05$ between 35 and 140 dosing. As an irreversible inhibitor of GABA-transaminase (GABA-T), vigabatrin is predicted to elevate both GABA and β-alanine (the latter a substrate for GABA-T). Carnosine is the L-histidine dipeptide of β-alanine. Animal numbers (n) were: n = 21 for vehicle; n = 8 for 35 mg/kg/d; n = 6 for 70 mg/kg/d; and n = 8 for 140 mg/kg/d.

serine was a significant decrease at the high-dose (140 mg/kg/d; Table 2). A similar decrease was observed for arginine (arg) with low dose (35 mg/kg/d; Table 2). All other amino acids were unaffected by VGB administration (data not shown).

3.3. Prefrontal cortex

VGB prefrontal cortext levels increased with dose in an approximate linear fashion. For prefrontal cortex (PFC), every amino acid evaluated was impacted by VGB administration. For amino acids predicted to be impacted by VGB (GABA, β-ala, carn), concentrations revealed approximately linear increases with VGB administration until high-dose, at which time contents returned roughly to the level of vehicle (Figs. 4 and 5 and Table 3). A similar effect was observed for taurine (tau), AADA, glutamic acid (glu), lys and gly (Figs. 4 and 5). For aspartic acid (asp) and ser, the content in PFC with low-dose VGB was not significantly different from vehicle, but was decreased with further VGB increase, and a similar high-dose effect with VGB was observed for phosphoethanolamine (PEA) and ethanolamine (EA; Fig. 2).

For PFC, high-dose VGB significantly decreased the large neutral amino acids (LNAAs; including tyrosine (tyr), methionine (met), valine (val), isoleucine (ile), leucine (leu), phenylalanine (phe) and tryptophan (trp) (Table 3). The high-dose effect of VGB was also observed for neutral amino acids (asparagine (asn), glutamine (gln), thr, ala, proline (pro)), as well as for the basic amino acids histidine (his) and arg (Table 3). It should be noted that some literature citations consider both his and gln as LNAAs (Pardridge and Oldendorf, 1975). Finally, in a situation similar to VC, both ala and orn showed significant increases with intermediate VGB dosing. The data of Figs. 4 and 5, coupled with Table 3, highlights the total of 27 amino acids quantified in all tissues.

