Biochemical Pharmacology, Vol. 31, No. 23, pp. 3917–3923, 1982.
Printed in Great Britain.

0006–2952/82/233917–07 $03.00/0
© 1982 Pergamon Press Ltd.

STUDIES ON THE RELATION OF γ-HYDROXYBUTYRIC ACID (GHB) TO γ-AMINOBUTYRIC ACID (GABA)

EVIDENCE THAT GABA IS NOT THE SOLE SOURCE FOR GHB IN RAT BRAIN

O. CARTER SNEAD, III,* CHUN-CHE LIU and LARRY J. BEARDEN
Department of Pediatrics and The Neuroscience Program, University of Alabama in Birmingham, School of Medicine, Birmingham, AL, U.S.A.

(*Received* 27 *November* 1981; *accepted* 27 *April* 1982)

Abstract—The effects of γ-aminobutyric acid (GABA)-α-oxoglutarate aminotransferase (GABA-T) inhibitors, L-glutamic acid decarboxylase (GAD) inhibitors, and antipetit mal anticonvulsants on γ-hydroxybutyric acid (GHB) and GABA were studied. Treatment with anticonvulsants and GABA-T inhibitors resulted in an increase in steady-state brain levels of both GHB and GABA. GAD inhibitors produced markedly decreased levels of brain GABA but no change in GHB concentrations. Studies of GHB derived exclusively from GABA showed that GABA-T inhibitors which produced an elevation of steady-state levels of GHB in brain also resulted in a decrease in GABA-derived GHB. Intracerebroventricular (i.c.v.) administration of GABA, putrescine, and 1,4-butanediol all produced significant elevations in brain GHB, but GABA-T inhibitors blocked this effect of GABA and putrescine. These data suggest that there may be another source for GHB in brain in addition to GABA and raise the possibility that 1,4-butanediol may be that source.

γ-Hydroxybutyric acid (GHB) is a four-carbon compound that occurs naturally in mammalian brain [1] and possesses a number of diverse neuropharmacologic and neurophysiologic properties [2]. The parent compound for GHB in brain is usually considered to be γ-aminobutyric acid (GABA) [3–5]. GABA is formed from glutamic acid by L-glutamic acid decarboxylase (GAD; EC 4.1.1.1.5) and then transaminated by GABA-α-oxoglutarate aminotransferase (GABA-T; EC 2.6.1.19) to succinic semialdehyde which is reduced to GHB by an NADPH-dependent oxido-reductase [6–9]. GHB is then metabolized back to succinic acid which enters the Krebs cycle [10–12].

We have demonstrated recently that anticonvulsant drugs used specifically in the treatment of petit mal epilepsy produce a dose-dependent increase in steady-state levels of GHB in rat brain [13]. One of these drugs, sodium valproate, would be expected, by virtue of what is known of its action on the above described metabolic pathway of GABA, to result in a decrease in GHB [13]. Therefore, the object of these experiments was to examine possible mechanisms of anticonvulsant–GHB interaction, to ascertain whether or not GABA is the only precursor for GHB and, if not, to examine alternative sources for GHB.

MATERIALS AND METHODS

Drugs. Ethosuximide and sodium valproate were supplied by Parke, Davis & Co. (Detroit, MI) and Abbott Laboratories (North Chicago, IL) respectively. γ-Vinyl GABA (GVG) and γ-acetylenic GABA (GAG) were provided by Merrell National Laboratories (Cincinnati, OH). GABA, GHB, 1,4-butanediol, isonicotinic hydrazide (INH), DL-allylglycine, 3-mercaptopropionic acid (3-MP), aminooxyacetic acid (AOAA), and putrescine were all obtained from the Sigma Chemical Co. (St. Louis, MO). Sterile saline was used as the vehicle for all drugs. γ-{2,3-^{3}H}Aminobutyric acid, 29.3 Ci/mmole, was obtained from the New England Nuclear Corp. (Boston, MA). The radioisotope was lyophilized and reconstituted at a concentration of 1 μCi/ml in normal saline as described by Gold and Roth [4]. The ammonium salt of γ-{2,3-^{3}H}hydroxybutyric acid, 20.3 Ci/mmole, was synthesized by the Amersham Corp. (Arlington Heights, IL).

