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EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON RAT PLASMA AMINO ACIDS AND RELATED COMPOUNDS

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Abstract—Using high performance liquid chromatography (HPLC) with fluorometric detection, thirty-three amino acids (AA) and related compounds were measured in plasma obtained from catheterized rats over a 3-hr period following a 2 g/kg, i.p., injection of ethanol. The concentrations of twenty-three of these compounds had decreased significantly 15 min after the injection, and twenty of the twenty-three remained depressed for the 3-hr period. Marked reductions were noted for alanine and arginine. Glutamic acid, 1-methylhistidine and 3-methylhistidine were unaffected by ethanol. During these studies individual differences were observed in that some rats showed marked biochemical changes, whereas other rats showed only minimal responses. These observations indicate that ethanol administration may have a significant and long-lasting impact on plasma amino acid biochemistry.

Amino acids (AA) are key biochemicals in virtually every organism and changes in basal levels could provide early warning signals of pathology for certain diseases. For this reason, plasma AA have received a large amount of attention and have been well studied in rats during the resting state as well as during exposure to many drugs including ethanol. In the case of ethanol, it has been shown that ethanol decreases certain plasma AA in rats [1–5].

Unfortunately, these animal studies of AA suffer from certain deficiencies which may affect the validity of their results. First, blood was obtained by decapitation which we have shown to produce artifacts in the levels of some AA [6]. While this was minimal and nonsignificant for some AA (e.g. serine, lysine), it was significant for other AA (e.g. aspartic acid, glycine). Second, reports on the effects of ethanol on AA levels generally measure plasma AA values at only one time point. A single time point measurement may be insufficient since AA may increase or decrease during ethanol exposure over time. Third, studies on ethanol have reported mean values for groups of animals. Individual values could not be further evaluated due to the fact that the animals were decapitated and provided only one data point. The existence of individual differences could only be inferred from the resulting large standard deviations. Recent work with individual rats has shown that indeed large biochemical differences can be found among members of the same group of "inbred" animals in response to an alcohol–stress situation [7].

Thus, the exact responses of plasma AA concentrations in individual rats to ethanol over a longer time period are still unknown. For this reason, we measured the effect of ethanol (2 g/kg, i.p.) on the plasma levels of thirty-three amino acids and related compounds, determined by ion-exchange high performance liquid chromatography (HPLC) with fluorometric detection [6], in catheterized rats able to move freely. The data obtained represent more exact values for these AA in response to ethanol in individual rats over a 3-hr period.

METHODS

Animals and catheterization. Eleven male Sprague–Dawley rats (Perfection Breeders, Douglasville, PA), 230–270 g, were used in this study. The animals were fitted with indwelling silastic tubing catheters in the jugular vein as previously described [8]. Catheters were kept patent with a dilute heparin solution (100 units/ml of saline). The animals were allowed to recover from surgery for 24 hr before testing; this had been found to be sufficient for recovery from surgery. They were housed individually with free access to food and water.

Alcohol and saline injections and preparation of plasma samples. Blood (0.3 ml) was drawn from the animals through the catheter into a heparin-containing syringe (about 200 units/ml blood) while undisturbed in the home cage ($t = 0$ min). Eight rats were then injected with ethanol (20%, w/v, solution, 2 g/kg, i.p.), and three animals were injected with an equivalent volume of saline (i.p.). For each rat, blood samples were then drawn 15, 30, 60, 90, 120 and 180 min after either ethanol or saline injection. All samples were centrifuged to obtain plasma which was stored frozen at −80°.

At the time of analysis, plasma samples were thawed, and an aliquot (0.15 ml) was deproteinized immediately as previously described [8].

Amino acid analysis. The levels of AA and related compounds in deproteinized plasma samples were determined by HPLC as previously described [6].

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Table 1. Effect of ethanol (2 g/kg) on plasma amino acids and related compounds of eight rats