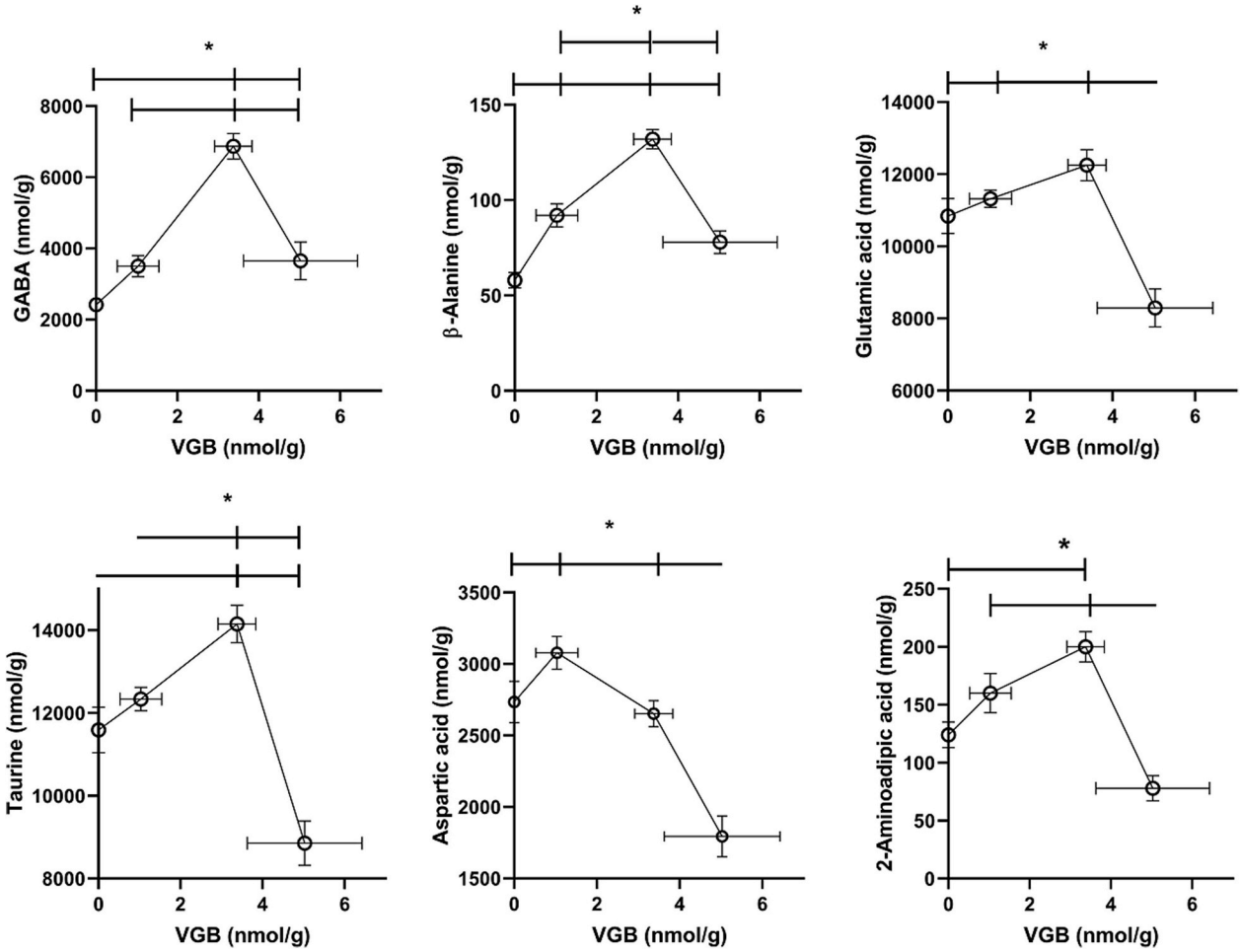

Fig. 4. Selected amino acids in prefrontal cortex as a function of vigabatrin dose. Amino acids shown included GABA, β-alanine, glutamic and aspartic acids, taurine and 2-aminoadipic acid. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

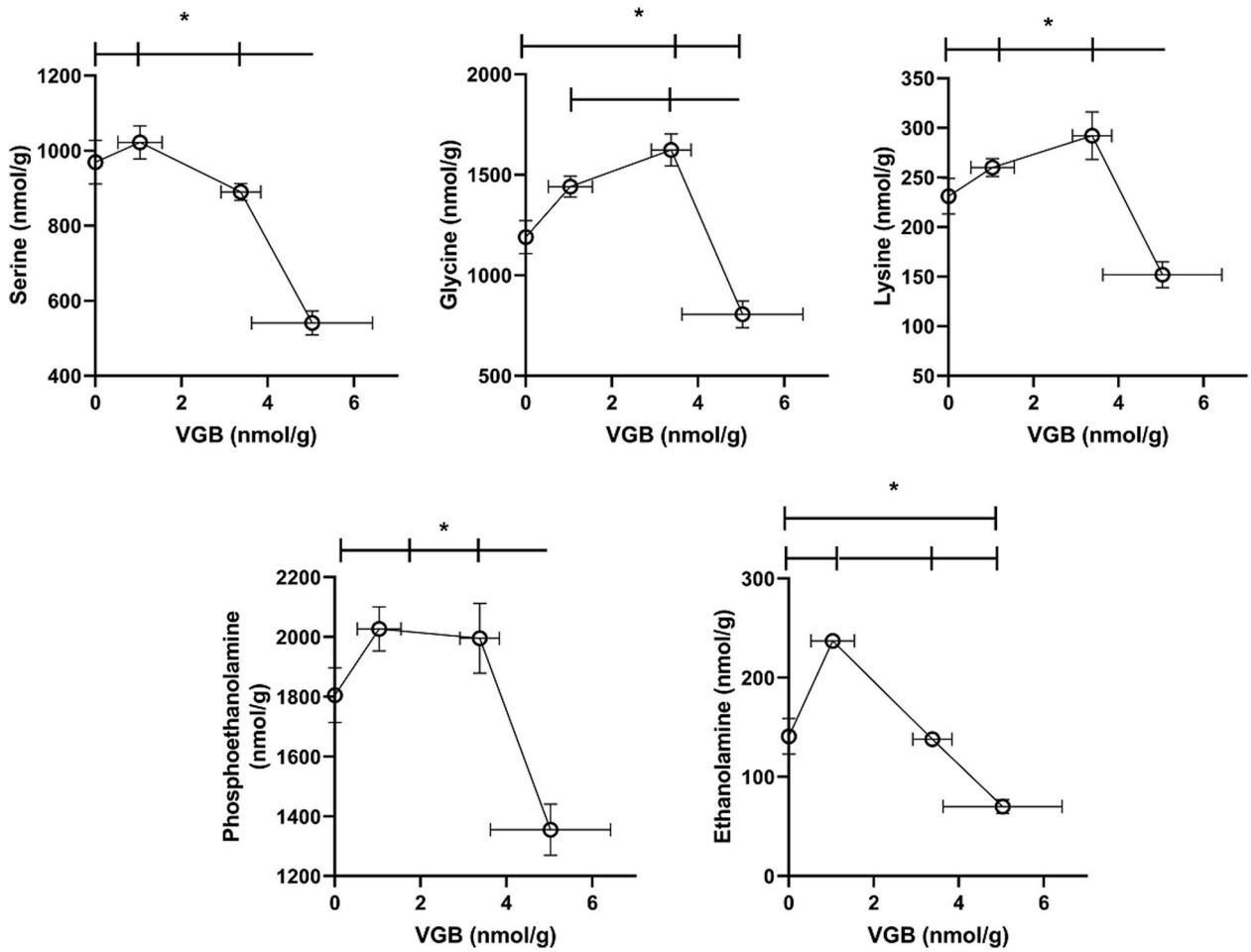

Fig. 5. Additional selected amino acids in prefrontal cortex as a function of vigabatrin dose. Amino acids shown included serine, glycine, lysine, phosphoethanolamine and ethanolamine. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

Table 3
Amino acid content in prefrontal cortex as a function of increasing vigabatrin administered subcutaneously.