Animals. Male Sprague–Dawley rats [Crl:CD(SD)BR, Charles River] weighing 200–350 g were used for all experiments. The animals were watered and fed *ad lib.* and were maintained on a 12-hr light–dark cycle. For experiments involving intracerebroventricular (i.c.v.) administration of drug, cannulas were implanted stereotactically in the lateral ventricle under pentobarbital anesthesia. Injections (i.c.v.) were made by hand not less than 48 hr post operatively, in a volume of 10 μl.

Assays. All animals were decapitated, and the brains were rapidly excised. For the comparative studies of GHB and GABA, the cerebellum was dissected within 5–10 sec of brain excision and placed in liquid nitrogen. GHB was assayed in whole brain and cerebellum by a modification of an electron capture gas–liquid chromatographic (GLC) technique described previously [13, 14]. Derivatization of the sample was as described, but a 25-meter fused

* Address reprint requests and correspondence to: O. Carter Snead, M.D., Room 202, The Children's Hospital, 1601 6th Avenue South, Birmingham, AL 35233, U.S.A.

silica capillary column coated with liquid methyl silicone was utilized for separation. A splitless injection of 1–3 μl of derivatized sample was made with the following conditions: injection port temperature, 250°; detection port temperature, 300°; and oven temperature, 65°. Argon–methane was used as the carrier gas with the following flow rates; column flow, 1 ml/min; make-up flow 36 ml/min; column head flow, 58 ml/min.

GABA content of cerebellum was determined by the flame ionization GLC technique of Löscher [15].

GABA-T inhibitor and anticonvulsant experiments. The effects of both the nonspecific GABA-T inhibitor AOAA and the specific, irreversible GABA-T inhibitors GAG and GVG on whole brain GHB were determined. The doses of these drugs and time from dose to sacrifice are listed in Table 1. Since sodium valproate and ethosuximide have the most pronounced effects on brain GHB levels of the antipetit mal anticonvulsants [13], they were selected for use in these experiments. The doses and times to sacrifice for the GABA-T inhibitors were those reported to be associated with maximum inhibition of GABA-T and elevation of brain GABA concentration [16–19]. To be certain, however, comparative studies of the effects of these compounds, as well as the anticonvulsants, on GHB and GABA content in cerebellum were also done.

Once the biochemical responses of GHB and GABA to pharmacologic manipulation with GABA-T inhibitors and anticonvulsants were determined, combination experiments were done. Animals were pretreated with GABA-T inhibitors prior to administration of anticonvulsants, and brain levels of GHB were determined. The reverse experiments were also done. Drug design, doses, and time from last drug to sacrifice are shown in Table 3.

Use of GAD inhibitors. The GAD inhibitors used were 3-MP, INH, and DL-allylglycine. Drugs were given in doses indicated in Table 4, and the animals were killed at the onset of generalized convulsive seizures, the time when GAD activity and GABA levels have been reported to be significantly depressed [15, 20–22]. Again, in order to assess the effect of this treatment modality on GABA concentration, we examined concentrations of GABA and GHB in the cerebellum after GAD inhibition.

GABA-derived GHB experiments. {^{3}H}GABA (10 μCi) was administered i.c.v., and then the disintegrations per min of {^{3}H}GHB were determined at various times after i.c.v. administration [4]. From the curve generated by these experiments (Fig. 1), a time of 3 min after administration of {^{3}H}GABA was selected for sacrifice following i.c.v. administration of the radioisotope. Animals were then treated with either saline, GAG, GVG, ethosuximide, or sodium valproate in the doses indicated in Table 6, and the conversion of {^{3}H}GABA to {^{3}H}GHB was determined 3 min after i.c.v. {^{3}H}GABA.

