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EFFECT OF ETHANOL ON GLUCOSE AND AMINO ACID METABOLISM IN BRAIN*

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Abstract—Ethanol administration in hamsters altered glucose metabolism in the brain, resulting in increased glucose content and decreased incorporation of labeled carbon from ^{14}C-glucose into the amino acids derived via the citric acid cycle and into lactate. In general, amino acid content of the brain changed little after ethanol, but there was a significant decrease in aspartate and an increase in glutamine. These changes in glucose and amino acid metabolism are enhanced by increasing ethanol doses. However, they seem to be an indirect effect of ethanol, since they do not appear until 20–30 min after ethanol injection, despite high blood ethanol levels throughout this period.

THE METABOLIC consequences of ethanol ingestion have been the subject of extensive study. Much of this work has been in areas other than the central nervous system, and as a result little is known about the effect of ethanol on intermediary metabolism in brain. It is known that large doses of ethanol and many other anesthetic agents cause a marked increase in brain glucose concentration when administered *in vivo*.[1–3] Furthermore, barbiturates and several other neurotropic drugs decrease the rapid incorporation of carbon from ^{14}C-labeled glucose into the free amino acids of brain.[4,5] We have shown previously that ethanol has a similar effect.[6] It is generally assumed that these effects are the result of impaired oxidative metabolism of glucose, although other explanations for the increase in cerebral glucose concentration have been suggested.[2]

Several investigators have examined the effect of ethanol on brain amino acid levels.[6–9] The experimental conditions of these studies varied greatly and possibly contributed to the fact that no consistent alteration in amino acids was observed.

In an effort to define more clearly the action of ethanol on cerebral amino acid and glucose metabolism, our previous studies[6] with ^{14}C-glucose have been extended to include dose and time relationships.

MATERIALS AND METHODS

Male, golden Syrian hamsters (90–110 g) were injected intraperitoneally with ethanol (25%, v/v, in saline), followed immediately by 5 μc [U-^{14}C]-glucose (specific activity, 5 mc/m-mole, New England Nuclear). Control animals received saline instead of ethanol. The animals were killed by immersion in liquid nitrogen for 5 min. The bodies were placed in a cryostat at $-15°$ for 90 min to facilitate removal of the brains. The solidly frozen brains were chiselled out in small fragments and weighed without thawing. The frozen brains were homogenized in 10 ml of ice-cold 5% perchloric acid, and the homogenates centrifuged for 20 min at 10,000 *g* and 4°C.

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Blood was collected as it thawed from hearts excised from the frozen animals and was deproteinized with 5% $ZnSO_4$ and 0·3 N $Ba(OH)_2$.

The perchloric acid extracts of brain were neutralized with KOH and centrifuged to remove $KClO_4$. Glucose in the brain was determined by the hexokinase method of Slein,[10] and lactic acid by the method of Barker and Summerson.[11] Blood glucose was determined by the glucose oxidase method of Bergmeyer and Bernt.[12] Brain and blood ethanol levels were measured by the gas chromotagraphic procedure of Roach and Creaven.[13]

Five-ml aliquots of the brain extracts (adjusted to pH 2 with HCl) were chromatographed by a modification of the ion exchange method of Spackman *et al.*[14] In this modified procedure, pH 5·28, 0·2 N citrate buffer was the second eluent for the 150 cm column, and eluted γ-aminobutyric acid (GABA) soon after phenylalanine. Thus a single-column operation separated the major amino acids of brain as well as glucose and lactic acid.[15]

Aliquots of the 2-ml chromatographic fractions containing glucose, lactic acid, and the amino acids were assayed for radioactivity by liquid scintillation spectrometry. Amino acid content of the fractions was determined manually with the ninhydrin reagent described by Spackman *et al.*[14]

In general, extracts from single brains were chromatographed, except for the study in which brains were examined at various times after ethanol injection. In this study, brain extracts from three to five animals were pooled for chromatographic analysis. Two control and two experimental pools were examined at each time. Brain glucose concentration was determined for each extract before pooling.

RESULTS

Dose response. Brain glucose concentration in hamsters was increased significantly over the control value at 40 min after 0·62 g/kg of ethanol (Table 1). The glucose level continued to rise with increasing ethanol dose and, at 2·50 g/kg, was twice the control value. Blood glucose levels increased slightly (Table 1).