Description	Metabolite	Vehicle	35 mg/kg/d	70 mg/kg/d	140 mg/kg/d
Drug	*Vigabatrin*	–	1 (0.5)	3.4 (0.5)	5 (1.4)
Drug-Related	Carnosine	84 (7)	94 (5)	161 (34)[a,b]	100 (18)
Neutral Amino Acids	Asparagine	76 (4)	77 (3)	77 (2)	53 (4)[d]
	Glutamine	5014 (300)	5379 (210)	5287 (60)	3172 (218)[d]
	Threonine	454 (45)	494 (35)	583 (49)	230 (41)[a,f]
	Alanine	1041 (130)	804 (38)	1996 (117)[a,b]	479 (27)[a,c]
	Proline	115 (7)	121 (4)	134 (6)	73 (6)[d]
Basic Amino Acids	Histidine	95 (7)	100 (5)	95 (6)	53 (4)[d]
	Arginine	142 (9)	153 (5)	160 (9)	98 (7)[d]
	Ornithine	20 (2)	17 (2)	34 (2)[a,b]	int
Large Neutral Amino Acids	Tyrosine	76 (6)	74 (5)	69 (5)	47 (4)[a]
	Methionine	58 (4)	53 (5)	58 (5)	39 (3)[a]
	Valine	94 (8)	104 (8)	103 (7)	50 (4)[d]
	Isoleucine	38 (4)	40 (3)	40 (2)	18 (1)[d]
	Leucine	88 (7)	101 (4)	95 (7)	47 (4)[a,f]
	Phenylalanine	70 (6)	76 (3)	75 (5)	37 (3)[d]
	Tryptophan	27 (3)	38 (4)[a]	17 (1)[b]	16 (1)[e]

Legend: nmol/g tissue (mean ± (SEM)).
Abbreviations: int, interference. As an irreversible inhibitor of GABA-transaminase (GABA-T), vigabatrin is predicted to elevate both GABA and β-alanine (the latter a substrate for GABA-T). Carnosine is the L-histidine dipeptide of β-alanine. Animal numbers (n) were: n = 21 for vehicle; n = 8 for 35 mg/kg/d; n = 6 for 70 mg/kg/d; and n = 8 for 140 mg/kg/d.

[a] $p < 0.05$ compared to vehicle.
[b] $p < 0.05$ between 35 and 70 dosing.
[c] $p < 0.05$ between 70 and 140 dosing.
[d] $p < 0.05$ compared to all other dosing (including vehicle).
[e] $p < 0.05$ between 35 and 140 dosing.
[f] $p < 0.05$ compared to ***both*** 35 and 70 dosing.