This method of determining GABA-derived GHB depends on the lactonization of GHB to γ-butyrolactone (GBL). Hence, the tracer radioactivity represents {^{3}H}GBL. The method utilizes a cation exchange resin to remove residual GABA and other organic acids that would be protonated in an extremely acid medium. However, to ascertain whether any {^{3}H}GABA contributed to the final counts present, we also performed the following experiments. We injected cold GABA i.c.v. in amounts ranging from 10 to 500 μg, decapitated the animals 3 min later, and carried the brains through the same procedure as the {^{3}H}GABA experiments. The aqueous effluent from the resin was titrated to a pH of 6.5 and 100 μl was assayed for GABA by the radioreceptor assay of Enna and Snyder [23]. Similarly, the final benzene extract was evaporated under nitrogen to 1 ml and reextracted with 3 ml of water, 100 μl of which was assayed for GABA. These experiments were done on four animals at each dose and four control animals injected with sterile water.

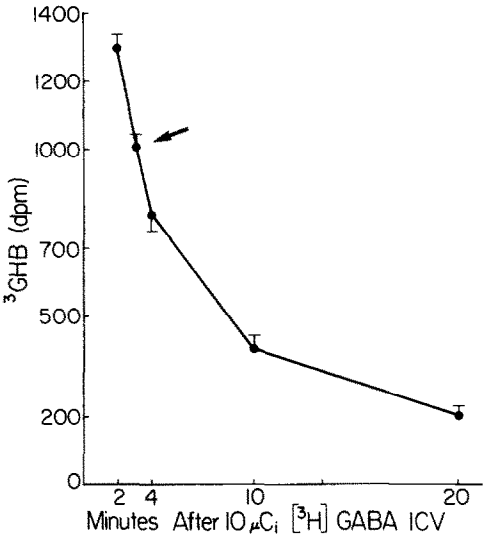

Fig. 1. Recovery of {^{3}H}GHB at various times after i.c.v. administration of 10 μCi {^{3}H}GABA. Each value is the mean ± S.E.M. of six determinations. The arrow designates the time after {^{3}H}GABA administration and the dpm of recorded {^{3}H}GHB that were used for the GABA-derived GHB experiments shown in Table 5.

To confirm that the radioactivity on the final benzene extract represents {^{3}H}GBL, we added authentic {^{3}H}GHB to an aqueous solution and carried it through the procedure outlined above. The final benzene extract was evaporated to 10 μl and spotted onto the preabsorbant area of an LK6DF, 5 × 20 cm channeled thin-layer chromatographic plate (Whatman Inc., Clifton, NJ). The thin-layer chromatography was also done on brain homogenate developed as above in animals killed 3 min after receiving 10 μCi of {^{3}H}GABA. The benezene effluent from these brains was evaporated to 10 μl and spotted as above. The chromatograms were developed in one of two separate solvent systems for 10 cm. The solvent systems used were either ethanol and water (5:5) or ethanol and methanol (6:2). Each cm was cut out, suspended in Liquifluor (New England Nuclear), and counted.

GHB precursor experiments. Possible precursors for GHB examined in these experiments included putrescine, GABA, and 1,4-butanediol. These drugs were given i.c.v. in the doses indicated in Table 6. Animals were killed 5 min later, and whole brain

GHB was determined. Once a response of GHB to precursor administration was ascertained, the experiments were repeated in animals pretreated with AOAA 60 min prior to administration of the precursor.

Statistics. Statistical analysis was performed by comparing each treated group with the control group, using the unpaired *t*-test.

RESULTS

GHB assay. For the GHB determinations, the capillary column provided better resolution and sensitivity than the 6 m packed column we had used previously for the assay (Fig. 2). The increased sensitivity afforded by the capillary column allowed us to assay a single, rather than pooled, cerebellum for GHB.

GABA-T and anticonvulsants. Each drug in these studies produced a significant increase in whole brain concentration of GHB (Table 1). The most dramatic change in brain GHB was the 60–80% increase brought about by the irreversible GABA-T inhibitor, GVG, and the anticonvulsants. AOAA produced a lesser, but still significant, rise of 17%. Studies of GHB and GABA in cerebellum (Table 2) showed that GVG, GAG, and sodium valproate all produced significant increases of GABA as well as GHB, while ethosuximide produced an increase in GHB but no change in GABA levels.

Pretreatment with either GABA-T inhibitor or anticonvulsant prior to treatment with one or the other type of drug resulted in a potentiation of the GHB increase with the combination producing a greater increase of brain GHB than either drug separately (Table 3).

