Effect of Ethanol on γ-Aminobutyric Acid (GABA) and Other Amino Acids in Rat Brains* (33843)

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The possibility that the neurologic changes which occur in alcohol intoxication might be related to changes in GABA levels in the cerebellum was suggested by the observation by Gordon (1) that these levels decreased markedly in rats at 3 and 5 hr after administration of an intoxicating dose of ethanol (4.3 mg/g).

Other investigators have found variable effects of alcohol on the concentration in whole brain of GABA and other amino acids synthesized from glucose in the brain. Häkkinen and Kulonen (2) found an increase in GABA, glutamic acid, and aspartic acid and a decrease in glutamine in ethanol-intoxicated rats. Ferrari and Arnold (3) found a decrease in GABA in some rats after an oral dose of alcohol. Higgins (4) did not find a significant decrease in GABA in rats after intraperitoneal injection of alcohol. Hagen (5) found no change in free or bound GABA in rats after prolonged ethanol administration.

This is a report of our study of the effect of ethanol on the concentrations of GABA and of its immediate precursor, glutamate, and other related amino acids in the cerebrum and hindbrain of normal rats.

Materials and Methods. Male rats of the Sprague-Dawley strain, weighting 275 to 345 g and maintained on Rockland rat diet, were used in these experiments. With the rats under ether anesthesia, polyethylene tubing (PE no. 10) was inserted in the tail vein for intravenous injections; polyethylene tubing (PE no. 50) was inserted in the femoral artery of some of the rats for collection of blood.

Ethanol was administered as a 33 or 25% solution (v/v) in doses of 4.3 or 3.3 mg/g of body weight by stomach tube to fed rats and to rats fasted 25 hr or by intraperitoneal injection to fed rats. Ethanol-treated rats and normal rats not given alcohol (control rats) were placed in restraining cages and infused with physiologic saline (1.25 mg/hr) for 2.5 hr. At the end of the 2.5 hr, the rats were anesthetized with 20 mg of sodium pentobarbital exactly 30 sec before they were immersed, head first, in liquid nitrogen (6). In some experiments, control rats were decapitated and the heads were immersed immediately in liquid nitrogen, because Gordon (1) had used this procedure. The brain and heart were chiseled out of the rats. The frozen brains were usually split into cerebrum and rhombencephalon (hindbrain) (7) which were analyzed separately. In one experiment the frozen hindbrains of four normal rats were separated into cerebellum and brain stem and portions of each were pooled. Blood was collected from the frozen hearts as they thawed or was collected from the femoral artery 1 min or less before the rats were killed.

Brains were homogenized in ice-cold 0.1 *N* NCl and deproteinized with picric acid for amino acid determinations or homogenized in 0.33 *M* $HClO_4$ for ethanol determinations. Blood or plasma was deproteinized with either $HClO_4$ or Somogyi reagents.

Unfrozen brains from four rats were dissected into cerebrum and hindbrain. The hindbrains were dissected into cerebellum and brain stems. Each portion was weighed. Other unfrozen brains were dissected into cerebral hemispheres, midbrain, cerebellum, and brain stem and each portion was weighed.

The concentrations of amino acids in brain were measured with the Beckman-Spinco amino acid analyzer by the method of Spackman *et al.* (8) as accelerated by Benson and Patterson (9). The columns for neutral and acidic amino acids were eluted through α-ami-

* Supported in part by Research Grant NB-4004 from the National Institutes of Health, Public Health Service.

TABLE I. Weights of Cerebrum and Hindbrain (mean ± SE).

Item	Brains	
	Frozen	Unfrozen
No. of rats	22	8
Body wt (g)	304 ± 6	306 ±10
Brain wt (g)[a]	1.59 ± 0.02	1.68 ± 0.02
Cerebrum (% of brain wt)[b]	73.3 ± 0.05	71.1 ± 0.2
Midbrain (% of cerebrum wt)	—	30.6 ± 1.7[c]
Hindbrain (% of brain wt)	26.7 ± 0.5	28.6 ± 0.7[c]
Cerebellum (% of hindbrain wt)	—	51.9 ± 1.4
Brain stem (% of hindbrain wt)	—	49.0 ± 2.4[c]

[a] Without olfactory bulbs.

[b] Cerebrum included monolfactory telencephalon, diencephalon, and mesencephalon (midbrain) (7). Hindbrain or rhombencephalon included cerebellum and brain stem.

[c] Only four rats represented in this mean.

no-*n*-butyric acid (added as an internal standard) and the basic columns were eluted through GABA. Ethanol in brain, blood, or plasma was measured with alcohol dehydrogenase and DPN by the method of Bonnichsen (10).