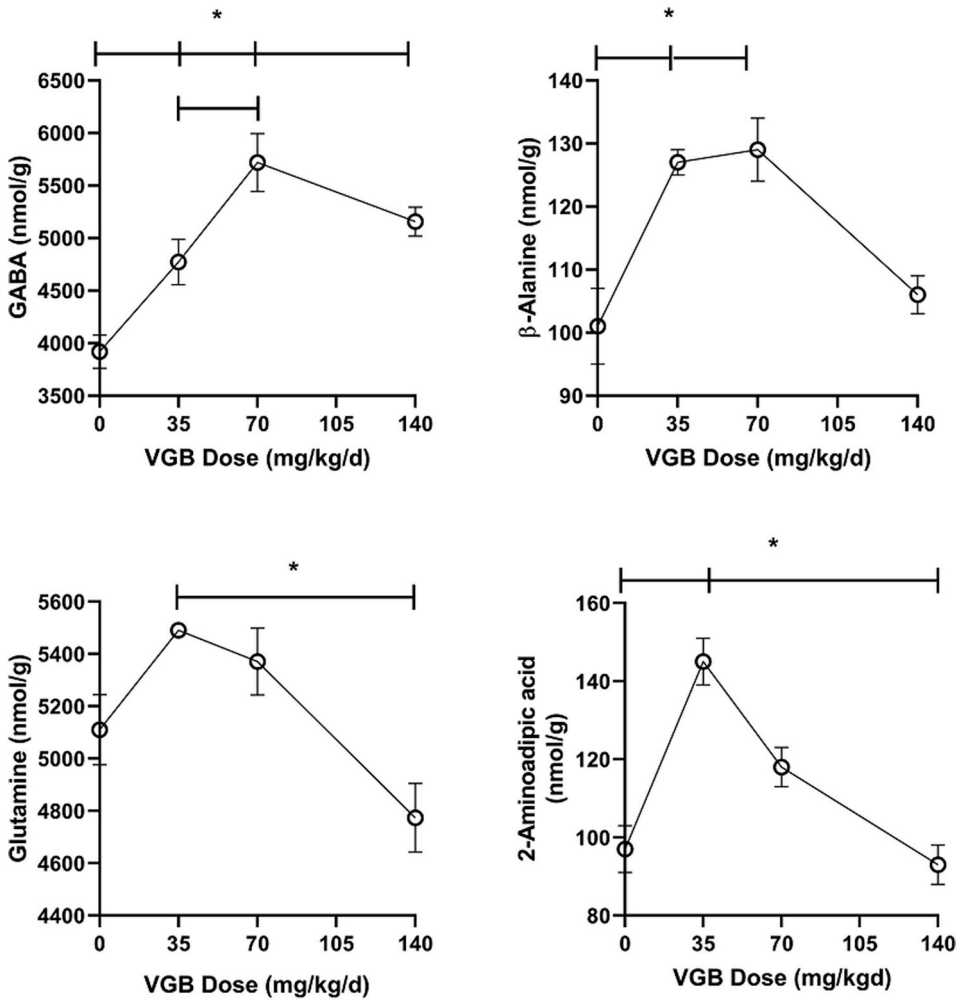

Fig. 6. Selected amino acids in brain (without prefrontal and visual cortex) as a function of vigabatrin dose. Amino acids shown include GABA, β-alanine, glutamine and 2-aminoadipic acid. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8). For both Figs. 6 and 7, the actual tissue VGB concentration for the 70 mg/kg/d dose was unavailable, and thus we presented x-axes values as dose, not concentration.

3.3.1. Brain (following removal of VC and PFC)

VGB brain levels increased with dose (35 mg/kg/d, 0.4 ± 0.1 nmol/g, mean ± SEM; 140 mg/kg/d, 6.8 ± 1.2 nmol/g). VGB data for the 70 mg/kg/d dose was unavailable, necessitating presentation of data in Figs. 6 and 7 for VGB as dose, and not actual concentration (x-axis). Amino acids were quantified in the remainder of brain homogenate, absent the dissected regions of VC and PFC (Figs. 6 and 7). Overall, brain extract revealed the fewest alterations with VGB administration, perhaps a reflection of the admixing of many different brain subsections. For both GABA and β-ala, high-dose VGB again revealed waning effects, with a similar effect for AADA and gln (Fig. 6). A similar high-dose effect was observed for gly, asp, arg and EA (Fig. 7), and there was a significant increase at low-dose VGB only for EA. All other amino acids were unaffected by VGB administration (data not shown).

3.4. Liver

In the liver VGB levels increased with dose in a supra dose-proportional manner. As predicted, both GABA and β-ala were increased with increasing VGB administration, however the effect waned with increasing VGB for β-ala and appeared to move toward saturation for GABA (Fig. 8). For several other amino acids, including tau, gln, PEA, lys, gly and his, low dose VGB led to a significant elevation which significantly waned with intermediate and high-dose administration (Figs. 8 and 9). This waning effect was also observed with a number of neutral amino acids (asparagine (asn), ser, thr, ala, pro) and acidic asp (Table 4). As was the case for the LNAAs in PFC, including tyr, met, val, ile, leu and phe, the effects of intermediate and high-dose VGB resulted in significant decreases in liver (Table 4). All other amino acids were unaffected by VGB administration (data not shown).

3.5. Plasma

VGB plasma levels increased with dose (35 mg/kg/d, 3 ± 0.8 μM, mean ± SEM; 70 mg/kg/d, 15 ± 1.4; 140 mg/kg/d, 34.6 ± 3.2). With the exception of his, ala and citrulline (cit), all amino acids in plasma were within normal limits when comparing VGB against vehicle. Similar to other tissues studied, both his and ala increased with increasing VGB, and waned with high-dose VGB administration (Fig. 10). The converse was observed for cit, which showed a near linear increase with VGB administration.

4. Discussion

Pharmacological investigations comparable to the present study frequently omit measurement of drug level. Nonetheless, Pitkänen et al. (1999) evaluated the effect of VGB administration on status epilepticus-