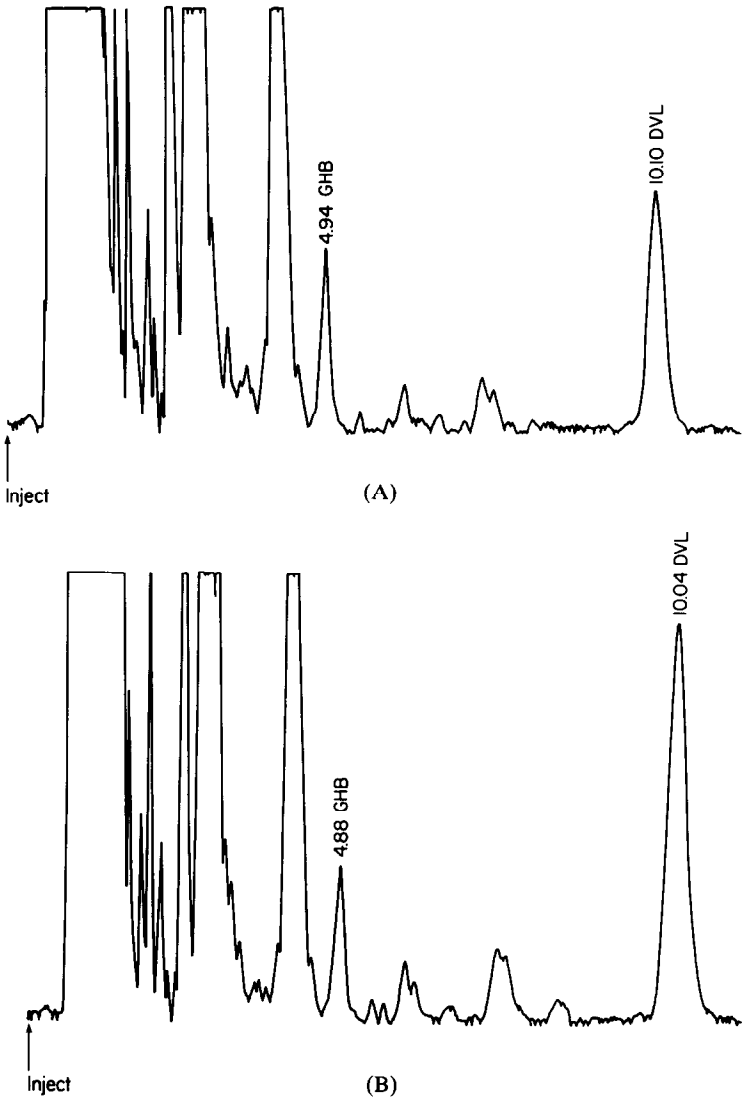

Fig. 2. Gas chromatograms obtained from heptofluorobutyryl methyl diester derivatives of: (A) 1 μg of GHB and 2 μg deltavalerolactone (DVL), the internal standard: and (B) a single rat brain derivatized in the same fashion as the standard in (A), again with DVL added as the internal standard. The samples were separated with a capillary column. The numbers designate retention time in minutes.

Table 1. Effects of GABA-T inhibitors and anticonvulsants on whole brain concentrations of GHB*

Drug	Dose (mg/kg)	Time from dose to sacrifice (hrs)	GHB concn (nmoles/g) ± S.E.M.	% Change of GHB concn from control	P
Control			2.08 ± 0.26		
Aminooxyacetic acid	25	1	2.56 ± 0.28	+17	< 0.05
Gamma-vinyl GABA	1200	18	3.55 ± 0.29	+63	< 0.05
Gamma-vinyl GABA	1200	60	3.66 ± 0.41	+68	< 0.05
Gamma-acetylenic GABA	120	18	3.71 ± 0.35	+70	< 0.05
Sodium valproate	400	1	3.90 ± 0.42	+79	< 0.05
Ethosuximide	300	1	3.64 ± 0.31	+67	< 0.05

* N = 8–10 for each experiment.