Ethanol administration of 0·62 g/kg produced a small but significant increase in glutamine concentration and had little effect on the levels of the other amino acids in the brain (Table 1). A dose of 1·25 g/kg decreased the concentration of aspartate and lactate significantly while glutamine remained elevated. At 2·50 g/kg, the aspartate concentration was further depressed, but changes in lactate and glutamine, though still evident, were not statistically significant.

The effect of ethanol on the distribution of the label from [U-^{14}C]-glucose in the brain, expressed as the percentage of total radioactivity in the acid-soluble fraction, is shown in Table 2. The percentage of the label remaining as glucose at 40 min increased with the ethanol dose, and at 2·50 g/kg, was 3-fold greater than the control value. The total radioactivity in brain did not change significantly (control, 178,000 ± 45,000 counts/min/g; 2·50 g/kg, 167,000 ± 25,000 counts/min/g).

The accumulation of radioactive glucose was accompanied by a decrease in the percentage of ^{14}C-incorporation into alanine and the amino acids derived via the citric acid cycle (Table 2). The labeling of glutamine decreased significantly at 0·62 g/kg of ethanol, and at 1·25 g/kg both glutamine and glutamate incorporated less ^{14}C. At the highest ethanol dose (2·50 g/kg), aspartate also showed significantly less ^{14}C-incorporation.

TABLE 1. EFFECT OF ETHANOL DOSE ON THE CONCENTRATION OF GLUCOSE, LACTATE AND AMINO ACIDS IN HAMSTER BRAIN

		Ethanol dose (g/kg)		
	Saline (12)	0·62 (8)	1·25 (8)	2·50 (7)
Glucose	1·06 ± 0·25	1·56 ± 0·20†	1·94 ± 0·76‡	2·24 ± 0·43‡
Lactate	2·54 ± 0·32	2·36 ± 0·31	2·14 ± 0·23†	2·20 ± 0·52
Glutamate	8·98 ± 0·59	8·94 ± 0·46	8·89 ± 0·76	8·62 ± 0·40
Glutamine	5·14 ± 0·43	5·81 ± 0·39§	5·75 ± 0·50‖	5·54 ± 0·35
Aspartate	2·77 ± 0·16	2·71 ± 0·23	2·50 ± 0·16‖	2·37 ± 0·31‡
GABA	1·93 ± 0·12	1·97 ± 0·09	1·94 ± 0·18	1·99 ± 0·20
Alanine	0.43 ± 0·05	0·35 ± 0·05†	0·40 ± 0·07	0·42 ± 0·07
Blood glucose	6·68 ± 1·09(6)	8·44 ± 0·82(6)	9·08 ± 0·92(4)†	8·60 ± 2·26(8)†

* Values are means ± S.D. as μmoles/g of wet brain or μmoles/ml of blood. Numbers of animals are indicated in parentheses. Animals were killed 40 min after injection of ethanol or saline. P values were calculated by Dunnett's *t*-test.[16]
† $P \leq 0{\cdot}05$.
‡ $P \leq 0{\cdot}005$.
§ $P \leq 0{\cdot}01$.
‖ $P \leq 0{\cdot}25$.

TABLE 2. EFFECT OF ETHANOL DOSE ON THE PERCENTAGE DISTRIBUTION OF RADIOACTIVITY FROM ^{14}C-GLUCOSE IN HAMSTER BRAIN*

		Ethanol dose (g/kg)		
	Saline (12)	0·62 (8)	1·25 (8)	2·50 (7)
Glucose	6·80 ± 2·36	10·70 ± 3·19	15·92 ± 7·02†	20 21 ± 5·59†
Lactate	9·36 ± 2·73	8·78 ± 2·24	7·57 ± 2·43	6·46 ± 1·82
Glutamate	36·71 ± 1·81	35·02 ± 1·51	33·13 ± 4·27‡	29·30 ± 2·61†
Glutamine	15·99 ± 1·42	13·84 ± 1·69§	13·28 ± 2·09‖	12·66 ± 1·54†
Aspartate	7·85 ± 0·75	7·79 ± 1·09	7·34 ± 1·50	6·24 ± 1·07‡
GABA	4·51 ± 0·34	4·33 ± 0·53	4·07 ± 0·85	4·23 ± 0·41
Alanine	1·42 ± 0·30	1·23 ± 0·14	1·15 ± 0·24	1·16 ± 0·21

* Values are means ± S.D. as per cent of total ^{14}C in acid-soluble fraction of brain. Numbers of animals are indicated in parentheses. Animals were killed 40 min after injection of ethanol or saline followed immediately by [U-^{14}C]-glucose. P values were calculated by Dunnett's *t*-test.[16]
† $P \leq 0{\cdot}005$.
‡ $P \leq 0{\cdot}025$.
§ $P \leq 0{\cdot}05$.
‖ $P \leq 0{\cdot}01$.