Results. Weights of brains. The mean weight of the frozen brains as they were chiseled out, without the olfactory bulbs, was 1.59 g (Table I). The cerebrum accounted for 73.3% of the brain weight and the hindbrain, 26.7%. The cerebellum accounted for 51.9% of the hindbrain and the midbrain accounted for 30.6% of the cerebrum.

Alcohol concentrations. After administration of ethanol by stomach tube to fed rats, blood ethanol level varied from 118 to 182 mg/100 ml at 30 min and from 84 to 189 mg/100 ml at 2.5 hr (Fig. 1). [Gordon (1) found a mean concentration of ethanol of 154 mg/100 ml at 3 hr.] Ethanol concentrations in blood were much higher when the ethanol was given orally to fasted rats or intraperitoneally to fed rats. [Maling and co-workers (11) also found higher levels after administration to fasting rats.] Blood ethanol was at its highest concentration in 30 min after intraperitoneal injection. [Duritz and Truitt (12) reported a similar observation.]

Brain/blood ratios of ethanol concentrations averaged 1.00 (range, 0.83–1.18) at 2.5 hr after ethanol administration. Brain water/plasma water ratios were similar because plasma/blood ratio was 1.20 and the water content of normal brains is 78.0% [Gordon found that ethanol had no effect on the water content of brain. Davson (13) previously demonstrated very rapid equilibration of blood ethanol with cerebrospinal fluid and thus brain tissue and plasma of the rabbit.]

Concentrations of amino acids and taurin in cerebrum and hindbrain of control rats. The concentrations of GABA and taurine were almost 40% smaller in hindbrain than in cerebrum (Table II). The concentrations of alanine, glutamic acid, and glutamine were decreased about 25% and the concentration of aspartic acid 15% in the hindbrain compared to the cerebrum. In contrast, the con-

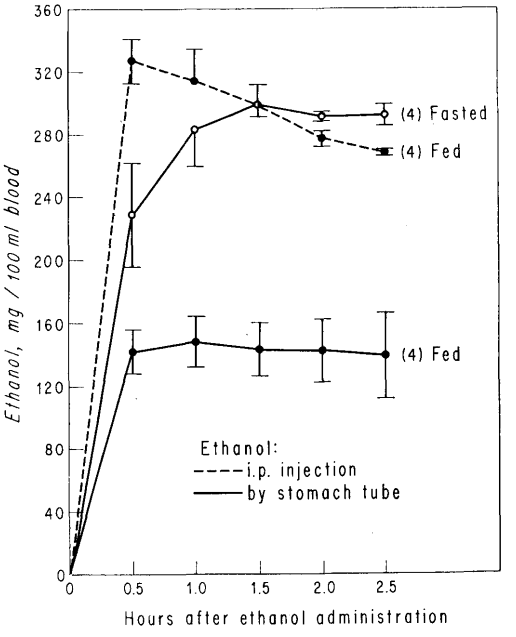

FIG. 1. Concentrations of ethanol in blood of rats after administration of ethanol by intraperitoneal injection (- - -) or by stomach tube (—); data are shown as means ± SE (4 in each group).

TABLE II. Concentrations[a] of Amino Acids in Cerebellum and Brain Stem of Control Rats (not given alcohol).

Amino acids	Rat brain frozen *in situ* (no.)				Frozen after decapitation	
			Hindbrain (4)			
	Cerebrum (8)	Hindbrain (8)	Cerebellum	Brain stem	Cerebrum (9)	Hindbrain (9)
Alanine	0.38 ± 0.02	0.28 ± 0.02[b]	0.35	0.20	0.44 ± 0.02	0.32 ± 0.05[c]
Glutamate	11.9 ± 0.4	9.14 ± 0.14[d]	11.9	7.76	12.7 ± 0.42	9.31 ± 0.11[d]
Glutamine	6.31 ± 0.14	4.68 ± 0.13[d]	6.70	4.41	6.52 ± 0.16	5.24 ± 0.19[d]
Aspartate	2.93 ± 0.11	2.50 ± 0.08[b]	2.52	2.82	2.90 ± 0.09	2.52 ± 0.02[d]
GABA	2.06 ± 0.03	1.28 ± 0.03[d]	1.17	1.52	1.93 ± 0.05	1.27 ± 0.04[d]
Threonine	0.52 ± 0.04	0.54 ± 0.15	0.56	—	0.54 ± 0.02	0.54 ± 0.02
Glycine	0.74 ± 0.02	1.76 ± 0.08[d]	0.67	3.28	0.85 ± 0.08	1.82 ± 0.15[d]
Taurine	4.50 ± 0.15	2.90 ± 0.15[d]	4.74	2.07	5.07 ± 0.07	3.69 ± 0.33[d]

[a] Means ± SE, as μmoles/g of wet wt.
[b] Difference from cerebrum, $p < 0.01$.
[c] Difference from cerebrum, $p < 0.05$.
[d] Difference from cerebrum, $p < 0.001$.

centration of glycine in the hindbrain was 238% of that in the cerebrum.

