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Effects of Chronic Oral Treatment with GABA-Transaminase Inhibitors on the GABA System in Brain, Liver, Kidney, and Plasma of the Rat

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ABSTRACT. The inhibitory neurotransmitter γ-aminobutyric acid (GABA) is not solely located in the CNS, it and the enzymes responsible for its synthesis (glutamic acid decarboxylase, GAD, EC 4.1.1.15) and catabolism (GABA-transaminase, GABA-T, EC 2.6.1.19) are also present in non-neuronal organs. Following 2, 8 and 21 day oral administration of ethanolamine-O-sulphate (EOS) and γ-vinyl GABA (GVG), two irreversible inhibitors of GABA-T, the GABA content and activities of GAD and GABA-T in rat brain, liver and kidney, and the GABA content of plasma were determined. GABA-T activity was significantly decreased (over 80%) in liver, brain and kidney, although there was 2–3 times the residual activity left in the brain compared with the peripheral organs. GABA content was subsequently significantly elevated in the liver (300–1500%), plasma (200–300%) and brain (200–300%), although, surprisingly, the kidney GABA content was reduced (by 60–70%) compared with control. GAD activity was decreased following 8 day treatment in liver and brain. Kidney GAD was reduced at all time points. These two compounds are anticonvulsant, GVG is used clinically for the treatment of epilepsy but it seems that these drugs have significant peripheral effects. BIOCHEM PHARMACOL 52;9:1355–1363, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. GABA; GVG; EOS; epilepsy; GABA-T; GAD

GABA† is the major inhibitory neurotransmitter in the CNS; however, it is widely distributed in nonneuronal tissue, e.g. the presence of GABA in various tissues of the cat, including kidney, liver, urinary bladder, and whole pancreas, has been reported [1]. Since then, the presence of GABA and the enzymes responsible for its synthesis (GAD, 1 glutamate 1-carboxy-lyase, EC 4.1.1.15) and its catabolism (GABA-T, 4-aminobutyrate 2-oxoglutarate aminotransferase, EC 2.6.1.19) have been demonstrated in several mammalian organs other than the CNS [2, 3].

GABA

Immunocytochemical evidence suggests that GABA-like immunoreactivity is located in nonneuronal cells of different organs, including hepatocytes [4], kidney tubular epithelium [3, 5], and platelets and erythrocytes [6].

GAD

Different forms of GAD have been located in nonneuronal mammalian cells [7, 8] including erythrocytes [6] and hepatocytes [4]. However, it is unclear whether (as in the CNS) the GAD pathway constitutes the major metabolic origin of GABA in peripheral tissues [9–12].

GABA-T

Evidence suggests that GABA-T is the most important catabolic enzyme of GABA in nonneuronal tissue [12]. This enzyme has been found in many nonneuronal mammalian cells including platelets and lymphocytes [6], kidney tubular epithelial cells [8], and hepatocytes [8, 13].

GABAergic Elements in the Mammalian Liver

Liver GABA content has been documented in many animals including cat, rabbit, and rat, with concentrations of 15–100 nmol/g tissue. Human liver, however, has been reported to be much higher, at 252 nmol/g [14]; the reason for the elevated levels is unclear, but the differences in the time taken to obtain postmortem samples from humans and experimental animals and the differences in analysis techniques employed [15, 16] may explain these findings in part. The liver has a relatively low GAD activity [17] compared with organs such as brain and reproductive system, but it

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† *Abbreviations:* GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GABA-T, GABA-transaminase; EOS, ethanolamine-O-sulphate; GVG, γ-vinyl GABA; HPLC, high pressure liquid chromatography; CNS, central nervous system; GAG, γ-acetylenic GABA.

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has been reported to possess the highest GABA-T activity outside the CNS [18].

In addition, [^{3}H]-GABA binding to hepatocytes, which are sensitive to bicuculline, indicated the presence of $GABA_A$ binding sites [19], and a GABA transport system has been identified and characterised [20] as a result of experiments investigating the role of GABA in the pathogenesis of hepatic encephalopathy. Subsequent studies have shown that interference with this transport system (e.g. cirrhosis, hepatitis, partial hepatectomy) leads to impaired hepatic clearance and/or elevation in serum GABA content [21–23].

GABAergic Elements in the Mammalian Kidney

All elements of the GABA system (GABA-T, GABA, GAD etc) are present in the mammalian kidney [13]. The presence of binding sites for the specific $GABA_A$ and $GABA_B$ ligands, muscimol, and baclofen have also been shown [24, 25]. A high-affinity GABA transport system has also been identified [26]. GABA may also be released from cortical and medullary areas by ouabain and K^+ [5]. Details of the GABA system in the mammalian kidney have been summarised [27], although the function is poorly understood.

Many workers have investigated the *in vivo* effect of treatment with the specific GABA-T inhibitors EOS and GVG (4-aminohexenoate, vigabatrin) on brain GABA content and GABA-T and GAD activity [28–32]. Because these drugs cross the blood–brain barrier poorly [29, 33], they need to be administered in relatively high doses and would be expected to affect peripheral enzymes. Almost complete inhibition of liver GABA-T following 2 and 9 days of EOS administration in rabbits has been reported [34]. GVG is now a widely used antiepileptic drug, so it is of interest to document the effect of this drug and the related GABA-T inhibitor EOS on GABA-T and GAD in liver and kidney as well as the content of GABA and other amino acids of liver, kidney, and plasma. The use of these inhibitors may assist understanding of the role of GABA in the periphery. Two, 8, and 21 days of treatment were employed, a regime we previously reported to produce a maximum of 85% inhibition of brain GABA-T activity and an elevation of 150% in brain GABA content [35].

MATERIALS AND METHODS

GVG was the gift of the Marion Merrell-Dow Research Centre (Winnersh, Berkshire, U.K.). EOS was purified as previously described [36].

Fifty-four male Wistar rats (255 ± 5 g) were randomised into three even groups. Group 1 (control) received 1 g/L sucrose in the drinking water (vehicle), group 2 received 3 g/L GVG, and group 3 received 3 g/L EOS. Body weights and fluid consumption were monitored regularly. After 2, 8, and 21 days of treatment, six animals from each group were killed by stunning prior to decapitation. Blood was collected into 1-mL plasma microtainers and stored on ice until centrifugation at 13,000*g* for 10 min and stored at −20°C until subsequent analysis. The brain was rapidly dissected out and homogenised (teflon-glass homogeniser) in 3-mL ice-cold distilled water. Liver samples and one kidney (de-encapsulated) were also homogenised. Samples were aliquoted into Eppendorf tubes and stored at −20°C until required.