The percentage of ^{14}C in lactate also tended to decrease with increasing ethanol dose. At 2·50 g/kg, the average figure for incorporation into lactate was 30 per cent lower than the control value, but this difference was not statistically significant because of the large standard deviation.

Because the ^{14}C content of the different brains varied greatly, relative rather than absolute specific activities were calculated. Relative specific activities (RSA) are defined as the ratios of the specific activities of the glucose metabolites to the specific activity of glucose ($RSA_{glucose}$) or to the specific activity of glutamate ($RSA_{glutamate}$). At 1·25 g/kg, only the specific activity of glutamine decreased significantly relative to glucose, but at 2·50 g/kg the activities of all the amino acids studied, except GABA, were depressed significantly (Table 3). At the highest dose, the $RSA_{glucose}$ of lactate

TABLE 3. EFFECT OF ETHANOL DOSE ON THE RELATIVE SPECIFIC ACTIVITY (RSA) OF METABOLITES OF ^{14}C-GLUCOSE IN HAMSTER BRAIN*

		Ethanol dose (g/kg)		
	Saline (12)	0·62 (8)	1·25 (8)	2·50 (8)
		$RSA_{glucose}$†		
Lactate	0·59 ± 0·15	0·55 ± 0·08	0·47 ± 0·22	0·36 ± 0·14‡
Glutamate	0·67 ± 0·15	0·61 ± 0·17	0·49 ± 0·17	0·41 ± 0·15§
Glutamine	0·51 ± 0·13	0·38 ± 0·15	0·31 ± 0·12‡	0·29 ± 0·14§
Aspartate	0·46 ± 0·10	0·46 ± 0·15	0·39 ± 0·14	0·31 ± 0·09‖
GABA	0·39 ± 0·11	0·37 ± 0·16	0·28 ± 0·14	0·26 ± 0·09
Alanine	0·55 ± 0·17	0·52 ± 0·13	0·39 ± 0·17	0·31 ± 0·05§
		$RSA_{glutamate}$¶		
Glucose	1·58 ± 0·38	1·75 ± 0·47	2·26 ± 0·69	2·75 ± 0·91**
Lactate	0·90 ± 0·21	0·94 ± 0·19	0·94 ± 0·17	0·88 ± 0·18
Glutamine	0·76 ± 0·06	0·60 ± 0·08**	0·62 ± 0·05**	0·65 ± 0·05§
Aspartate	0·70 ± 0·09	0·74 ± 0·06	0·79 ± 0·10	0·77 ± 0·10
GABA	0·58 ± 0·05	0·60 ± 0·09	0·56 ± 0·10	0·66 ± 0·10
Alanine	0·82 ± 0·16	0·93 ± 0·14	0·78 ± 0·11	0·83 ± 0·22

* Values are means ± S.D. of ratios of specific activities (counts/min/μmole). Numbers of animals are indicated in parentheses. Animals were killed 40 min after injection of ethanol or saline followed immediately by [U-^{14}C]-glucose. P values were calculated by Dunnett's *t*-test.[16]

† Specific activity of glucose = 1.

‡ $P \leq 0{\cdot}025$.

§ $P \leq 0{\cdot}01$.

‖ $P \leq 0{\cdot}05$.

¶ Specific activity of glutamate = 1.

** $P \leq 0{\cdot}005$.

was also significantly lower. [The absolute specific activities (counts/min/μmole) of glucose were: control, 11,800 ± 5100; 0·62 g/kg, 14,200 ± 3800; 1·25 g/kg, 15,600 ± 5800; and 2·50 g/kg, 15,600 ± 6500.] With the exception of glutamine, the specific activities of the amino acids relative to glutamate ($RSA_{glutamate}$) changed little after ethanol (Table 3). Glutamine activity was lower at all dose levels, however. The $RSA_{glutamate}$ of glucose increased with dose, but the change was statistically significant only at 2·50 g/kg.

Blood and brain ethanol levels at 40 min are shown in Table 4.

