TABLE 2. EFFECT OF 5-METHOXYINDOLE-2-CARBOXYLIC ACID ON ALLOXANIZED MICE*

Expt.	No. of mice/group	Median blood sugar (mg/100 ml)		
		Control	Tolbutamide (50 mg/kg, i.p.)	5-Methoxyindole-2-carboxylic acid (100 mg/kg, i.p.)
1	3	478	483	330
2	6	504	530	378
3	10	412	479	354

* Alloxanized male mice, dosed as indicated, were bled 60 min after dosing. Medians are used to avoid large changes in mean from occasional "tolbutamide-responders" and occasional hypoglycemic moribund mice. However, analysis of variance, without correcting for or discarding outlying values, shows 5-methoxyindole-2-carboxylic acid to lower blood sugar significantly ($P = 0{\cdot}02$), while tolbutamide does not.

at the 2-position or a potential carboxyl group at the 2-position; (3) the 1-position must be unsubstituted, as must be the 6- and 7-positions. A variety of electron-releasing groups seem to be suitable for the 5-position.

Studies on the mechanism of action of these compounds suggest that they inhibit gluconeogenesis.[8–10]

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The effect of γ-hydroxybutyric acid on amino acid levels in brain

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γ-HYDROXYBUTYRIC acid (GHB), a general anesthetic with certain unusual properties, has recently been extensively studied in experimental animals and in man.[1–4] The chemical structure of GHB is quite similar to that of γ-aminobutyric acid (GABA), which is a normal constituent of brain with

several actions suggesting a possible role as an inhibitory neurotransmitter. These similarities have led to speculation that the central depressant effects of GHB may in some way be mediated through GABA, and this idea has received support from two reports of increased levels of GABA in brain after administration of GHB.[5, 6] However, other investigators have reported that GHB does not alter GABA levels in brain,[7, 8] although labeled GHB is converted by brain to GABA,[8] and labeled GABA to GHB.[9] In the present study we report the effects of anesthetic doses of GHB alone and in combination with insulin on the concentration of GABA, glutamic acid, aspartic acid and glutamine in rat brain.

MATERIALS AND METHODS

Adult Sprague–Dawley rats were given food and water *ad libitum* except in experiments involving the effects of insulin. In this case, control and experimental animals were fasted for 20 hr before administration of drugs. Sodium γ-hydroxybutyrate (Wy-3478) was obtained from Wyeth Laboratories, Philadelphia, Pa., and was injected i.p. as a 20% (w/v) aqueous solution. Crystalline zinc insulin (Lilly) was also administered i.p. Preliminary experiments demonstrated that a dose of 0·8 to 1 g/kg of GHB* was required to consistently produce sleep, as judged by loss of the righting reflex. Rats were sacrificed 40 min after injection of 1 g per kg of GHB and 3 hr after insulin administration. When the lower dose (800 mg/kg) of GHB was used, either alone or in combination with insulin, rats were sacrificed 1 hr after administration of GHB.

Amino acids in a 5% trichloroacetic acid extract of brain were isolated by column chromatography, using a previously described modification[10] of the method of Berl *et al.*[11]

RESULTS AND DISCUSSION

In agreement with other investigators who have determined GABA quantitatively, either by a specific enzymatic assay,[7] or by isolation of the amino acid,[8] we found no change in GABA levels during GHB-induced sleep (Table 1). Previous reports of small increases in GABA produced by GHB are probably attributable to the semiquantitative methods used in those studies,[5, 6] which were based on estimating the density of the GABA spot on paper strips after electrophoretic separation of amino acids.

Although no changes were observed in the concentrations of GABA, glutamic acid and glutamine after administration of GHB to unfasted animals, there was a significant increase in the concentration of aspartic acid in brain (Table 1).

TABLE 1. EFFECT OF γ-HYDROXYBUTYRATE (GHB) ON THE CONCENTRATION OF AMINO ACIDS IN RAT BRAIN

Treatment	N	(μmoles amino acid/g brain ± S.E.M.)			
		GABA	Glutamine	Glutamic acid	Aspartic acid
Control	6	2·78 ± 0·08	7·34 ± 0·28	10·78 ± 0·10	2·68 ± 0·06
GHB (1 g/kg)	10	2·80 ± 0·05	7·34 ± 0·09	10·73 ± 0·01	3·52 ± 0·06
Change (%)		0	0	0	+31

Laborit[1] has reported that fasting potentiates the anesthetic action of GHB and that small doses of insulin having no hypnotic action by themselves can induce sleep in rats when administered together with subanesthetic doses of GHB. It is of interest that insulin is reported to produce increases in brain aspartic acid levels[12, 13] similar to those found by us to accompany GHB anesthesia, and that GHB produces an electrographic pattern of seizure activity[14] similar to that preceding insulin coma. In an attempt to determine whether the observed increase in brain aspartic acid levels was indicative of a common factor leading to or resulting from the loss of consciousness produced by these two agents, amino acid levels were analyzed in fasted rats given insulin and GHB separately and in combination. In confirmation of previous reports,[12, 13] insulin in doses of 10–100 U per kg (i.p.)