GABA-T and GAD activity were assayed by the fluorimetric methods of Salvador and Albers [37] and Lowe *et al.* [38] respectively. Tissue homogenates were analysed for protein content by the method of Bradford [39]. For high pressure liquid chromatography (HPLC) analysis, 10 μL of 100% trichloracetic acid was added to 90 μL tissue homogenate and the resultant suspension centrifuged at 11,000*g* for 10 min. Ten microlitres of the supernatant were neutralised with 90 μL 0.2 M $NaHCO_3$, pH 9; this was diluted with distilled water as necessary. Analysis of the amino acid content of samples was carried out by gradient, reverse-phase HPLC [40] following precolumn derivatisation with *o*-pthaldialdehyde. The gradient conditions used did not completely resolve the serine and histidine doublet or the glycine, threonine, and arginine triplet.

Statistical investigation was carried out by the application of Student's unpaired *t*-test. Statistical significance was determined at the 5% level.

RESULTS

Body Weight and Fluid Consumption

Animals receiving GVG did not gain weight, and EOS consumption caused no significant difference in weight gain compared with control animals, which supports results reported previously [41]. The mean (±SD) drug consumption of treated animals throughout the study for those receiving GVG was 185 ± 27 mg/kg/day versus 280 ± 28 mg/kg/day for EOS.

GVG-treated animals drank 45% less fluid than did control animals (90 ± 13 mL/day, cf. 164 ± 12, $P < 0.0001$). EOS-treated animals drank 93% of controls' intake (153 ± 12, $P < 0.005$).

Tissue Determination

GABA-T ACTIVITY. The GABA-T activity of liver, brain, and kidney was found to be significantly decreased (Fig. 1). In the peripheral tissues, a decrease of more than 93% was observed at all time points versus the 75% (GVG) and 60% (EOS) inhibition produced in the brain following 2 days' treatment. Enzyme activity decreased with further treatment to leave 14–18% remaining after 21 days.

GAD ACTIVITY. Figure 2 illustrates the change in GAD activity observed after treatment. Liver and brain possessed the same profile: 2-day treatment with both compounds had no effect, 8-day treatment caused a significant decrease in brain (30%) and liver (40–50%), and 21-day treatment

TABLE 1. Effect of 2-, 8-, and 21-day treatments with 3 g/L GVG, EOS, or vehicle (CTL) on whole brain amino acid content (nmol/mg protein)

Amino acid	2 Days treatment			8 Days treatment			21 Days treatment		
	CTL	GVG	EOS	CTL	GVG	EOS	CTL	GVG	EOS
ASP	71.75 ± 5.26	68.00 ± 3.48	63.03 ± 4.65	69.19 ± 4.77	63.02 ± 5.11	76.84 ± 4.67	73.62 ± 8.36	71.63 ± 6.89	61.41 ± 7.19
GLU	158.15 ± 9.28	146.76 ± 2.24	138.38 ± 4.61	157.72 ± 7.88	142.66 ± 11.48	175.59 ± 4.72	144.16 ± 4.91	154.98 ± 6.06	156.98 ± 4.98
ASN	8.77 ± 0.52	8.21 ± 0.33	6.89 ± 0.4*	8.42 ± 0.59*	5.54 + 0.31*	6.60 ± 0.74	8.18 ± 0.83	7.02 ± 0.58	5.26 ± 0.54
SER/HIS	68.83 ± 7.00	54.25 ± 3.79	39.41 ± 1.23*	59.37 ± 8.21	41.73 ± 10.86	49.75 ± 5.39	55.26 ± 13.4	57.93 ± 17.13	55.61 ± 30.31
GLN	81.73 ± 9.15	80.76 ± 2.14	67.27 ± 3.91	77.82 ± 3.72	56.58 ± 4.86*	78.86 ± 2.56	78.81 ± 4.62	64.45 ± 1.70*	68.13 ± 1.70*
A/G/T†	31.67 ± 4.50	32.13 ± 1.33	26.92 ± 1.46	30.94 ± 2.85	25.65 ± 4.31	27.80 ± 1.32	27.93 ± 3.97	36.44 ± 6.01	21.06 ± 0.57
TAU	91.51 ± 4.14	89.80 ± 2.49	88.59 ± 4.52	90.69 ± 3.50	86.76 ± 5.95	113.81 ± 7.03*	88.54 ± 3.72	92.13 ± 2.54	96.89 ± 3.44
ALA	46.64 ± 5.72	33.73 ± 2.08	27.05 ± 1.00*	30.48 ± 2.30	26.46 ± 6.26	29.97 ± 2.91	30.12 ± 5.89	35.44 ± 7.29	31.11 ± 13.50
TYR	7.69 ± 0.73	7.24 ± 1.02	6.25 ± 0.49	6.39 ± 1.31	5.65 ± 1.42	5.50 ± 0.59	4.82 ± 1.10	6.35 ± 1.58	5.56 ± 0.54
GABA	59.79 ± 4.43	107.08 ± 4.73*	69.88 ± 3.68‡	55.33 ± 1.65	128.02 ± 8.36*	109.20 ± 5.07*	52.95 ± 3.37	133.23 ± 6.47*	91.11 ± 4.74*

Values are mean ± SEM, n = 6 animals/group for each time point.

* Difference from control relevant to that time point, $P < 0.05$, Student's t-test.

† The arginine-glycine-threonine triplet.

‡ Although this is not statistically significant at 95% confidence interval, the trend is evident ($P = 0.1$).