Time course. At 5 min after 2·50 g/kg of ethanol, the brain glucose concentration increased slightly over the control value, but the difference was not statistically sig-

TABLE 4. CHANGES IN BLOOD AND BRAIN ETHANOL LEVELS WITH DOSE AND TIME*

	Dose (g/kg)		
	0·62	1·25	2·50
Blood (mg/ml)†	0·33 ± 0·08	1·10 ± 0·20	2·84 ± 0·58
Brain (mg/g)†	0·38 ± 0·13	1·21 ± 0·38	2·95 ± 0·84

	Time (min after injection)				
	5	10	20	30	40
Blood (mg/ml)‡	2·36 ± 0·95	3·42 ± 0·85	4·08 ± 0·48	3·32 ± 0·18	2·87 ± 0·12

* Values are means ± S. D.
† At 40 min after ethanol injection.
‡ Ethanol dose was 2·5 g/kg.

nificant (Table 5). By 20 min the difference was significant, and by 30 min the glucose level was twice the control value. Blood glucose levels changed little with time after ethanol administration (Table 5).

Variations in amino acid and lactate concentrations are shown in Fig. 1. The glutamate, GABA, and alanine levels did not change, but glutamine increased and

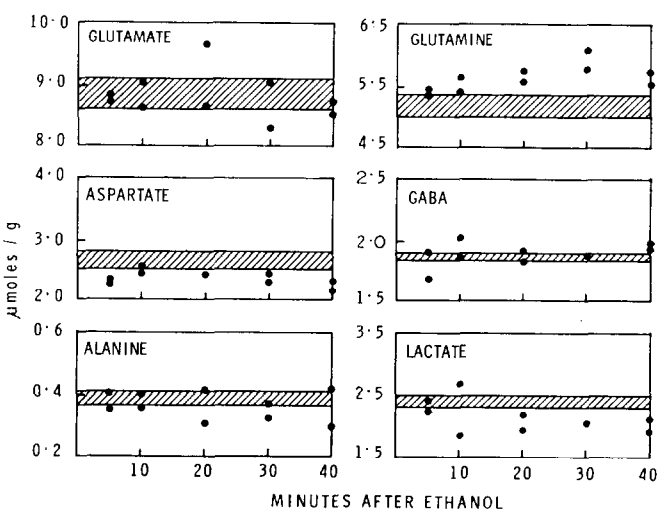

FIG. 1. Change in amino acid and lactate content of hamster brain after ethanol (2·50 g/kg). Horizontal bars represent mean control values ± S.D. Values of ethanol-treated animals are shown as individual points. Two control and two experimental pools of brain extracts, containing three to five brains per pool, were examined at each time.

TABLE 5. EFFECT OF ETHANOL ON BRAIN AND BLOOD GLUCOSE CONCENTRATION*

	Time (min)				
	5	10	20	30	40
Brain					
Control	1·27 ± 0·32 (7)	1·24 ± 0·22 (7)	1·30 ± 0·36 (7)	1·17 ± 0·26 (7)	1·04 ± 0·14 (5)
Ethanol	1·71 ± 0·29 (7)	1·61 ± 0·36 (8)	1·94 ± 0·31 (7)†	2·30 ± 0·44 (7)†	2·17 ± 0·26 (7)†
Blood					
Control	8·98 ± 1·78 (7)	7·16 ± 0·77 (6)	6·59 ± 1·56 (7)	7·55 ± 0·79 (6)	6·68 ± 1·09 (6)
Ethanol	8·50 ± 0·72 (6)	7·56 ± 1·41 (8)	8·53 ± 1·41 (7)	9·52 ± 1·52 (7)	8·60 ± 2·26 (8)‡

* Values are means ± S.D. as μmoles/g of wet brain or μmoles/ml of whole blood. Numbers of animals are indicated in parentheses. Animals were killed at the times indicated after injection of ethanol (2·50 g/kg) or saline. P values were calculated by Dunnett's *t*-test,[16]

† $P \leq 0{\cdot}005$.

‡ $P \leq 0{\cdot}05$.

aspartate decreased with time. Lactate concentration also decreased slightly with time after ethanol.

The time course of ethanol's effect on the $RSA_{glucose}$ is shown in Fig. 2. Through 20 min, the specific activities of the control and ethanol-treated groups were similar. At 30 min and 40 min, the relative specific activities in the ethanol-treated animals were markedly lower than in the control animals. This decrease in relative specific activity was most pronounced with glutamate and glutamine.

The change in blood ethanol concentration after a dose of 2·50 g/kg is shown in Table 4.