Amino acids and related compounds	Time after injection (min)						
	0 (Baseline)	15	30	60	90	120	180
Taurine	61.3 ± 10.8	51.1 ± 3.1*	42.5 ± 7.5†	41.1 ± 6.7†	40.3 ± 8.0†	35.9 ± 5.6†	43.0 ± 6.2†
Phosphoethanolamine	4.5 ± 0.5	4.5 ± 0.4	3.6 ± 0.5	3.4 ± 0.3	3.3 ± 0.4	3.4 ± 0.2	4.2 ± 0.5
Aspartic acid	7.3 ± 1.0	5.9 ± 0.6*	5.7 ± 0.7†	5.4 ± 0.6†	5.4 ± 0.8†	4.6 ± 0.5†	5.0 ± 0.8†
Threonine	292 ± 32	207 ± 21†	182 ± 22†	186 ± 17†	193 ± 26†	182 ± 25†	199 ± 21†
Serine	194 ± 18	134 ± 11†	108 ± 11†	111 ± 8†	117 ± 18†	105 ± 11†	121 ± 13†
Asparagine	89.3 ± 11.8	60.5 ± 7.9†	53.5 ± 9.3†	51.9 ± 7.6†	57.3 ± 10.6†	51.4 ± 7.9†	57.2 ± 8.8†
Glutamic acid	51.7 ± 4.6	51.9 ± 6.0	48.4 ± 6.6	46.9 ± 6.8	44.8 ± 7.4	36.7 ± 3.8†	43.2 ± 7.4
Glutamine	399 ± 27	320 ± 24*	290 ± 20†	326 ± 16*	380 ± 32	376 ± 30	459 ± 38
α-Aminoadipic acid	13.2 ± 1.3	11.8 ± 1.2	9.72 ± 1.00†	11.2 ± 0.9	11.0 ± 1.1	10.5 ± 0.8*	12.4 ± 0.8
Glutathione (oxidized)	0.45 ± 0.15	0.23 ± 0.10	0.39 ± 0.20	0.65 ± 0.22	0.39 ± 0.14	0.43 ± 0.15	0.55 ± 0.09
Glycine	271 ± 32	193 ± 23†	174 ± 27†	192 ± 26†	208 ± 34†	189 ± 24†	223 ± 35†
Alanine	422 ± 41	305 ± 29†	240 ± 30†	233 ± 28†	242 ± 37†	222 ± 32†	243 ± 36†
Citrulline	71.8 ± 9.2	57.1 ± 7.4†	49.9 ± 8.1†	52.3 ± 6.8†	56.1 ± 6.6†	50.8 ± 4.5†	60.1 ± 6.2*
α-Amino-*n*-butyric acid	5.6 ± 0.5	4.2 ± 0.5*	4.0 ± 0.3†	4.8 ± 0.53	5.4 ± 0.5	5.0 ± 0.2	6.0 ± 0.4
Valine	244 ± 25	191 ± 23†	166 ± 19†	171 ± 17†	166 ± 18†	143 ± 10†	151 ± 8.7†
Cystine	25.8 ± 3.3	17.7 ± 1.7†	16.1 ± 2.1†	14.0 ± 1.8†	13.7 ± 1.9†	17.5 ± 2.7†	18.2 ± 2.3†
Methionine	58.3 ± 5.9	45.7 ± 4.2†	37.7 ± 4.5†	37.2 ± 3.2†	36.4 ± 4.3†	33.1 ± 2.9†	37.0 ± 3.1†
Cystathionine	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	0.95 ± 0.12	1.1 ± 0.1	0.92 ± 0.10	1.1 ± 0.2
Isoleucine	116 ± 12	90.1 ± 7.8†	84.6 ± 9.3†	85.6 ± 8.7†	86.5 ± 9.2†	74.1 ± 6.5†	83.0 ± 6.6†
Leucine	221 ± 29	171 ± 19†	139 ± 16†	142 ± 13†	148 ± 18†	127 ± 9.4†	131 ± 9†
Tyrosine	91.5 ± 10.2	66.5 ± 5.9†	60.2 ± 7.5†	59.6 ± 6.7†	55.7 ± 7.4†	47.9 ± 4.9†	49.2 ± 4.7†
Phenylalanine	74.2 ± 6.2	57.7 ± 4.0†	50.6 ± 4.6†	47.4 ± 4.3†	48.9 ± 5.2†	45.8 ± 4.0†	55.8 ± 4.3†
β-Alanine	0.97 ± 0.15	0.78 ± 0.18	0.75 ± 0.17	0.94 ± 0.24	0.95 ± 0.22	0.90 ± 0.18	0.94 ± 0.20
Tryptophan	75.5 ± 6.1	53.7 ± 4.7†	46.6 ± 5.7†	49.5 ± 5.3†	48.2 ± 5.5†	43.3 ± 4.1†	52.1 ± 3.7†
Ethanolamine	7.39 ± 0.78	7.25 ± 1.81	6.17 ± 1.89	6.10 ± 1.82	5.76 ± 0.66*	5.28 ± 0.49*	5.70 ± 1.80*
Ammonia	65.0 ± 6.4	52.8 ± 3.8†	54.8 ± 4.0	60.2 ± 5.8	48.7 ± 82.7	40.4 ± 3.8*	38.8 ± 9.2†
Hydroxylysine	3.8 ± 0.5	2.7 ± 0.3	2.6 ± 0.53	2.7 ± 0.5	3.24 ± 0.4	2.4 ± 0.4	3.3 ± 0.5
Ornithine	57.9 ± 5.3	41.2 ± 3.1†	40.7 ± 3.9†	36.5 ± 4.0†	39.5 ± 5.0†	36.6 ± 5.8†	33.8 ± 2.8†
Lysine	404 ± 35	254 ± 28†	274 ± 35†	244 ± 30†	282 ± 35†	264 ± 30†	247 ± 30†
Histidine	65.8 ± 3.8	51.3 ± 2.6†	45.0 ± 3.9†	49.8 ± 3.2†	54.5 ± 5.3†	49.1 ± 3.1†	52.3 ± 3.2†
1-Methylhistidine	3.5 ± 0.4	2.9 ± 0.3	2.7 ± 0.3	2.9 ± 0.3	3.4 ± 0.4	3.1 ± 0.4	3.6 ± 0.4
3-Methylhistidine	3.3 ± 0.5	2.7 ± 0.3	2.6 ± 0.3	3.1 ± 0.4	3.0 ± 0.3	2.6 ± 0.3	3.4 ± 0.3
Arginine	212 ± 18	143 ± 16†	118 ± 15†	126 ± 17†	126 ± 15†	111 ± 14†	105 ± 9†