TABLE 2. Effect of 2-, 8-, and 21-day treatments with 3 g/L GVG, EOS, or vehicle (CTL) on liver amino acid content (nmol/mg protein)

Amino acid	2 Days treatment			8 Days treatment			21 Days treatment		
	CTL	GVG	EOS	CTL	GVG	EOS	CTL	GVG	EOS
ASP	9.94 ± 0.68	9.84 ± 0.64	8.27 ± 0.97	7.04 ± 0.39	7.23 ± 0.30	7.40 ± 0.40	6.44 ± 0.92	6.67 ± 0.48	5.84 ± 0.37
GLU	36.87 ± 2.98	33.07 ± 1.81	31.55 ± 2.42	30.53 ± 2.17	28.57 ± 1.00	29.62 ± 2.22	25.56 ± 1.70	25.04 ± 2.38	21.93 ± 1.55
ASN	9.54 ± 0.29	9.52 ± 0.74	8.02 ± 1.00	6.83 ± 0.38	5.51 ± 0.28*	6.10 ± 0.41	5.25 ± 0.70	6.04 ± 0.38	4.62 ± 0.11
SER/HIS	18.40 ± 0.45	19.26 ± 1.38	16.33 ± 2.04	11.42 ± 0.57	12.12 ± 0.51	12.96 ± 0.61	10.78 ± 0.88	12.66 ± 0.68	10.09 ± 0.74
GLN	38.03 ± 1.08	39.74 ± 2.64	37.88 ± 3.27	35.87 ± 2.21	32.88 ± 2.19	32.98 ± 2.23	26.76 ± 1.71	31.93 ± 2.03	29.84 ± 0.64
A/G/T†	13.60 ± 1.30	12.13 ± 1.06	10.90 ± 1.22	7.93 ± 1.47	8.50 ± 0.35	8.36 ± 0.54	7.00 ± 0.48	7.83 ± 0.34	6.89 ± 0.43
TAU	8.28 ± 0.14	7.51 ± 0.55	8.51 ± 0.61	8.10 ± 1.28	6.49 ± 0.23	8.86 ± 1.19	9.44 ± 1.88	5.99 ± 0.31	7.45 ± 0.86
β-ALA	2.00 ± 0.10	2.46 ± 0.48	2.28 ± 0.20	1.59 ± 0.11	2.52 ± 0.26*	2.51 ± 0.29*	1.33 ± 0.11	2.20 ± 0.37*	1.95 ± 0.21*
ALA	41.53 ± 2.18	32.77 ± 3.33	35.65 ± 3.20	30.24 ± 2.09	23.79 ± 2.26	31.47 ± 2.24	28.66 ± 2.65	25.46 ± 0.50	24.73 ± 1.85
TYR	5.68 ± 0.33	5.75 ± 0.59	4.84 ± 0.57	3.91 ± 0.11	3.49 ± 0.22	3.74 ± 0.30	3.29 ± 0.34	3.62 ± 0.23	2.93 ± 0.17
GABA	0.58 ± 0.11	1.81 ± 0.23*	1.75 ± 0.30*	0.43 ± 0.05	3.26 ± 0.27*	1.33 ± 0.36*	0.22 ± 0.03	3.57 ± 0.58*	1.26 ± 0.24*

Values are mean ± SEM, n = 6 animals/group for each time point.

* Difference from control relevant to that time point, $P < 0.05$, Student's t-test.

† Arginine-glycine-threonine triplet.

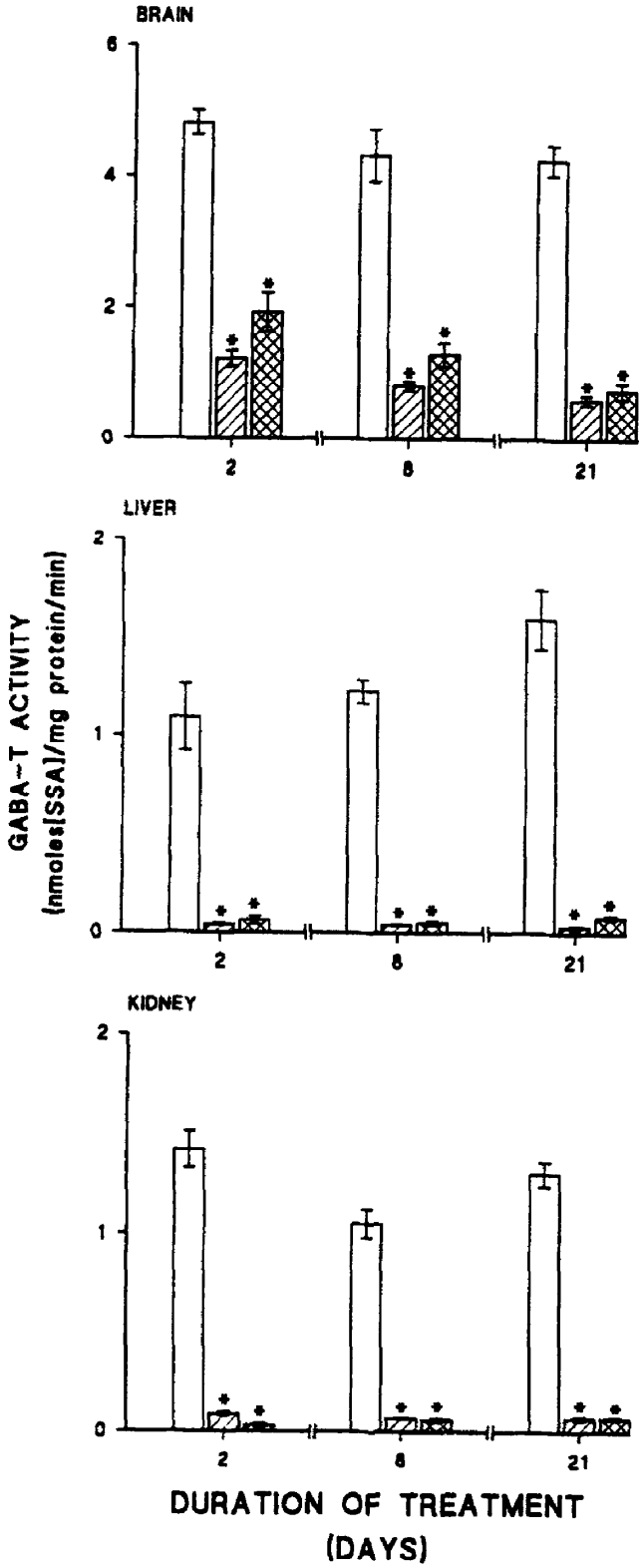

FIG. 1. The effect of treatment with 3 g/L GVG (striped bar), EOS (cross-hatched bar), or vehicle (white bar) on GABA-T activity. Units are nanomoles of succinic semildehyde (SSA) produced per mg protein/min, expressed as mean ± SEM. *$P < 0.05$, Student's *t*-test, n = 6 animals/group for each time point.