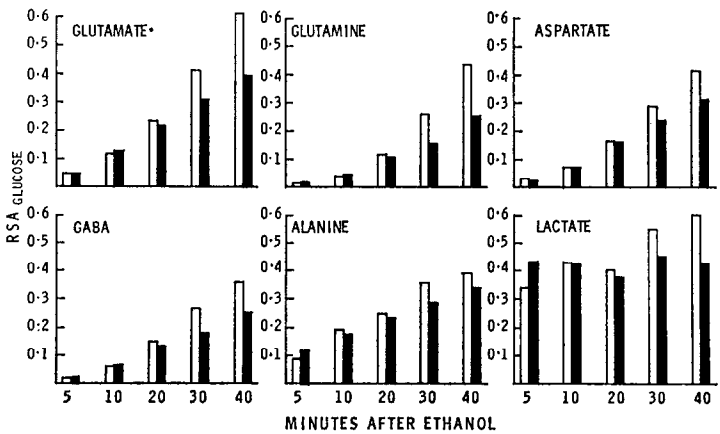

FIG. 2. Change in relative specific activities (specific activity of glucose = 1) of amino acids and lactate in hamster brain after ethanol (2·50 g/kg). Open bars represent control animals; solid bars, ethanol-treated animals. Two control and two experimental pools of brain extracts, containing three to five brains per pool, were examined at each time.

DISCUSSION

Brain glucose has been reported to increase in rats after a very large ethanol dose (4·1 g/kg),[3] as well as after anesthetizing doses of other depressants.[1,2] This increase is generally attributed to diminished oxidative metabolism of glucose during narcosis, although Mayman *et al.*[2] have suggested that it could be caused by changes in glucose transport or intracellular distribution. Although in these reports narcosis accompanied increased brain glucose, in the present study brain glucose increased 50–80 per cent at low ethanol doses, while the animals remained alert, showing only slight evidence of intoxication.

As indicated by the studies with ^{14}C-glucose, the higher cerebral glucose level after ethanol administration is at least partly due to decreased glucose utilization by the brain. The alteration in glucose metabolism does not seem to be produced by ethanol acting directly on the enzymes involved in glucose oxidation, however, since there is a 20-min period during which blood ethanol levels are quite high, but inhibition of of glucose utilization is not evident.

The effect of ethanol on brain amino acid levels has been studied by several investigators with variable results. Häkkinen and Kulonen[8] reported increased glutamate and GABA and decreased glutamine in rats at 60 min after ethanol. Our results in hamsters differ from these in that we found an increase in glutamine, a decrease in

aspartate, and no changes in glutamate and GABA. In another study with rats, Sutherland and Rikimaru[9] found a significant decrease in aspartate in two of three brain areas and a significant increase in GABA in only one area. They reported no change in the concentration of glutamine or glutamate in any of the three areas studied. The reason for these varying reports of brain amino acid levels after ethanol is not clear, but may be caused by differences in experimental conditions.

The results reported here indicate that, in hamster brain, the labeling of glutamine by ^{14}C from glucose is affected by ethanol at a lower dose than the other amino acids. A possible explanation for this is suggested by the concept of compartmentation of amino acid metabolism in brain.[17] Glutamate seems to exist in two pools, one smaller than the other. The major portion of glutamine is derived from the smaller glutamate pool. Metabolism of ^{14}C-glucose, which labels both glutamate pools, gives a specific activity ratio of glutamine-to-glutamate that is less than one. Other labeled substrates, including acetate, preferentially enter the small, glutamine-precursor pool of glutamate and yield a glutamine-to-glutamate ratio that is greater than one. Lundquist *et al.*[18] have shown that after ethanol ingestion more than 50 per cent of the hepatically metabolized ethanol leaves the liver as acetate to be metabolized elsewhere. If acetate formed from unlabeled ethanol were metabolized in brain concomitantly with ^{14}C-glucose, the labeling of the smaller glutamate pool should be diluted, and subsequently, the specific activity of glutamine lowered. Since the labeling of the larger pool of glutamate should not be diminished appreciably by acetate metabolism, the relative specific activity of glutamine-to-glutamate should be lower after ethanol. The data presented here for hamsters are consistent with this premise, showing a decrease in the specific activity of glutamine relative to glutamate after ethanol administration. Preliminary studies of the incorporation of ^{14}C from labeled ethanol into brain amino acids support this hypothesis.

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