Table 2. Effects of GABA-T inhibitors and anticonvulsants on cerebellar concentrations of GHB and GABA*

	Mean concentrations ± S.E.M.	
Drug	GHB (nmoles/g)	GABA (μmoles/g)
Control	5.37 ± 0.44	1.12 ± 0.03
Gamma-vinyl GABA	9.13 ± 0.83†	4.57 ± 0.76†
Sodium valproate	8.97 ± 0.94†	2.40 ± 0.25†
Ethosuximide	9.23 ± 0.11†	1.23 ± 0.05

* Dose and time from dose to sacrifice = dose as in Table 1 with an 18-hr time from dose to sacrifice for GVG. N = 6–8 for all experiments.
† $P < 0.05$.

Table 3. Effects of anticonvulsant–GABA-T inhibitor combinations on whole brain GHB concentrations

GABA-T inhibitor	Time to anticonvulsant (hr)	Anticonvulsant	Time to sacrifice (hr)	GHB concn (nmoles/g ± S.E.M.)	% Change from control
Saline	1	Saline	1	2.31 ± 0.28	
Aminooxyacetic acid	1	Saline	1	2.78 ± 0.25	+19
Saline	1	Ethosuximide	1	3.93 ± 0.41	+70
Aminooxyacetic acid	1	Ethosuximide	1	4.67 ± 0.42	+102*
Saline	1	Sodium valproate	1	4.18 ± 0.44	+81
Aminooxyacetic acid	1	Sodium valproate	1	5.20 ± 0.49	+125*
Gamma-vinyl GABA	18	Saline	1	3.81 ± 0.44	+65
Saline	18	Ethosuximide	1	3.69 ± 0.32	+67
Gamma-vinyl GABA	18	Ethosuximide	1	4.30 ± 0.34	+86*
Gamma-vinyl GABA	18	Sodium valproate	1	6.65 ± 0.69	+188*

* Significantly ($P < 0.05$) increased over control, GABA-T-inhibitor, or anticonvulsant.

GAD inhibitors. All three GAD inhibitors that were used produced no change in the steady-state whole brain or cerebellar concentrations of GHB in the face of a significant 50–75% decrease in cerebellar GABA levels (Table 4).

GABA-derived GHB. The radioreceptor assay, sensitive to 10^{-12} M concentrations of GABA, showed no detectable GABA in either the resin effluent or the aqueous extract of the final benzene phase. This eliminates the possibility that the radioactivity in the final benzene extract represented residual {^{3}H}GABA. The presence of {^{3}H}GBL in the final benzene effluent was confirmed by thin-layer chromatography. The R_f of the {^{3}H}GBL, in those experiments in which {^{3}H}GHB was carried through acidification and heating and then was passed through the resin and extracted with benzene, was 0.9 for the ethanol–water system and 0.75 for the ethanol–methanol system. Radioactivity was concentrated in those same areas on the thin-layer chromatographic plate in the benzene extract of effluent from brain extracts in animals that had received i.c.v. {^{3}H}GABA.

The GABA-T inhibitors used in this study produced a 50% inhibition of formation of {^{3}H}GHB from {^{3}H}GABA. Ethosuximide-treated animals showed no change in the amount of {^{3}H}GHB formed, while those treated with sodium valproate had a 36% increase in formation of {^{3}H}GHB (Table 5).

Precursors. Putrescine, GABA, and 1,4-butanediol all produced significant elevations of whole brain

Table 4. Comparison of the effects of GAD inhibitors on brain concentrations of GABA and GHB

Drug	Dose (mg/kg)	Generalized convulsive seizures	% Change in concentration from control (N)		
			Whole brain GHB	Cerebellar GHB	Cerebellar GABA
Saline					
Isonicotinic hydrazide	250	+	−4.8 (6)	−3.9 (6)	−53* (6)
DL-Allylglycine	300	+ +	0 (4)	0 (14)	−67* (4)
3-Mercaptoproprionic acid	60	+	−2.3 (4)	−6.1 (4)	−73* (4)

* $P < 0.05$.