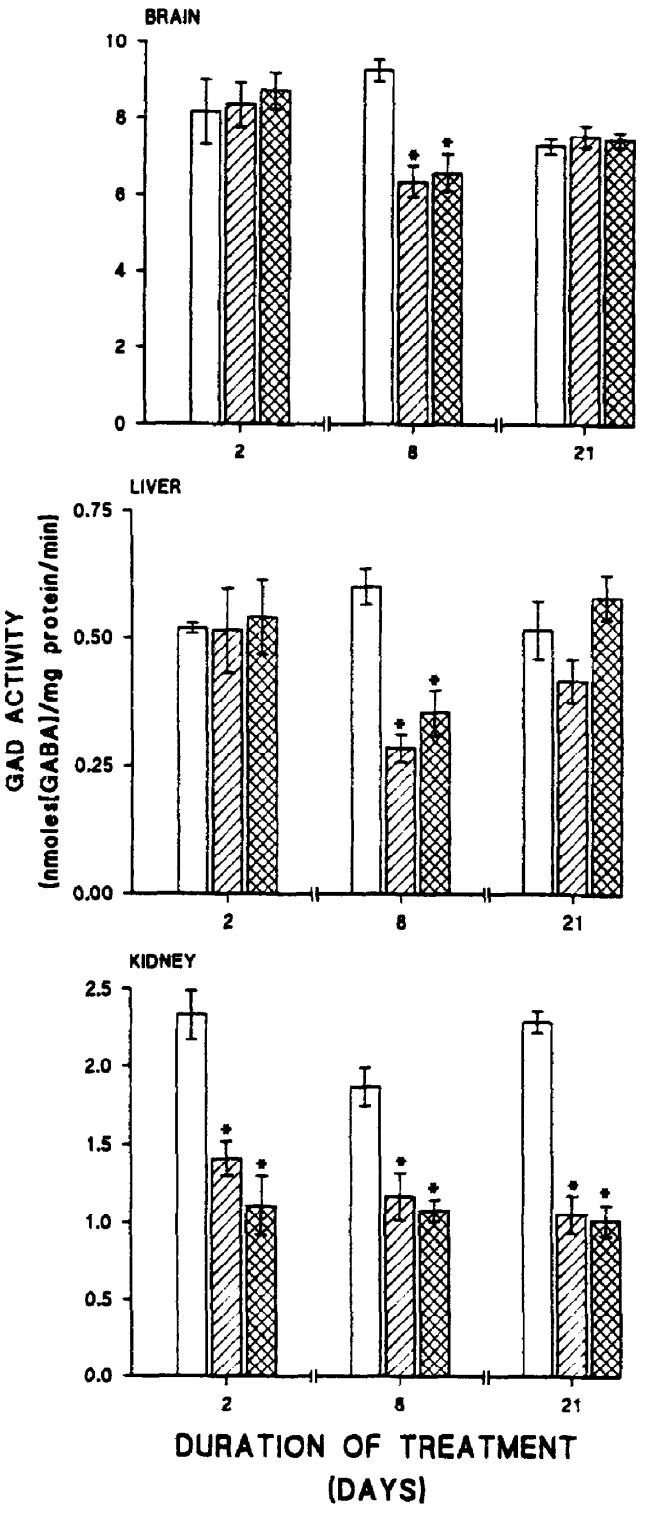

FIG. 2. The effect of treatment with 3 g/L GVG (striped bar), EOS (cross-hatched bar), or vehicle (white bar) on GAD activity. Units are nanomoles of GABA produced per mg protein/min, expressed as mean ± SEM. *$P < 0.05$, Student's *t*-test, n = 6 animals/group for each time point.

TABLE 3. Effect of 2-, 8-, and 21-day treatments with 3 g/L GVG, EOS, or vehicle (CTL) on whole kidney amino acid content (nmol/mg protein)

Amino acid	2 Days treatment			8 Days treatment			21 Days treatment		
	CTL	GVG	EOS	CTL	GVG	EOS	CTL	GVG	EOS
ASP	65.47 ± 2.75	54.76 ± 5.10	51.06 ± 1.73*	44.94 ± 3.31	53.65 ± 5.53	55.26 ± 5.91	44.16 ± 1.31	47.58 ± 3.42	45.49 ± 3.76
GLU	151.88 ± 5.98	138.10 ± 9.35*	132.82 ± 5.94	108.20 ± 5.94	125.6 ± 10.64	147.43 ± 8.91*	121.26 ± 3.27	134.33 ± 9.38	137.50 ± 5.43
ASN	51.92 ± 2.74	50.38 ± 3.93	45.48 ± 2.34	36.6 ± 2.12	39.89 ± 4.09	49.88 ± 7.36	50.34 ± 7.39	41.28 ± 4.00	44.63 ± 4.00
SER/HIS	69.68 ± 3.23	64.70 ± 6.53	62.19 ± 1.12	44.64 ± 2.40	51.32 ± 5.11	50.15 ± 2.59	41.95 ± 0.98	54.05 ± 4.85*	49.12 ± 2.88*
GLN	22.65 ± 1.79	23.41 ± 2.42	21.72 ± 0.57	18.03 ± 0.72	18.73 ± 1.55	21.80 ± 2.21	17.73 ± 0.53	20.51 ± 1.80	21.00 ± 1.53
A/G/T†	43.00 ± 2.88	41.74 ± 3.92	40.50 ± 1.36	30.84 ± 1.96	40.56 ± 3.79*	50.34 ± 5.53*	35.71 ± 0.86	43.64 ± 4.06	41.38 ± 2.96
TAU	104.07 ± 4.00	101.67 ± 8.03	92.47 ± 5.03	91.08 ± 4.95	116.34 ± 7.41*	121.90 ± 3.68*	109.06 ± 3.78	115.78 ± 8.02	112.33 ± 5.16
β-ALA	3.98 ± 0.15	2.67 + 0.27*	3.43 ± 0.22	3.17 ± 0.15	2.83 + 0.25	3.57 ± 0.24	3.64 + 0.19	3.07 + 0.33	3.00 ± 0.15
ALA	91.39 ± 4.39	86.17 ± 7.54	77.59 ± 2.44*	59.83 ± 3.06	69.91 ± 6.29	73.66 ± 2.52*	60.00 ± 2.34	67.03 ± 5.50	65.78 ± 3.72
TYR	26.61 ± 1.65	24.5 ± 1.96	21.17 ± 0.69*	17.44 ± 1.25	19.80 ± 1.11	22.28 ± 1.90	16.62 ± 0.74	19.30 ± 2.17	17.39 ± 1.44
GABA	4.02 ± 0.55	0.71 ± 0.08*	0.96 ± 0.27*	1.37 ± 0.15	1.09 ± 0.12	0.68 ± 0.08*	1.18 ± 0.04	0.72 ± 0.10*	0.41 ± 0.04*

Values are mean ± SEM, n = 6 animals/group for each time point.