* All doses of GHB refer to the sodium salt.

was found to produce a significant increase (40–170 per cent) in aspartic acid and decreases in the levels of GABA, glutamic acid and glutamine in brain. However, 2·5 U per kg of insulin increased the aspartic acid concentration by only 26 per cent and no significant further increase was produced when this dose of insulin was given together with a minimum anesthetic dose (800 mg/kg) of GHB (Table 2). It would therefore appear that the increase in aspartic acid produced by insulin and by

TABLE 2. EFFECT OF INSULIN AND γ-HYDROXYBUTYRATE (GHB) ON THE CONCENTRATION OF AMINO ACIDS IN RAT BRAIN

Treatment	N	(μmoles amino acid/g brain ± S.E.M.)			
		GABA	Glutamine	Glutamic acid	Aspartic acid
Control (fasted)	4	2·75 ± 0·04	5·15 ± 0·24	11·40 ± 0·12	3·18 ± 0·05
+GHB (0·8 g/kg)	3	2·47 ± 0·03	6·45 ± 0·16	11·27 ± 0·07	3·81 ± 0·05
+Insulin (2·5 U/kg	5	2·49 ± 0·04	5·15 ± 0·31	10·17 ± 0·25	3·99 ± 0·18
+Insulin and GHB	4	2·49 ± 0·08	5·49 ± 0·15	9·89 ± 0·19	4·12 ± 0·05

GHB involves a common biochemical mechanism affecting aspartic acid metabolism, since otherwise one would expect an additive effect on aspartic acid levels when both insulin and GHB are administered together. The fact that brain aspartic acid levels are capable of increasing to a much greater extent than observed here is demonstrated by the considerably larger increases (up to 8·7 μmoles/g of brain) produced by insulin at a dose of 25 U per kg. Hypoglycemia is apparently not a factor in the GHB-induced rise in brain aspartic acid levels since, like many other anesthetics, GHB increases the glucose concentration in blood[15] and brain.[8, 15, 16]

Fasting alone produced a 30 per cent decrease in glutamine and increased aspartic acid levels by 19 per cent. Although GHB produced no change in glutamine in unfasted rats, when administered to fasted animals, the glutamine concentration rose by 25 per cent to reach levels almost the same as those found in unfasted normal controls (Table 2). The ability of GHB to restore glutamine levels in fasted animals is probably due to its hyperglycemic action. Other changes in brain amino acid levels in fasted rats before or after administration of drugs were generally small (less than 10 per cent) and are not considered to be of biological significance.

It is not yet possible to evaluate the extent to which the pharmacological effects of GHB may be mediated through alterations in brain amino acid metabolism. Godin and Mark[16] have reported that 500 mg/kg of γ-butyrolactone decreases the incorporation of labeled glucose into free aspartic acid, glutamic acid, GABA and glutamine relative to the specific activity of brain glucose and in the absence of any change in amino acid concentration. Pentobarbital anesthesia has been reported to have a similar effect on the incorporation of glucose into the free amino acids of brain, but differs from GHB in that it does not decrease the formation of GABA from glutamic acid.[16, 17] Since the first step in GHB metabolism is β-oxidation,[18] the effects of GHB on amino acid metabolism cannot be due to the provision of additional 4-carbon units at the succinate level.

The reported conversion of GABA to GHB *in vivo* and *in vitro* again raises the possibility that GHB is a normal metabolite in brain, albeit in very low concentrations. Mitoma and Neubauer[8] have demonstrated the conversion of labeled GHB to GABA in brain homogenates and conclude that this does not occur by way of glutamic acid, since the specific activity of the GABA formed was considerably higher than that of glutamic acid. However, the well demonstrated compartmentation of glutamic acid metabolism in brain is known to give rise, under certain circumstances, to glutamine and GABA of a higher specific activity than that of the precursor glutamic acid.[19, 20] The results of Mitoma and Neubauer[8] would therefore suggest that GHB may be converted to GABA by way of this small but highly labeled pool of glutamic acid, leading to GABA with a much higher specific activity than that of the total glutamic acid.

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The effect of Metronidazol on the toxicity of ethanol

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METRONIDAZOL (hydroxy-2-ethyl,1-methyl-2-nitroimidazol) (M) was recently introduced in the treatment of alcoholism without offering experimental or clinical data, concerning its influence on the toxicity of ethanol (E).[1, 2, 5, 9–11]

The purpose of this paper is to present results concerning the acute and subacute toxicity of E in Metronidazol treated rats. Because Disulfiram (D) is also a drug widely used in the treatment of alcoholism, the experiments were carried out comparatively.

Materials and methods

Male rats, weighing 120–140 g, were used. The LD_{50} of a 50% E solution, administered by i.p.