Values are mean ± S.E.M., N = 8.

The following amino acids were not detectable at any time: phosphoserine, glutathione (reduced), β-aminoisobutyric acid, γ-aminobutyric acid, anserine, carnosine and homocarnosine.

* $P < 0.05$ with respect to $t = 0$ min.

† $P < 0.01$ with respect to $t = 0$ min.

This procedure utilized a meter long microbore HPLC column containing spherical cation-exchange resin and fluorescence detection following post-column reaction with *o*-phthalaldehyde. Lithium citrate elution buffers were used to separate the AA and related compounds. This technique has been fully validated in a previous study [6].

Evaluation. Individual AA were quantitatively determined by relating their chromatographic peak heights to the peak heights from a known amount of a physiological AA standard and an internal standard (*m*-fluorophenylalanine). Statistical analysis of the data was performed by an analysis of variance for repeated measurements with a Newman–Keuls *post hoc* test. Values of $P < 0.05$ or less were considered significant.

RESULTS

Injection of saline had no effect on the levels of plasma AA and related compounds during repeated blood sampling over 3 hr. This is in agreement with earlier findings that nine repeated blood drawings over 2 hr have no effect on plasma AA levels [6].

Table 1 shows the levels of plasma AA and related compounds before ($t = 0$ min) and after ($t = 15$–180 min) a single injection of ethanol. None of the AA levels increased significantly after ethanol administration. However, the levels of the majority of the detectable plasma AA and related compounds (twenty out of thirty-three) decreased significantly during the first 15 min after ethanol injection and remained depressed for at least 3 hr. Alanine and arginine showed the greatest reduction from baseline plasma levels (approx. 40%). Glutamine and α-aminobutyric acid decreased significantly after 15 min but returned to baseline values after 60 min. Finally, some AA levels were not altered after ethanol administration (glutamic acid, β-alanine, hydroxylysine, 1-methylhistidine and 3-methylhistidine).

During this study, individual animals varied in the responses of certain AA to ethanol. Table 2 shows, as an example, the response of plasma tryptophan to ethanol in three of the rats. Although the baseline values of tryptophan differed for rats 1 and 2, both animals showed about the same marked response to ethanol (about a 40% decrease from baseline levels). However, the responses of rats 2 and 3 to ethanol were very different even though their baseline values of tryptophan were about the same.

Table 2. Individual differences of tryptophan levels in response to ethanol (2 g/kg, i.p.)

	Tryptophan (nmoles/ml plasma)				
	Time after injection (min)				
	0 (Baseline)	15	30	60	120
Rat 1	89.4	48.2	37.3	46.3	46.9
Rat 2	60.1	35.1	30.3	30.1	23.1
Rat 3	61.8	56.4	38.9	42.6	42.9

DISCUSSION

Most of the measured plasma AA levels decreased markedly 15 min after a 2 g/kg sample of ethanol was injected, i.p., into a rat. Neither sequential blood collection nor a saline solution affected measured plasma AA levels. Most of these AA remained at these reduced levels for the period of 3 hr. Two amino acids, glutamine and α-aminobutyric acid, however, displayed a sharp drop in concentration (evident 15 min after injection) which then returned to baseline levels after 60 min. No changes were seen with the methylhistidines. Since 3-methylhistidine, a known indicator of muscle degradation [9], did not change significantly, we believe that little or no muscle damage occurred due to ethanol exposure.