* Difference from control relevant to that time point, $P < 0.05$, Student's t-test.

† Arginine-glycine-theonine triplet.

TABLE 4. Effect of 2-, 8-, and 21-day treatments with 3 g/L GVG, EOS, or vehicle (CTL) on plasma amino acid content (nmol/mL)

Amino acid	2 Days treatment			8 Days treatment			21 Days treatment		
	CTL	GVG	EOS	CTL	GVG	EOS	CTL	GVG	EOS
ASP	66.83 ± 5.90	74.03 ± 9.65	79.91 ± 8.94	92.91 ± 10.08	69.66 ± 5.59	49.32 ± 4.69*	40.38 ± 2.70	47.55 ± 4.55	39.92 ± 7.71
GLU	168.30 ± 14.09	154.69 ± 12.27	176.0 + 24.40	184.86 ± 35.47	131.85 ± 16.11	130.63 ± 6.08	70.93 + 13.13	70.12 ± 5.80	91.72 ± 7.07
ASN	112.68 ± 14.29	97.91 ± 4.48	93.32 ± 11.06	73.43 ± 11.64	61.77 ± 6.89	55.39 ± 4.40	75.14 ± 15.63	75.17 ± 11.438	83.14 ± 8.52
SER/HIS	344.68 ± 33.34	407.52 ± 19.46	308.46 ± 25.18	357.7 ± 29.59	279.17 ± 27.43	240.01 ± 45.38	254.35 ± 25.13	284.23 ± 30.44	271.23 ± 30.03
GLN	456.15 ± 21.34	453.32 ± 12.33	382.02 ± 41.77	425.81 ± 59.91	360.39 ± 30.75	359.56 ± 28.33	373.48 ± 44.55	365.91 ± 38.11	426.11 ± 19.44
ARG	294.76 ± 37.20	268.01 ± 19.49	210.02 ± 14.05	230.03 ± 75.59	161.69 ± 19.98	162.88 ± 17.95	203.98 ± 55.00	216.48 ± 33.88	246.71 ± 38.59
GLY	318.70 ± 25.21	361.56 ± 11.65	352.70 ± 37.22	333.67 ± 35.76	212.73 ± 34.14	151.53 ± 15.39*	160.98 ± 32.40	188.50 ± 29.75	180.31 ± 17.31
THR	213 ± 22.59	206.46 ± 7.01	160.20 ± 12.10	166.22 ± 36.44	118.10 ± 12.41	113.77 ± 11.55	120.86 ± 24.33	141.52 ± 22.33	112.81 ± 24.93
TAU	282.87 ± 34.67	341.90 ± 18.07	347.42 ± 49.13	310.90 ± 64.62	274.02 ± 30.21	281.60 ± 30.43	135.78 ± 8.74	147.04 ± 18.31	155.45 ± 12.76
ALA	581.72 ± 70.00	556.12 ± 16.84	440.67 ± 55.14	416.56 ± 104.62	303.68 ± 34.28	374.21 ± 49.61	390.26 ± 75.66	370.15 ± 47.58	430.14 ± 49.18
TYR	166.40 ± 18.06	159.71 ± 7.35	119.37 ± 15.67	148.10 ± 44.51	91.58 ± 10.12	94.52 ± 11.22	108.43 ± 20.28	113.18 ± 14.63	109.30 ± 18.03
GABA	0.79 ± 0.12	1.88 ± 0.42*	2.06 ± 0.34*	1.12 ± 0.12	3.37 ± 0.27*	2.04 ± 0.40*	0.83 ± 20	1.81 ± 0.36*	1.71 ± 0.21*

Values are mean ± SEM, n = 6 animals/group for each time point.

* Difference from control relevant to that point, $P < 0.05$, Students's t-test.

showed a return to control values. Kidney GAD activity, however, showed a decrease, compared with control, of 40–50% after 2 days, and a decrease of 40% and 55% after 8 and 21 days, respectively.

AMINO ACID CONTENT. The amino acid content of control and treated brain, liver, kidney, and plasma are summarised in Tables 1–4.

In brain, GABA levels were significantly elevated at all time points after treatment with GVG, and 8 and 21 days following EOS treatment ($P < 0.0001$). Two-day treatment with EOS elevated GABA levels by 17%, but this was not significant at the 95% confidence level.

In liver, GABA content was elevated at all time points, showing an increase of 1500% after 21-day GVG treatment and 469% with EOS. β-Alanine content was also elevated following treatment, but the 14–23% increase observed following 2-day treatment was not statistically significant.

In kidney, GABA content was significantly decreased upon treatment (except after 8 days with GVG, where the 20% decrease was not statistically significant), which was in sharp contrast to that observed for liver and brain. β-Alanine content followed the same trend as GABA, i.e. a decrease, but this reached significance only after 2 days of GVG treatment.

In plasma, GABA content showed a significant increase after treatment at all time points, ranging from 180 to 300% of control values.

DISCUSSION

Body Weight and Fluid Consumption

Animals receiving GVG in a drinking solution are reported to show resistance to drinking [42], showing an almost 50% statistically significant decrease in fluid intake. EOS-treated animals had only a slightly lower fluid intake compared with controls. The lack of an increase in body weight following GVG treatment was in line with the anorexic effect of GABA-T inhibitors [43]; interestingly, EOS caused no change in body weight. The 50% decrease in fluid intake of GVG-treated animals may have contributed to the lack of weight gain.