GHB concentration after i.c.v. administration (Table 6). The greatest increase, 80–100%, was seen with putrescine and 1,4-butanediol, with GABA producing a less dramatic, but still significant, increase of 30%. The GABA and putrescine-induced changes in whole brain GHB were blocked by pretreatment with AOAA. However, pretreatment with AOAA potentiated the 1,4-butanediol-induced increase in GHB.

DISCUSSION

These data suggest that sodium valproate and ethosuximide may have different mechanisms of action in their effects on brain GHB. Both are capable of elevating GHB in brain, but sodium valproate also elevates GABA while ethosuximide has no effect on GABA in dosages that result in a considerable increase in GHB (Table 2). Further, sodium valproate treatment was associated with increased formation of GHB from GABA, but ethosuximide had no effect on the flux of GABA to GHB. These actions of sodium valproate suggest that this drug may produce an increase in GHB by its inhibition of succinic semialdehyde dehydrogenase (succinate-semialdehyde:NAD oxidoreductase; EC.1.2.1.16) and thus of GHB catabolism. Sodium valproate is also known to inhibit GABA-T, succinic acid dehydrogenase, and an NADPH-dependent aldehyde reductase responsible for reduction of succinic semialdehyde, with the inhibition of the aldehyde reductase being considerably stronger than that of the other two enzymes [24]. On the surface then, it would seem that sodium valproate, in inhibiting the synthetic enzyme of GHB, should produce a decrease rather than an increase of brain GHB [13]. Evidence [25–29] has recently accumulated, however, indicating that there are several aldehyde reductases in mammalian brain. Of these multiple reductases, the NADPH-dependent oxidoreductase which is specific for the reduction of succinic semialdehyde to GHB is resistant to inhibition by sodium valproate [8, 9, 28]. Hence, although the succinic semialdehyde dehydrogenase catalyzes a reaction that competes with that catalyzed by the reductase, the effect of valproate on GHB metabolism seems to be limited to GABA-T and succinic semialdehyde dehydrogenase. Our radioisotope studies suggest that the latter enzyme is predominantly affected.

With regard to the {^{3}H}GABA experiments, there exists a possibility that these data may represent compartmental changes of GABA rather than real changes in the flux of GABA to GHB. For example, the increase in GABA produced by pretreatment with GVG could decrease the specific activity of {^{3}H}GABA in brain. The apparent decrease in counts from control observed in the {^{3}H}GABA–GVG experiments could thus, theoretically, reflect a low specific activity of the GABA pool rather than decreased GHB formation from GABA. However, this thesis is not supported by the sodium valproate data. This drug also produces an increase in brain GABA. Using the above line of reasoning, sodium valproate would also be expected to decrease the specific activity of the GABA pool. However, the final dpm in those experiments were increased by

Table 5. Effects of GABA-T inhibitors or anticonvulsants on conversion of {^{3}H}GABA to {^{3}H}GHB

Drug	Dose (mg/kg)	Time to administration of {^{3}H}GABA (hr)	Disentegrations/min of {^{3}H}GHB 3 min after {^{3}H}GABA i.c.v. (mean ± S.E.M.)	N
Saline			1100 ± 96	15
Gamma-vinyl GABA	1200	18	464 ± 32*	8
Gamma-acetylenic GABA	120	18	497 ± 53*	7
Sodium valproate	300	1	1500 ± 123†	7
Ethosuximide	300	1	1053 ± 102	8

* Significantly ($P < 0.05$) decreased from control.
† Significantly ($P < 0.05$) increased from control.

36% over control, indicating that this experimental maneuver does reflect the flux from GABA to GHB.

Another consideration in trying to draw conclusions from the GABA-derived GHB experiments is the question of whether or not the final radioactivity represents {^{3}H}GHB or some other compound. Possible candidates for sources of radioactivity other than GABA or GHB would be succinic acid or the Krebs cycle intermediates [10–12]. The presence of {^{3}H}GABA was excluded by the radioreceptor assay experiments and the presence of {^{3}H}GHB was confirmed by the thin-layer chromatography. Thus, the final radioactivity does represent {^{3}H}GHB.