Although our results in general agree with those of other investigators [2, 3, 5], that an acute dose of ethanol decreases most rat plasma AA, there were some exceptions. Eriksson and coworkers [3] reported that the values of aspartic acid and cystine in controls and ethanol-treated rats are the same; however, we found significant differences between baseline values and those after ethanol injection. The differences may have been due to the fact that the fluorescent HPLC procedure we used is more sensitive [6]. Those authors also reported that ethanol did not alter glutamine concentration at 60 min. We found, however, that ethanol decreased glutamine levels before returning to baseline levels after 60 min. The discrepancies between these studies may have resulted from the different methods of blood collection since the catheterized plasma gives more valid plasma AA levels than the decapitated animal. The observation that ethanol decreased most plasma AA in the rat is in agreement with human studies which show that an acute dose of ethanol decreases plasma AA in healthy and alcoholic individuals [10–13].

There are several possible reasons for an ethanol-induced decrease in most plasma AA. Since ethanol is oxidized to acetaldehyde via NAD^+, the coenzyme of alcohol dehydrogenase (EC 1.1.1.1), an increase in the NADH/NAD^+ ratio resulting in a corresponding increase in lactate/pyruvate ratio is observed [14, 15]. However, because it is necessary to regenerate NAD^+ to further oxidize the ethanol load, other oxidizing agents such as pyruvate or other ketoacids would be necessary to convert NADH back to NAD^+. It is possible that AA could serve as a source of ketoacids by undergoing oxidative deamination to ketoacids and, thus, the levels of plasma AA would decrease after acute ethanol administration. Second, ethanol could inhibit transport of AA from the small intestine to the blood stream resulting in reduced plasma levels [16]. Third, acute administration of ethanol can result in hyperglycemia [15]. Insulin levels may increase and result in an increase in the uptake of AA into muscle and adipose tissue and a decrease in plasma AA [17]. Finally, Eriksson and coworkers [2] found that propranolol, a β-adrenergic antagonist, partially blocked the ethanol-induced decreases in plasma AA. They reasoned that increases in epinephrine and norepinephrine levels, by high doses of ethanol,

stimulated β-adrenergic receptors in the liver [18] which produced increased blood flow through the liver and caused an increase in AA metabolism in the liver and a decrease in the plasma AA [19].

It has been generally accepted that the availability of certain brain AA (e.g. tryptophan) depends on their plasma levels [20, 21] as well as on their competition with other plasma AA (e.g. isoleucine, leucine, tyrosine, phenylalanine, tryptophan and valine [22–24]) for the same transport carrier through the blood–brain barrier [25, 26]. In this study, we found an ethanol-induced decrease in plasma tryptophan which could affect the level of brain tryptophan, the precursor of brain serotonin [27, 28]. We also observed an ethanol-induced decrease in plasma tyrosine and phenylalanine which may affect the levels of brain tyrosine and phenylalanine, the precursors of brain dopamine, norepinephrine and epinephrine [29, 30]. However, we found that, since the ratio of these plasma aromatic AA to their competing plasma AA does not change after ethanol administration, a slight decrease or no change in brain AA should be expected. This is in partial agreement with reports that central levels of tryptophan, tyrosine and phenylalanine levels after ethanol injection remained unchanged or slightly decreased [3, 31]. However Pohorecky and coworkers [5] found that brain tryptophan levels increased while brain tyrosine levels decreased 2 hr after ethanol (4 g/kg, i.p.) was injected into rats. Only Badawy and coworkers [32] showed that brain tyrosine level initially increased but decreased later after the ethanol administration.

By using the catheterized rat, time curves showed strong individual differences in the response of certain AA to ethanol. Table 2 shows just such an example. Some animals responded only minimally, whereas others showed marked responses to ethanol. These individual differences may help to explain why ethanol affects plasma tryptophan, tyrosine and phenylalanine and their corresponding brain AA more in some animals than in other animals. Similar individual variances may explain why some alcoholics experience more liver or brain damage than do others under comparable circumstances. Perhaps rats with low or high AA responses to ethanol could be bred selectively to see if these animals are selectively susceptible to changes in central neurotransmitter or to peripheral or central pathology. In particular, perhaps strains of rats could be bred with marked reduction in plasma tryptophan levels after ethanol administration since alcoholics with depression have been claimed to have decreased plasma tryptophan levels and decreased plasma ratios of tryptophan to other neutral AA relative to controls [33].

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