The concentration of GABA was low in both the cerebellum and brain stem compared to the cerebrum. However, the concentrations of alanine, glutamate, glutamine, threonine, glycine, and taurine in the cerebellum were very similar to those in the cerebrum. The concentrations of alanine, glutamate, glutamine, and taurine were much lower in the brain stem than in the cerebellum and the concentration of glycine was much greater.

The concentration of GABA in the hindbrain was not altered by freezing after decapitation but there was a small decrease in concentration of GABA in the cerebrum ($p<0.05$) (Table II). There was a small increase in the concentrations of alanine ($p<0.05$) and taurine ($p<0.01$) in the cerebrum. The concentrations of glutamine and taurine in the hindbrain were both increased ($p<0.05$).

Concentrations of amino acids and taurine in cerebrum and hindbrain of alcohol-treated rats. After oral or intraperitoneal administration of alcohol, differences in concentrations of amino acids and taurine between cerebrum and hindbrain (Table III) were similar to those found in normal rats without alcohol. The concentration of GABA was lower in the hindbrain than in the cerebrum in all three groups of alcohol-treated rats. No effect of alcohol on the concentrations of any of the amino acids studied was observed in the fed rats intubated with alcohol but the concentration of taurine was increased 16% in these rats. In fasted rats, glutamic acid concentration decreased approximately 10% in the hindbrain; after intraperitoneal injection of alcohol, it decreased this much in both cerebrum and hindbrain. Increases of about 15% in concentrations of glutamine were found in the cerebrum of the fasted rats after oral administration of alcohol and in both parts of the brain after intraperitoneal injection of alcohol. Decreases in concentration of aspartic acid of 13% were found in hindbrain of the fasted rats after oral administration of alcohol and of 14% in cerebrum after intraperitoneal injection of alcohol.

Comment. We found the concentration of GABA to be lower in hindbrain and cerebellum than in cerebrum of control rats but Gordon (1) found it to be the same in cerebellum and cerebral hemisphere. Difficulties arise in comparing the GABA concentrations found by different investigators partly because different areas of the brain were analyzed. Shaw and Heine (14) found concen-

TABLE III. Effect of Ethanol on Concentrations[a] of Amino Acids and Taurine in Brain.

	Alcohol by stomach tube to rats (no.)				Alcohol by ip injection; fed rats (5)	
	Fed (6)		Fasted (4)			
Amino acids	Cerebrum	Hindbrain	Cerebrum	Hindbrain	Cerebrum	Hindbrain
Alanine	0.34 ± 0.02	0.26 ± 0.01	0.39 ± 0.04	0.25 ± 0.02	0.36 ± 0.02	0.29 ± 0.04
Glutamic acid	11.5 ± 0.4	8.73 ± 0.18	12.2 ± 0.5	8.25 ± 0.24[b]	10.7 ± 0.1[b]	8.33 ± 0.18[b]
Glutamine	6.49 ± 0.34	4.85 ± 0.33	7.23 ± 0.37[c]	4.63 ± 0.19	7.26 ± 0.27[c]	5.15 ± 0.14[b]
Aspartic acid	2.92 ± 0.07	2.55 ± 0.09	2.84 ± 0.12	2.17 ± 0.03[b]	2.50 ± 0.06[c]	2.31 ± 0.07
GABA	2.04 ± 0.05	1.27 ± 0.05	2.12 ± 0.09	1.27 ± 0.07	2.00 ± 0.05	1.27 ± 0.05
Threonine	0.49 ± 0.02	0.52 ± 0.03	0.52 ± 0.05	0.50 ± 0.05	0.50 ± 0.02	0.54 ± 0.03
Glycine	0.73 ± 0.02	1.82 ± 0.08	0.85 ± 0.05	1.84 ± 0.09	0.78 ± 0.01	1.87 ± 0.08
Taurine	5.39 ± 0.28	3.33 ± 0.16	4.85 ± 0.40	2.66 ± 0.24	4.64 ± 0.39	3.16 ± 0.38