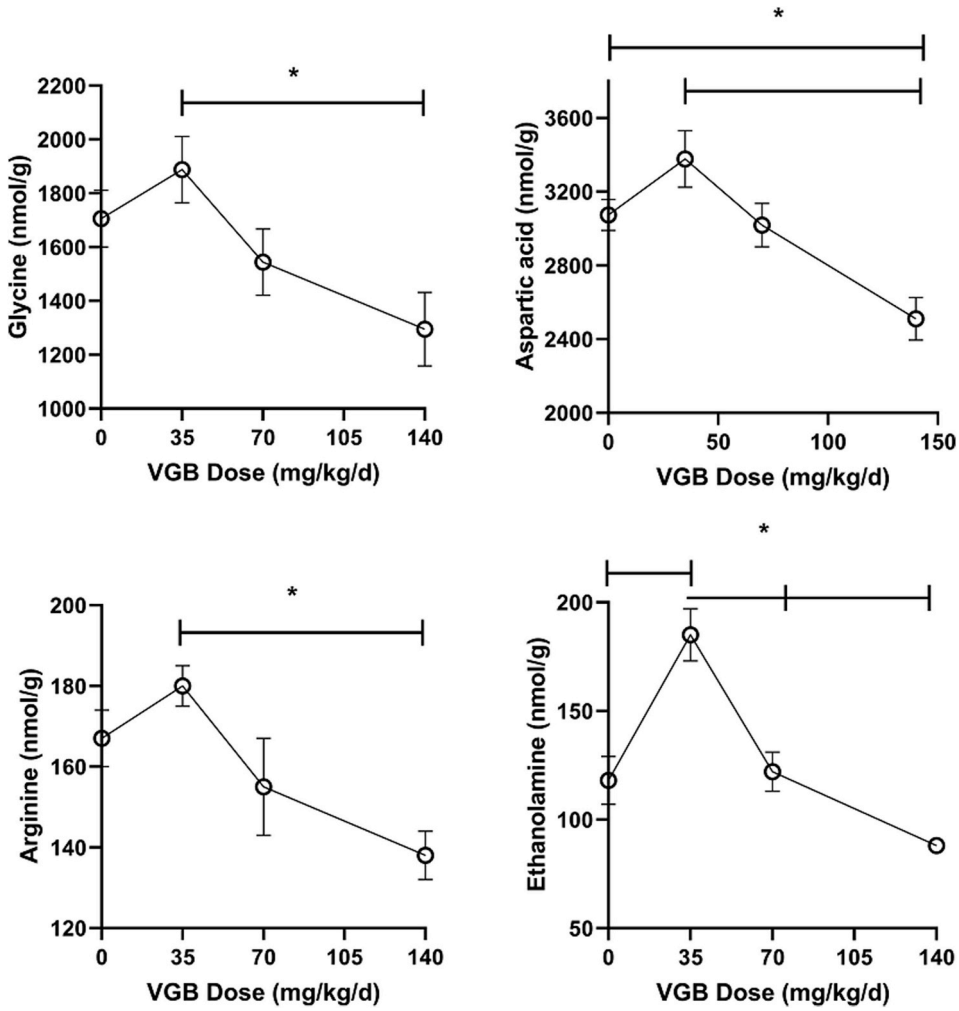

Fig. 7. Additional selected amino acids in brain (without prefrontal and visual cortex) as a function of vigabatrin dose. Amino acids shown include glycine, aspartic acid, arginine and ethanolamine. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

induced neuronal damage employing a pump infusion paradigm in rat (2 months, 75 mg/kg/d). Sera VGB, quantified by HPLC, was ~35–54 μM (days 2–14), while GABA content in hippocampus, also measured by HPLC, was ~ 4–5 μmol/g tissue. In our analyses, plasma VGB levels were ~15–35 μM (70–140 mg/kg/d, 12 days), and GABA content in VC and PFC (70 mg/kg/d) was ~7 μmol/g tissue. Despite obvious study differences (rat vs. mouse; plasma vs. sera; hippocampus vs. VC and PFC; HPLC vs. mass spectrometry methodology), the consistency of analyte measures suggests that VGB pharmacokinetics/tissue distribution were comparable with previous studies conducted using the continuous infusion dose paradigm we employed.

The most consistent amino acid elevations demonstrated in all tissues were GABA, β-alanine, carnosine and AADA. The literature predicts increase of the first two with VGB (Grove et al., 1981; Petroff et al., 1998), and carnosine elevation is reasonable since it is the histidine dipeptide of β-alanine (Blancquaert et al., 2016). However, AADA has never been demonstrated in mammalian tissues in association with VGB intake, although patients consuming VGB have shown 2-aminoadipic aciduria (Vallat et al., 1996) which may be associated with mutations in DHTKD1 (dehydrogenase E1 and transketolase domain containing 1; Danhauser et al., 2012). Patients with 2-aminoadipic aciduria do not manifest ocular features, however (OMIM 204750). Conversely, AADA has shown toxicity toward both astrocytes and Muller cells (Brown and Kretzschmar, 1998; Karlsen et al., 1982), the latter the predominant form of glial cell in the vertebrate retina. Conversely, AADA has the capacity to suppress the development of form deprivation myopia via intravitreal administration in guinea pigs (Mao and Liu, 2013). The six carbon homologue of glutamate (Fig. 1), AADA may exert its toxicity via enhanced release or transport of excitatory glutamate, or disruption of photoreceptors (Shen et al., 2011; Pannicke et al., 1994). Further exploration of the role of AADA, if any, in VGB ocular toxicity would appear warranted.

Our study demonstrated that VGB exerts numerous tissue alterations of neuroactive amino acids, including aspartic and glutamic acids, as well as serine and glycine. In the light/dark state, the signaling between retinal bipolar, ganglion, and amacrine cells (the latter the primary GABAergic cells of retina) is achieved via a series of graded potentials involving cyclic GMP (guanosine monophosphate) (Bloomfield and Dacheux, 2001; Tanaka and Tachibana, 2013). Glutamate and glycine are intimately involved in these potentials, as are D-serine (Gustafson et al., 2015) and aspartic acid (Bloomfield and Dowling, 1985). Interestingly, serine racemase is expressed in vertebrate retina (Stevens et al., 2003), and our system for amino acid determination would not differentiate D-from L-serine. Abnormalities in aspartic and glutamic acids were consistent in eye and PFC, but not in VC. Conversely, consistent alterations of glycine, alanine and serine across the majority of tissues surveyed may simply reflect some degree of disruption of intermediary metabolism by VGB.