Our data also strongly support the thesis that there exists in brain a source for GHB, additional to GABA. The demonstration of a 30–40% decrease in GABA-derived GHB in the face of a 60–80% increase in steady-state levels of GHB by GABA-T inhibitors and a significant decrease in GABA with no change in GHB by GAD inhibitors make it unlikely that GABA is the only source for GHB in brain.

There are additional lines of evidence developed by others that also support the presence of precursors for GHB other than GABA. The first of these consists of data showing the presence of GHB in extraneural sites such as liver, heart, kidney, and muscle, tissues notable for their absence of GABA [30, 31]. Similarly, liver and kidney have a concentration of $NADP^+$-dependent oxidoreductase (D-glucuronate reductase, EC 1.1.1.19), the primary catabolic enzyme for GHB, 10–20 times that in brain [32]. These data suggest that in the periphery, at least, there is an alternative source for GHB. Other supporting evidence can be found in recently published experiments dealing with GHB in developing brain [33] which demonstrate that the ontogeny of GHB differs significantly from that of GABA, GABA-T or GAD.

Of the three possible GHB precursors that we examined, 1,4-butanediol appears to be the most likely candidate. Putrescine appears to be converted to GHB via GABA [34] since the elevated GHB produced by putrescine was not seen in animals pretreated with a GABA-T inhibitor. Since the conversion of putrescine to GABA involves a transamination, the transaminase inhibition could block the conversion of putrescine to GABA as well as that of GABA to succinic semialdehyde. The problem with the hypothesis that 1,4-butanediol is a precursor for GHB in brain is that 1,4-butanediol has yet to be demonstrated as a natural constituent of brain, although its presence has been postulated in the form of "diol-lipids" [35].

Table 6. Changes in whole brain GHB after i.c.v. administration of possible precursors with and without GABA-T inhibitors

Drug	Dose (μg)	Time to sacrifice (min)	GHB concn (nmoles/g) ± S.E.M.	% Change in GHB	N
Saline		5	2.13 ± 0.14		20
Aminooxyacetic acid (25 mg/kg, i.p.)		60	2.53 ± 0.21	+20*	16
Putrescine	25	5	3.83 ± 0.41	+82*	6
Putrescine + aminooxyacetic acid†	25	5	2.15 ± 0.21	0	6
GABA	25	5	2.77 ± 0.25	+30*	6
GABA + aminooxyacetic acid	25	5	2.44 ± 0.29	+15	4
1,4-Butanediol	25	5	4.26 ± 0.39	+100*	4
1,4-Butanediol	100	5	4.28 ± 0.44	+100*	4
1,4-Butanediol + aminooxyacetic acid	25	5	5.26 ± 0.57	+147‡	6

* Significantly changed ($P < 0.05$) from saline control
† In all aminooxyacetic acid combination experiments, the aminooxyacetic acid was given 60 min prior to the second drug.
‡ Significantly ($P < 0.05$) changed from aminooxyacetic acid control.

An alternative explanation to the presence of a GHB precursor other than GABA which might explain our data deals with the kinetics of GHB dehydrogenase. This is a recently described enzyme which is an $NADP^+$-linked alcohol oxido-reductase which catalyzes interconversion of GHB and succinic semialdehyde [32]. Conceivably the pharmacologic manipulations carried out in the current experiments could have altered the activity of this enzyme such that the catabolism of GHB was slowed signifciantly. Similarly, since GHB oxidation is linked to D-glucuronate reduction and the availability of glucose [36], alterations of either of these conditions by the drugs used in our experiments could alter GHB catabolism.

Experiments are currently underway in our laboratory to address these questions as well as that of the presence of 1,4-butanediol in brain.

Acknowledgements—We are grateful to Parke, Davis & Co., Abbot Laboratories, and Merrell National Research Laboratories for the drugs used in these studies. This work was supported in part by NINCDS Grants K07 NS 00484-02 and R01 NS 17117-01.