GABA-T Activity

Liver and kidney GABA-T activity were almost completely abolished following 2-day treatment, and this was maintained with further treatment; this was in contrast with brain, whose GABA-T activity became progressively less with continuing treatment. This residual brain activity has been observed previously [29] and is presumably due to the fact that these GABA-T inhibitors do not cross the blood–brain barrier easily.

Brain GABA

As expected and published elsewhere [29, 30] brain GABA content was elevated following administration of GVG and EOS in a time-dependent manner due to the continuing inhibition of GABA-T.

Liver GABA

Liver GABA content was significantly elevated at all time points following treatment with GVG and EOS, leading to a maximum GABA content following 21-day GVG treatment of over 16 times control levels, compared with the sixfold increase following EOS administration. However, these large increases in liver GABA over control levels still represent only approximately 3–7% of the GABA concentration found normally in brain.

Intravenous administration of [^{3}H]-GABA to rabbits has been reported to result in rapid and efficient clearance by the liver [21]. Three to 4 min following administration, more than 90% of the [^{3}H]-GABA was not detectable as GABA within the systemic circulation, with increasing levels of hepatic GABA and GABA metabolites. Pretreatment with the hepatotoxin galactosamine hydrochloride impaired the GABA clearance [44], which would seem to indicate that the liver may have some function in GABA clearance, especially because 80% of all GABA-T in the human body (including the CNS) is found in this organ [18].

Although little is actually known about GABA's role in hepatobilliary function and disease, impaired hepatic clearance and elevation in serum GABA content has been observed following diseases interfering with the liver GABA transport system, such as cirrhosis and hepatitis [21–23]. Clinically, the suppression of normal neuronal activity is a symptom of hepatic encephalopathy, and preventing GABA synthesis has been shown to improve the encephalopathic state [45].

Kidney GABA

In sharp contrast to that observed in the other organs examined, an unexpected result was observed following GVG and EOS treatment in kidney: the GABA content was decreased. The decrease was significant at all time periods examined and tended to stay at the same absolute value throughout.

The reason for this alteration in GABA is not known, but a few suggestions may be offered. Although GABA-T is present in kidney, it does not necessarily operate in the direction demonstrated in brain and liver; the normal reaction may be the production of GABA from succinic acid semialdehyde and glutamate. In this case, inhibition of GABA-T would prevent the formation of GABA, leading to a decrease in the kidney content observed. However, the observed decrease in kidney GAD content is inconsistent with this hypothesis because brain GAD activity is thought to be reduced following "feed-back" inhibition of the elevated GABA.

As much as 50% of a bolus injection of GABA may be recovered in the urine [46]. Urinary excretion of GABA

may be observed following a substantial increase in plasma GABA content as in hyper-β-alaninemia [47]. It is possible, although not particularly feasible, that following the elevated GABA content peripherally, excess GABA is excreted into urine, with an overcompensation producing the lowered GABA content observed in kidney. A study investigating changes in urine GABA content following GABA-T inhibition would be of interest; preliminary investigations of control rat urine, where GABA is normally present in trace amounts, found that the interpretation of HPLC profile of urine was impossible without prior cleaning of the urine through ion-exchange columns. The administration of GVG to rats showed a marked increase in the urinary excretion of GABA [48], hypotaurine, and β-alanine; quantification of either the levels or the increases was not provided.

Plasma GABA

The GABA content of plasma was elevated following GVG and EOS administration at all time points. GABA levels in the plasma have been reported not to reflect CNS concentrations. The blood-brain barrier is practically impermeable to GABA, so plasma levels therefore reflect peripheral synthesis and absorption from enteric bacterial sources [49]. It has been reported that platelet GABA-T activity exhibited a similar profile of inhibition as brain GABA-T [50], although the platelet enzyme was inhibited more rapidly and to a greater extent.

Plasma and CSF GABA content following GVG administration has been studied [51]. Following 60 mg/kg GVG (a very low dose compared with almost 200 mg/kg/day used in these studies) administered intravenously to anaesthetised dogs, CSF GABA was elevated by a maximum of 38%, but no concomitant increase in plasma was observed. However, 20 and 60 mg/kg GAG produced dose-dependent elevations in GABA in both plasma and CSF. The reason for the discrepancy may be the very low dose of GVG administered and the observation that GAG is a much more potent GABA-T inhibitor than GVG [52].

β-Alanine Content

The structure of β-alanine is similar to that of GABA and as such is a suitable substrate for metabolism by GABA-T [53]. The β-alanine content of liver was found to be increased after 8 and 21 days of treatment with GVG and EOS; this is parallel (although not of the same magnitude) to the changes observed in GABA content. However, the β-alanine content of kidney was found to decrease significantly only after 2 days' treatment with GVG. A trend in decrease at other time points was found not to be significant. As with liver these changes parallel those observed in kidney GABA content, the reasons for the observed decline would supposedly be in line with those discussed previously for the change in GABA content.

GAD Activity

The GAD activity of brain and liver was reduced following 8 days of treatment, and kidney GAD was decreased at all time points analysed. Both GVG and EOS have been reported not to inhibit GAD *in vitro* [33, 54]; however, *in vivo* administration of EOS does lead to a small significant reduction on whole brain GAD activity [32], as does GVG [29, 48]. The results of these groups suggest that these drugs do not cause a direct inhibition of the enzyme, but that the elevated brain GABA levels induce the inhibition of existing enzyme and/or the synthesis of new enzyme [32, 52, 55].

An interesting observation was that, following 21 days' administration with GVG and EOS, both liver and brain GAD content returned to control. The reason for this is unclear; it may be that the synthesis of new GAD had been carried out to replenish that inhibited by the elevated GABA. As far as we can tell, this effect has not been observed by any other investigators.