VGB has been shown to inhibit pyridoxine-5′-phosphate oxidase (PNPO), an enzyme involved in the reactivation of pyridoxal-5′-

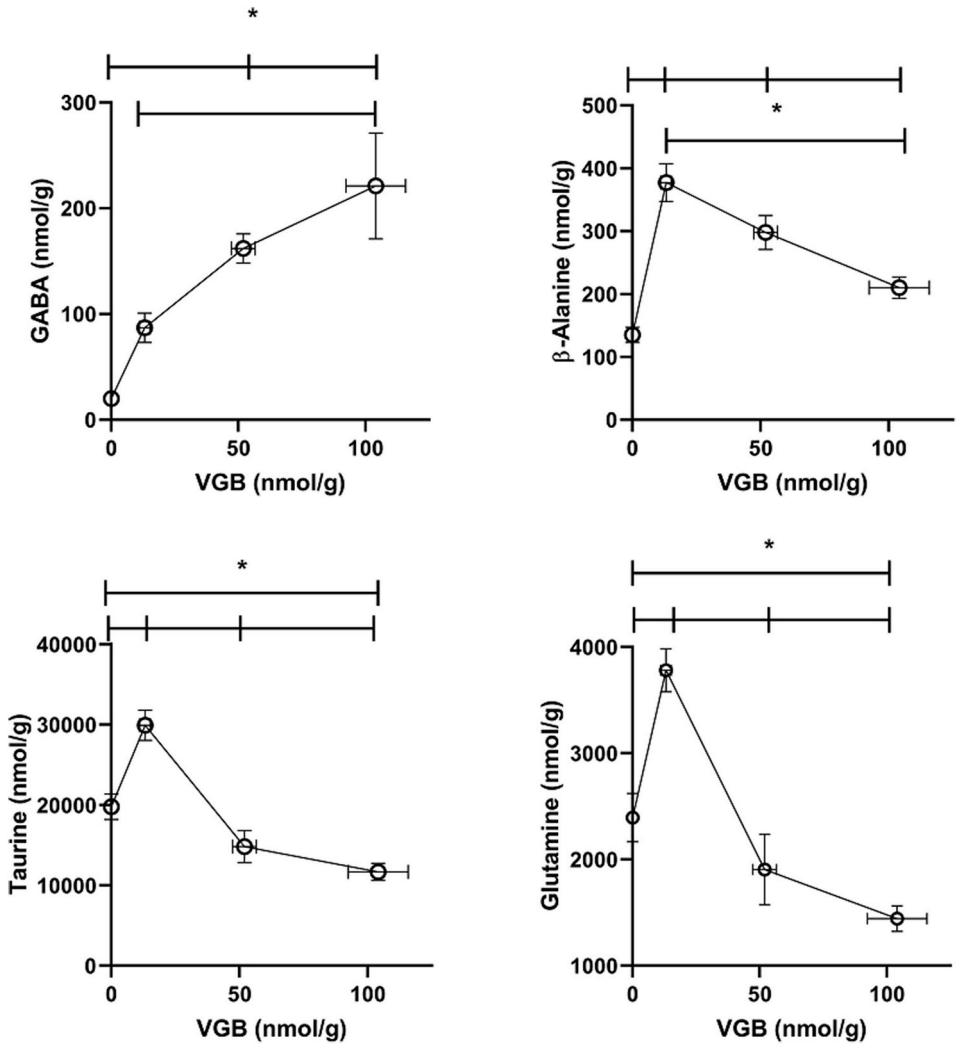

Fig. 8. Selected amino acids in liver as a function of vigabatrin dose. Amino acids shown include GABA, β-alanine, taurine and glutamine. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

phosphate (PLP; vitamin B_6) (An et al., 2004). The latter is a coenzyme in all mammalian transamination reactions. If this inhibition is occurring in our study, the off-target effect of VGB to disrupt PLP reactivation could well explain many of the amino acid elevations we documented, and especially in PFC, in which every amino acid was dysregulated to some degree. An off-target role for VGB in PNPO inhibition, or perhaps transporters involved in amino acid movement (Nøhr et al., 2014), would also serve to explain the waning effect of high-dose VGB in many tissues (VC, brain and liver), and prominently in PFC. As well, the presence of elevated 2-AADA, a component of the lysine degradative pathway associated with PNPO deficiency, raises the possibility that pyridoxine (or pyridoxal-5′-phosphate) might have a protective role for VGB ocular toxicity. Assuming VGB off-target effects are exerted via a competitive mechanism, high levels might well be predicted to lead to waning effects. Conversely, low to medium doses of VGB might exert inhibitory effects while higher doses may conceivably either activate the enzyme or induce its expression. Irreversible GABA-T inhibition (Fig. 1) would also disrupt GABA shunt activity (Sills et al., 2003), which utilizes 2-oxoglutarate as nitrogen acceptor from GABA to produce glutamic acid. This effect may also explain the alterations of glutamic acid-glutamine recycling by elevated GABA (Yang and Shen, 2009). All of these potential phenomenona would require experimental evaluation, most likely in cultured cell models.

Previous work indicated that taurine and ornithine may have a role in the ocular toxicity of VGB (Sorri et al., 2010; Gaucher et al., 2012; Rasmussen et al., 2015). In the current study, we did observe a consistent effect of VGB on ornithine in eye and VC, but not for taurine. The lack of effect on taurine suggests that it may not manifest a prominent protective role in VGB toxicity, but until we investigate amino acid parameters in isolated retina, this question will remain to be addressed. Indeed, we found a number of interesting findings for basic amino acids in the presence of VGB. At least at low-dose, VGB resulted in a significant reduction of arginine in VC, which would be consistent with the generation of 4-guanidinobutyrate from GABA (Fig. 1). Additionally, high-dose VGB significantly lowered histidine in PFC, consistent with the formation of homocarnosine from GABA conjugating to histidine. Although homocarnosine was not measured, it has been shown to be increased with VGB administration (Petroff et al., 1998, 1999). In addition, there was a very consistent dose-response effect of VGB on citrulline levels in plasma, one of the few changes in plasma for any amino acid, and a finding hitherto unreported with the use of VGB in a continuous administration paradigm.