REFERENCES

1. R. H. Roth and N. J. Giarman, *Biochem. Pharmac.* **19**, 1087 (1970).
2. O. C. Snead, *Life Sci.* **20**, 1935 (1977).
3. R. H. Roth and N. J. Giarman, *Biochem. Pharmac.* **18**, 247 (1969).
4. B. I. Gold and R. H. Roth, *J. Neurochem.* **28**, 1069 (1977).
5. E. Santaniello, A. Manzocchi and L. Tosi, *J. Neurochem.* **31**, 1117 (1978).
6. R. A. Anderson, R. F. Ritzmann and B. Tabakoff, *J. Neurochem.* **28**, 633 (1977).
7. J. F. Rumigny, M. Maitre, C. Cash and P. Mandel, *J. Neurochem.* **36**, 1433 (1981).
8. J. F. Rumigny, M. Maitre, C. Cash and P. Mandel, *Fedn Eur. Biochem. Soc. Lett.* **117**, 111 (1980).
9. C. D. Cash, M. Maitre and P. Mandel, *J. Neurochem.* **33**, 1169 (1979).
10. J. D. Doherty, R. W. Stout and R. H. Roth, *Biochem. Pharmac.* **24**, 469 (1975).
11. H. Möhler, A. J. Patel and R. Balázs, *J. Neurochem.* **27**, 253 (1976).
12. J. D. Doherty and R. H. Roth, *J. Neurochem.* **30**, 1305 (1978).
13. O. C. Snead III, L. H. Bearden and V. Pegram, *Neuropharmacology* **19**, 47 (1980).
14. J. D. Doherty, O. C. Snead III and R. H. Roth, *Analyt. Biochem.* **65**, 268 (1975).
15. W. Löscher, *Biochem. Pharmac.* **28**, 1397 (1979).
16. M. J. Jung, B. Lippert, B. W. Metcalf, P. Böhlen and P. J. Schechter, *J. Neurochem.* **29**, 797 (1977).
17. R. C. Collins and S. Mehta, *Brain Res.* **157**, 311 (1978).
18. W. Löscher, *Naunyn-Schmiedeberg's Archs Pharmac.* **315**, 119 (1980).
19. B. W. Metcalf, *Biochem. Pharmac.* **28**, 1705 (1979).
20. P. M. Beart and K. Bilal, *Biochem. Pharmac.* **28**, 449 (1979).
21. R. W. Horton, A. G. Chapman and B. S. Meldrum, *J. Neurochem.* **30**, 1501 (1978).
22. R. W. Horton, A. G. Chapman and B. S. Meldrum, *J. Neurochem.* **33**, 745 (1979).
23. S. J. Enna and S. H. Snyder, *J. Neurochem.* **26**, 221 (1976).
24. S. R. Whittle and A. J. Turner, *J. Neurochem.* **31**, 1453 (1978).
25. B. Tabakoff and J. P. Von Wartburg, *Biochem. biophys. Res. Commun.* **63**, 957 (1975).
26. P. L. Hoffman, B. Wermuth and J. P. Von Wartburg, *J. Neurochem.* **35**, 354 (1980).
27. P. L. Hoffman, B. Wermuth and J. P. Von Wartburg, *Adv. exp. Med. Biol.* **132**, 749 (1981).
28. S. R. Whittle and A. J. Turner, *Biochim. biophys. Acta* **657**, 94 (1981).
29. J. F. Rumigny, C. Cash, P. Mandel, G. Vincendon and M. Maitre, *Fedn Eur. Biochem. Soc. Lett.* **134**, 96 (1981).
30. R. H. Roth, *Biochem. Pharmac.* **19**, 3013 (1970).
31. T. Nelson, E. Kaufman, J. Kline and L. Sokoloff, *J. Neurochem.* **37**, 1345 (1981).
32. E. E. Kaufman, T. Nelson, C. Goochee and L. Sokoloff, *J. Neurochem.* **32**, 699 (1979).
33. O. C. Snead III and B. J. Morley, *Devl Brain Res.* **1**, 579 (1981).
34. N. Seiler, T. Schmidt-Glenewinkel and S. Sarhan, *J. Biochem., Tokyo* **86**, 277 (1979).
35. L. D. Bergelson, V. A. Vaver, N. V. Prokazova, A. N. Ushakov and G. A. Popkova, *Biochim. biophys. Acta* **116**, 511 (1966).
36. E. E. Kaufman and T. Nelson, *J. biol. Chem.* **256**, 6890 (1981).