In conclusion, it seems clear that the liver is a site for metabolism of GABA (and β-alanine) by the action of GABA-T, but the complete inactivation of the enzyme by GABA-T inhibitors does not result in large absolute concentrations of GABA in the periphery. One report [56] has shown that cirrhosis leads to elevated plasma GABA concentrations but that the impaired metabolism of peripheral GABA does not lead to cerebral dysfunctions. The changes in peripheral GABA metabolism brought about by the chronic use of GABA-T inhibitors may, however, be of significance in hepatic regenerative capacity. It has been found that GABA inhibits hepatic putrescine synthesis at a posttranscriptional level in rats after partial hepatectomy [57]. These investigators have suggested that this inhibition may help to explain why hepatic regenerative activity is impaired in patients with elevated serum GABA concentrations and fulminant hepatic failure. The absolute levels of plasma GABA and the increases following partial hepatectomy reported by these investigators are similar to the results we report here for the level of GABA in the plasma and its increase following GABA-T inhibition.

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References

1. Tallan HH, Moore S and Stein WH, Studies on the free amino acids and related compounds in the tissues of the cat. *J Biol Chem* **211**: 927–939, 1954.
2. Erdo SL and Bowery NG, *GABAergic Mechanisms in the Mammalian Periphery*. Raven Press, New York, 1986.
3. Erdo SL, *GABA Outside the CNS*. Springer-Verlag, New York, 1991.
4. Minuk GY, GABAergic mechanisms and their functional importance in the liver. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 325–337. Raven, New York, 1986.
5. Erdo SL, Dobo E, Parducz A and Wolff JR, Releasable GABA

in tubular epithelium of rat kidney. *Experentia* **47:** 227–229, 1991.
6. Oset-Gasque MJ, Launay JM and Gonzalez MP, GABAergic mechanisms in blood cells: their possible role. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 305–324. Raven, New York, 1986.
7. Wu JY, Chude O, Wein J and Roberts E, Glutamate decarboxylase from neural and non-neural tissues. *J Neurochem* **30:** 849–857, 1978.
8. Wu JY, Lin CT, Lin H, Xu Y, Liu JW, Hwang BH and Wei SC, Immunochemical characterization and immunohistochemical localization of glutamate decarboxylase and GABA transaminase in peripheral tissues. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 19–34. Raven, New York, 1986.
9. Andersson AC and Henningson S, *In vitro* metabolism of putrescine by diamine oxidase in tissues of the pregnant rat. *Agents Actions* **10:** 104–106, 1980.
10. Andersson AC, Henningson S and Jarhult J, Diamine oxidase activity and gamma-aminobutyric acid formation in medullary carcinoma of the thyroid. *Agents Actions* **10:** 299–301, 1980.
11. Caron PC, Kremzner LT and Cote LJ, GABA and its relationship to putrescine metabolism in the rat brain and pancreas. *Neurochem Int* **10:** 219–229, 1987.
12. Fogel WA, GABA and polyamine metabolism in peripheral tissues. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 35–56. Raven, New York, 1986.
13. Krause DN, Involvement of local GABA mechanisms in vascular regulation. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 193–203. Raven, New York, 1986.
14. Zachmann M, Tocci P and Nyhan WL, The occurrence of γ-aminobutyric acid in human tissues other than brain. *J Biol Chem* **241:** 1355–1358, 1966.
15. Waniewski RA and Suria A, Alterations in γ-aminobutyric acid content in the rat superior cervical ganglion and pineal gland. *Life Sci* **21:** 1129–1142, 1977.
16. Ferenci P, Ebner J and Zimmerman C, Overestimation of serum concentrations of γ-aminobutyric acid in patients with hepatic encephalopathy by the γ-aminobutyric acid-radioreceptor assay. *Hepatology* **8:** 69–72, 1988.
17. Erdo SL and Kiss B, Presence of GABA, glutamate decarboxylase, and GABA transaminase in peripheral tissues: A collection of quantitative data. In: *GABAergic Mechanisms in the Mammalian Periphery* (Eds. Erdo SL and Bowery NG), pp. 5–17. Raven Press, New York, 1986.
18. White HL and Sato TL, GABA-transaminases of human brain and peripheral tissues—kinetic and molecular properties. *J Neurochem* **31:** 41–47, 1978.
19. Minuk GY, Bear CE and Seargent EJ, Sodium independent bicuculline sensitive, [^{3}H] GABA binding to isolated rat hepatocytes. *Am J Physiol* **252:** 642–647, 1987.
20. Schafer DF, Fowler JM and Jones EA, Hepatic encephalopathy and the γ-aminobutyric acid neurotransmitter system. *Lancet* **1:** 18–19, 1982.
21. Ferenci P, Covell D, Schafer DF, Waggoner JG, Schrager R and Jones AE, Metabolism of the inhibitory neurotransmitter γ-aminobutyric acid in a rabbit model of fulminant hepatic failure. *Hepatology* **3:** 507–512, 1983.
22. Minuk GY, Winder A, Burgess ED and Sarjeant EJ, Serum γ-aminobutyric acid (GABA) levels in patients with hepatic encephalopathy. *Hepatogastroenterology* **37:** 171–174, 1985.
23. Maddison JE, Dodd PR, Johnston GA and Farrell GC, Brain γ-aminobutyric acid receptor binding is normal in rats with thioacetamide-induced hepatic encephalopathy despite elevated plasma γ-aminobutyric acid-like activity. *Gastroenterology* **93:** 1026–1068, 1987.
24. Amenta F, Cavallotti C, Iacopino L and Erdo SL, Autoradiographic localization of the $GABA_A$ receptor agonist [^{3}H]-muscimol within rat kidney. *Pharmacology* **36:** 390–395, 1988.
25. Erdo SL, Baclofen binding to $GABA_B$ receptor sites in rat kidney. *Eur J Pharmacol* **184:** 305–309, 1990.
26. Goodyer PR, Rozen R and Scriver CR, A gamma-aminobutyric acid-specific transport mechanism in mammalian kidney. *Biochim Biophys Acta* **818:** 45–54, 1985.
27. Dobo E, Parducz A, Wolff JR and Erdo SL, Gaba-immunoreactive structures in rat kidney. In: *GABA Outside the CNS* (Ed. Erdo SL), pp. 155–166. Springer-Verlag, Berlin, 1991.
28. Fowler LJ, Analysis of the major amino acids of rat brain after *in vivo* inhibition of GABA transaminase by ethanolamine O-sulphate. *J Neurochem* **21:** 437–440, 1973.
29. Jung MJ, Lippert B, Metcalf BW, Bohlen P and Schechter PJ, γ-Vinyl-GABA (4-amino-hex-5-enoic acid), a new selective inhibitor of GABA-T: effects on brain GABA metabolism in mice. *J Neurochem* **77:** 797–802, 1977.
30. Schechter PJ and Tranier Y, The pharmacology of enzyme-activated inhibitors of GABA-transaminase. In: *Enzyme-Activated Irreversible Inhibitors* (Eds. Seiler N, Jung MJ and Koch-Weser J), pp. 149–162. Elsevier/North-Holland Press, 1978.
31. Anzelark G, Horton RW, Meldrum BS and Sawaya MCB, Anticonvulsant action of ethanolamine-O-sulphate and di-n-propylacetate and the metabolism of γ-amino butyric acid (GABA) in mice with audiogenic seizures. *Biochem Pharmacol* **25:** 413–417, 1976.
32. Fletcher A and Fowler LJ, γ-Aminobutyric acid metabolism in rat brain following chronic oral administration of ethanolamine-O-sulphate. *Biochem Pharmacol* **29:** 1451–1454, 1980.
33. Fowler LJ and John RA, Active site directed irreversible inhibition of 4-aminobutyrate aminotransferase by ethanolamine-O-sulphate *in vitro* and *in vivo*. *Biochem J* **130:** 569–573, 1972.
34. Fowler LJ, Kennard DA, Kirby N, Aitken A and Southan C, Studies on liver GABA-transaminase. In: *Biochemistry of Vitamin B_6* (Eds. Korpela T and Christen P), pp. 161–164. Birkhauser Verlag, Basel, 1987.
35. Qume M, Davies J and Fowler JD, Chronic treatment with the GABA transaminase inhibitors ethanolamine-O-sulphate and vigabatrin enhance basal GABA release from cross chopped hippocampal slices. *Br J Pharmacol* **105:** 170P.
36. Phillips NI and Fowler LJ, The effects of sodium valproate on γ-aminobutyrate metabolism and behaviour in naive and ethanolamine O-sulphate pretreated rats and mice. *Biochem Pharmacol* **31:** 2257–2261, 1982.
37. Salvador RA and Albers RW, The distribution of glutamic-gamma-aminobutyric transaminase in the nervous system of the rhesus monkey. *J Biol Chem* **234:** 922–925, 1959.
38. Lowe IP, Robins E and Eyerman GS, The fluorometric measurement of glutamic acid decarboxylase and its distribution in brain. *J Neurochem* **3:** 8–18, 1958.
39. Bradford MM, A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. *Anal Biochem* **72:** 248–254, 1976.
40. Lindroth P and Mopper K, High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatisation with o-pthaldialdehyde. *Anal Chem* **51:** 1667–1674, 1979.
41. Qume M, Whitton PS and Fowler LJ, The effect of chronic treatment with the GABA transaminase inhibitors γ-vinyl GABA and ethanolamine O-sulphate on the in vivo release of GABA from rat hippocampus. *J Neurochem* **64:** 2256–2261, 1995.