[a] Means ± SE, as μmoles/g of wet wt; measured 2.5 hr after dose.
[b] Difference from normal, $p < 0.05$.
[c] Difference from normal, $p < 0.01$.

trations of GABA (by the amino acid analyzer) in cerebral hemisphere, midbrain, cerebellum, and pons medulla of rats to be 1.520, 3.059, 1.563, and 1.318 μmoles/g, respectively. Using the percentages of brain weight in Table I, we calculated the concentrations of GABA in the cerebrum and hindbrain of their rats to be 1.99 and 1.44 μmoles/g, respectively, which are very similar to our values. Gordon (1) and Baxter and Roberts (15), who used *Pseudomonas fluoresce* for the estimation of GABA (16), found higher concentrations in cerebral hemisphere and cerebellum than did Shaw and Heine (2.71 and 2.96 μmoles/g by Gordon; 2.52 and 2.71 μmoles/g by Baxter and Roberts).

The precursor of GABA, glutamate, also occurred in smaller concentration in the hindbrain than in the cerebrum in our rats as did two other metabolites of glutamate, glutamine, and aspartate. The ratios of the three metabolites to glutamate were similar in cerebrum and hindbrain: 0.53 and 0.51 for glutamine/glutamate, 0.25 and 0.27 for aspartate/glutamate, and 0.17 and 0.14 for GABA/glutamate.

We found no effect of ethanol on the concentration of GABA in the cerebrum or hindbrain, whether the alcohol was given by stomach tube to fed or fasted rats or by intraperitoneal injection to fed rats in the same dosage used by Gordon (1). The concentration of ethanol in blood in our rats was as high as or higher than in her rats. The concentrations of GABA in the hindbrains of our alcohol-intoxicated rats was much closer to the mean concentrations, 1.17 μmoles/g, found by Gordon in alcohol-intoxicated rats than to that in her control rats. This suggests the possibility that some other substance in brains of control rats gives a positive test for GABA by the enzyme technique and this substance is depleted by alcohol.

The lack of effect of ethanol on the concentration of GABA in the brain is in agreement with the observations by Higgins (4) and Hagen (5). Our observation of small decreases in the concentrations of glutamate and aspartate and small increases in the concentrations of glutamine in brains of alcohol-treated rats differs from those of Häkkinen and Kulonen (2) who found increases in concentrations of glutamate and aspartate and decreases in glutamine. It is possible that the decrease in concentrations of glutamate and aspartate in our rats reflects impaired utilization of glucose in brains of alcohol-intoxicated rats.

Summary. The concentration of GABA was less in the hindbrain (in both the cerebellum and brain stem) than in the cerebrum of normal rats not given alcohol (control rats). Smaller amounts of the immediate precursor of GABA, glutamate, and its metabolites, glutamine and aspartate, were also found in the hindbrain than in the cerebrum. Adminis-

tration of intoxicating doses of alcohol had no effect on the concentration of GABA in the hindbrain but produced small decreases in the concentrations of glutamate and aspartate.

The authors thank Mr. Donald Gustafson, Mrs. Jauneita Ogg, and Mr. Curtis Grabau for valuable technical assistance.

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Received Nov. 4, 1968. P.S.E.B.M., 1969, Vol. 131.

Lysosomal Enzymes in Regenerating Rat Liver* (33844)

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The possible role of the lysosomes or certain lysosomal enzymes in the process of cell division has been described by many authors. Adams (2) showed that acid phosphatase and acid DNase attain their highest concentration in regenerating rat liver about 3–5 hr before the DNA synthesis of the cell is at its maximum. Allison and Malluchi (4) observed that the lysosomes in phytohemagglutinin-stimulated lymphocytes were enlarged and considered this a primary phenomenon associated with the initiation of cell mitosis. The location of the lysosomes at various stages of cell division has been studied electron microscopically by Robbins and Gonatas (16) who observed acid phosphatase-positive particles during the prophase. During the metaphase this phenomenon was even more conspicuous. Kent *et al.* (14) reported that iron-laden lysosomes were localized according to a certain pattern during the early stages of mitosis.

During the first 24 hr of regeneration, minimal or no biochemical variations in the activity of free and total acid phosphatase have been described (20). The same applied to acid phosphatase and acid RNase during the first 8 days of regeneration (3).

Material and Methods. Rats of the Sprague-Dawley strain, weighing 200–300 g, were used. Hepatectomy was carried out by the method of Higgins and Andersson (12), about 70% of the liver was removed. The operation was performed at the same time of the day in order to eliminate the influence of

* Aided by grants from the National Research Council of Medical Sciences and the Sigrid Jusèlius Foundation.