Perusal of Fig. 1 reveals a significant number of basic amino acids whose levels are altered during chronic VGB treatment, including lys, AADA, and gln, in addition to the guanidino species orn, cit, arg and 4-GBA (the latter not characterized in the current study; Walters et al., 2019). The structural similarities of these species with VGB is apparent and may provide clues to many of its off-target effects. With regard to

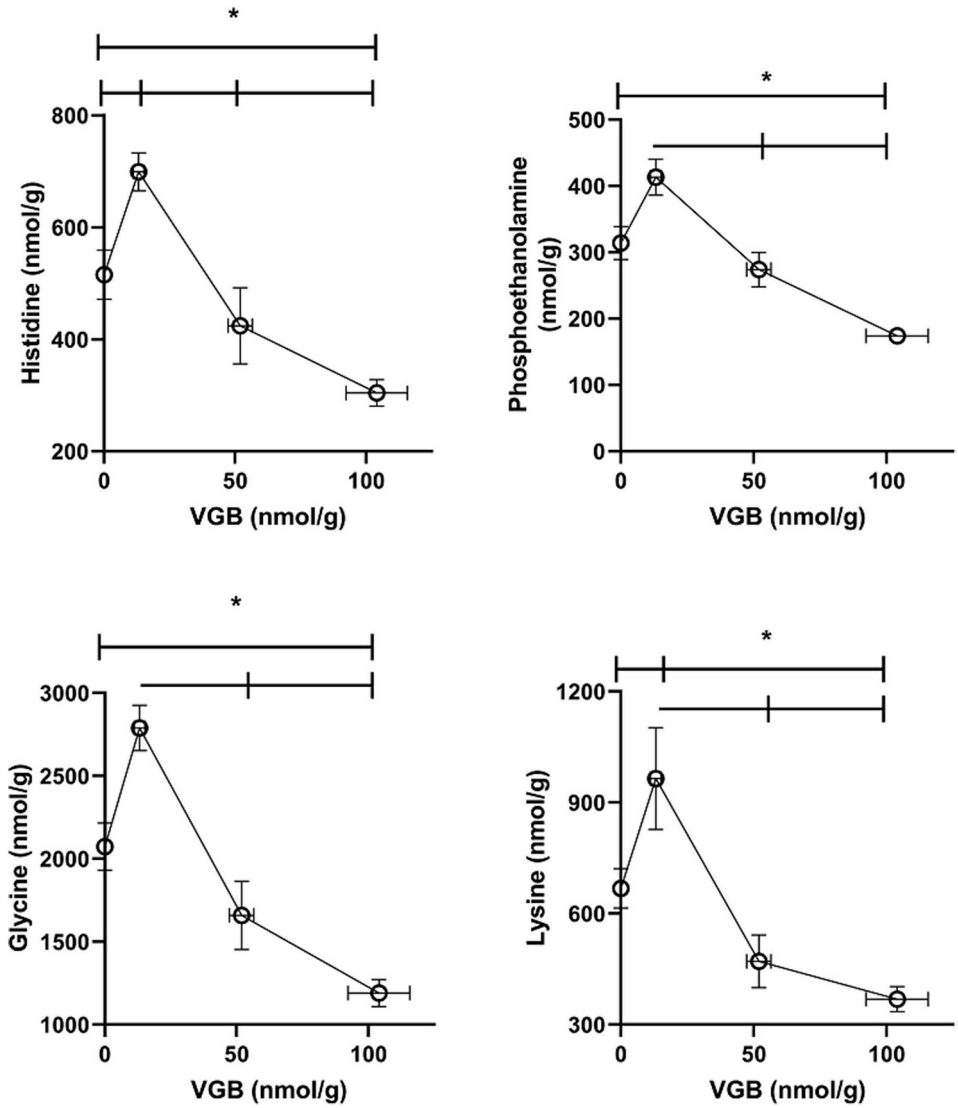

Fig. 9. Additional selected amino acids in liver as a function of vigabatrin dose. Amino acids shown include histidine, phosphoethanolamine, glycine and lysine. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

Table 4
Amino acid content in liver as a function of increasing vigabatrin administered subcutaneously.

Description	Metabolite	Vehicle	35 mg/kg/d	70 mg/kg/d	140 mg/kg/d
Drug	*Vigabatrin*	–	13 (2)	52 (5)	104 (12)
Neutral Amino Acids	Asparagine	203 (20)	292 (26)[a]	99 (10)[a,c]	136 (19)[e]
	Serine	438 (46)	583 (50)	259 (34)[b]	289 (42)[e]
	Threonine	333 (31)	461 (37)	205 (25)[b]	229 (30)[e]
	Alanine	3325 (229)	4693 (311)[a]	2457 (213)[b]	2342 (259)[e]
	Proline	287 (18)	359 (35)	254 (58)	224 (21)[e]
Acidic Amino Acids	Glutamic acid	1039 (114)	1421 (165)	575 (76)[b]	659 (51)[e]
Large Neutral Amino Acids	Tyrosine	207 (19)	310 (25)[a]	103 (13)[b]	136 (13)[e]
	Methionine	135 (13)	177 (15)	54 (5)[a,b]	108 (16)[e]
	Valine	332 (26)	444 (46)	265 (48)[b]	215 (15)[e]
	Isoleucine	178 (13)	275 (35)[d]	137 (26)	123 (10)
	Leucine	507 (182)	668 (61)	311 (44)[b]	408 (53)[e]
	Phenylalanine	199 (17)	260 (20)	113 (13)[a,b]	153 (23)[e]