42. John RA, Rimmer EM, Williams J, Cole G, Fowler LJ and Richens A, Microvacuolation in rat brains after long term administration of GABA-transaminase inhibitors. *Biochem Pharmacol* **36:** 1467–1473, 1987.
43. Olgiati VR, Netti C, Guidobono F and Pecile A, The central GABAergic system and control of food intake under different experimental conditions. *Psychopharmacology* **68:** 163–167.
44. Zeneroli ML, Iulano E, Racagni G and Baraldi M, Metabolism and brain uptake of γ-aminobutyric acid in galactosamine-induced hepatic encephalopathy in rats. *J Neurochem* **38:** 1219–1222, 1982.
45. Crossley IR and Williams R. Progress in the treatment of chronic porta-systemic encephalopathy. *Gut* **25:** 89–98, 1984.
46. van Gelder N and Elliott KAC, Disposition of γ-aminobutyric acid administered to mammals. *J Neurochem* **3:** 139–143, 1958.
47. Scriver CR, Pueschel S and Davies E, Hyper-β-alaninemia associated with ω-aminoaciduria and β-aminobutyricaciduria, somnolence and seizures. *New Engl J Med* **274:** 635–643, 1966.
48. Perry TL, Kish SJ and Hansen S, Gamma-vinyl-group: effects of chronic administration on the metabolism of group and other amino compounds in the rat brain. *J Neurochem* **32:** 1641–1645, 1979.
49. Schafer DF, Fowler JM and Jones EA, Colonic bacteria: a source of β-aminobutyric acid in blood. *Proc Soc Exp Biol Med* **167:** 301–303, 1981.
50. Bolton JB, Rimmer E, Williams J and Richens A, The effect of vigabatrin on brain and platelet GABA-transaminase activities. *Br J Clin Pharmacol* **27:** 35S–42S, 1989.
51. Loscher W, GABA in plasma and cerebrospinal fluid of different species. Effects of gamma-acetylenic GABA, gamma-vinyl GABA and sodium valproate. *J Neurochem* **32:** 1587–1591, 1979.
52. Schechter PJ, Tranier Y, Jung MJ and Bohlen P, Audiogenic seizure protection by elevated brain GABA concentrations in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. *Eur J Pharmacol* **45:** 319–328, 1977.
53. Fowler LJ and John RA, Studies on the self inactivation of rabbit brain GABA transaminase by β-alanine. In: *Chemical and Biological Aspects of Vitamin B_6 Catalysis* (Ed. Evangelopoulos AE), pp. 67–75. Alan R. Liss Inc, New York, 1984.
54. Lippert B, Metcalf BW, Jung MJ and Casara P, 4-Amino-hex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric acid aminotransferase in mammalian brain. *Eur J Biochem* **74:** 441–445, 1977.
55. Porter TG and Martin DL, Evidence for feedback regulation of glutamate decarboxylase by γ-aminobutyric acid. *J Neurochem* **43:** 1464–1467, 1984.
56. Loscher W, Kretz F-J, Karavius T and Dillinger U, Marked increases of plasma gamma-aminobutyric acid concentrations in cirrhotic patients with portacaval shunts are not associated with alteration of cerebral functions. *Digestion* **49:** 212–220, 1991.
57. Minuk GY, Gauthier T, Gahaire A and Murphy LJ, The effect of GABA on serum and hepatic polyamine concentrations after partial hepatectomy in rats. *Hepatology* **14:** 685–689, 1991.