Legend: nmol/g tissue (mean ± (SEM)).
[a] $p < 0.05$ compared to vehicle.
[b] $p < 0.05$ between 35 and 70 dosing.
[c] $p < 0.05$ between 70 and 140 dosing.
[d] $p < 0.05$ compared to all other dosing (including vehicle).
[e] $p < 0.05$ between 35 and 140 dosing. Animal numbers (n) were: n = 21 for vehicle; n = 8 for 35 mg/kg/d; n = 6 for 70 mg/kg/d; and n = 8 for 140 mg/kg/d.

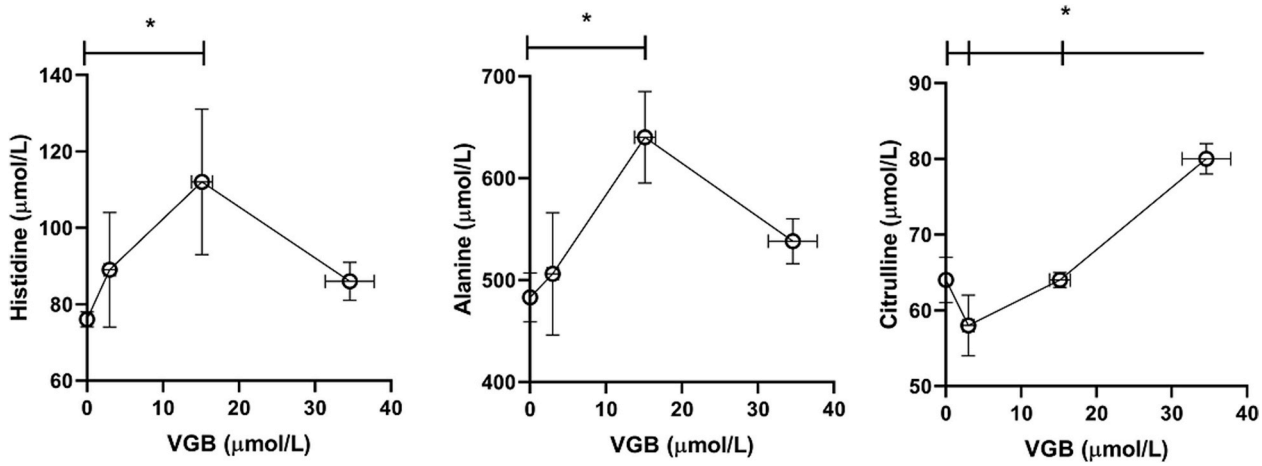

Fig. 10. Selected amino acids in plasma as a function of vigabatrin dose. Amino acids shown include histidine, alanine and citrulline. Statistical analysis employed a one way ANOVA with post-hoc analysis (significance set a $p < 0.05$). Data presented as mean ± SEM for both x and y-values. Animal numbers included vehicle (n = 21), 35 (n = 8), 70 (n = 6) and 140 mg/kg/d (n = 8).

guanidino species, there are unique transporters for these compounds across the blood-brain and blood-cerebrospinal fluid barriers, and some of these accumulated guanidino species may elicit epileptic discharges (Tachikawa and Hosoya, 2011). Further, alterations of guanidino species, especially arg, could suggest alterations in nitric oxide levels, a gaseous neurotransmitter shown to stimulate GABA release in retina while inhibiting release of glycine (Yu and Eldred, 2005). Finally, there was a tissue-specific effect of VGB on large neutral amino acids in both liver and VC, including tyr, met, val, ile, leu, phe and trp. These species, in addition to gln and his, move from the peripheral circulation across the blood brain barrier via LAT-1, the large neutral amino acid transporter (Vogel et al., 2013). Although VGB has not previously been reported to have an effect on LAT-1, this possibility seems plausible in relation to the current data, and the capacity of the LAT-1 to translocate the antiepileptic gabapentin (Furugen et al., 2017).

5. Conclusions

We have documented significant alterations of amino acid content in the eye, VC, brain, liver, PFC and plasma with continuous VGB administration to mice over 12 days. Like GABA, many amino acids (glycine, β-alanine, serine, aspartic and glutamic acids) are neuroactive, potentially inducing an imbalance in excitatory vs. inhibitory potentiation. This is the first identification of the glutamate homologue, AADA, in mammalian tissues associated with VGB intake. The known toxicity of AADA in glial cells, an important component of retina, provides new avenues for assessing VGB ocular toxicity. The effect of VGB on basic amino acids may point to the capacity of VGB to disrupt urea cycle function and nitrogen disposal. Future studies will investigate amino acids in retina, as well as aqueous and vitreous humors, using a continuous VGB administration paradigm.

Declaration of interest

None.

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