Decrease in plasma amino acids in rat after acute administration of ethanol

TOMAS ERIKSSON*, ARVID CARLSSON, STURE LILJEQUIST, MATS HAGMAN†, RUDOLF JAGENBURG†, * *Department of Pharmacology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden,* † *Department of Clinical Chemistry, Sahlgren's Hospital, University of Göteborg, S-413 45 Göteborg, Sweden*

We have recently reported that the passage of exogenously administered L-dopa through the blood-brain barrier in rat is increased after previous administration of ethanol (Eriksson et al 1979). To explain this phenomenon the possibility might be considered that ethanol causes a decrease in the plasma concentration of other amino acids competing with L-dopa for the same carrier system (Oldendorf 1975; Pardridge & Oldendorf 1977; Wurtman & Fernstrom 1976; Carlsson & Lindqvist 1978). In fact, Siegel et al (1964) reported that a single dose (not indicated) of ethanol to man, which gave rise to a blood ethanol concentration over 90 mg/100 ml caused a decrease in 13 plasma amino acids. However, this observation was not confirmed by Kreisberg et al (1972) in an investigation in which two doses of ethanol were given to man (19·6 g followed 1 h later by 9·8 g which led to a blood ethanol concentration less than 75 mg/100 ml). They found a decrease only in plasma alanine after this treatment. We thus deemed it necessary to investigate the effect of ethanol on the plasma amino acids under the conditions of our experiments quoted above.

Male Sprague Dawley rats (about 200 g) were purchased from Anticimex, Sollentuna, Sweden. Before use the animals were housed for at least one week in a room maintained on a 12:12 light/dark cycle and had free access to food and water.

Ethanol (20% w/v) 2 g kg^{-1} was injected intraperitoneally. Control rats received the equivalent volume of 0·9% NaCl (saline). After the injections no food or water was given. Sixty min after the ethanol injection the animals were decapitated and immediately about 5 ml blood was collected in a tube containing 0·5 ml of a 1% solution of EDTA and the brains were frozen on dry ice. The brains were homogenized and deproteinized and the plasma samples deproteinized (Bertler et al 1958). The preparations were stored at − 70 °C until analysis.

Amino acids were determined by ion exchange chromatography (Kontron Liquimat III, resin DC-4A and Pico-Buffer System IV from Durrum). The peak areas were integrated (Spectra Physics SP 4000).

Before analysis 0·1 ml sulphosalicylic acid (50% w/v), containing DL-norleucine as internal standard, was added to 1·0 ml of the preparations. The mixture was centrifuged in an Eppendorf centrifuge 3200 for 2 min and the clear supernatant was stored at + 4 °C until analysis (< 12 h). Determinations of aspartate, threonine, serine, glutamine and glutamate in the brain preparations were made on a sample diluted 10 times with Li-citrate buffer pH 2·2. The analysis were started immediately after the thawing of the preparations in order to get accurate determinations of glutamate and glutamine.

Statistical significances were assessed by Student's *t*-test.

Table 1 gives the values for the analysed amino acids in plasma 60 min after the injection of ethanol or saline, respectively. All amino acids except glutamine and glutamate were decreased in the ethanol-treated animals compared with the controls. The amplitude of the decrease varied between 12 and 53%.

The relative amount of each individual amino acid in per cent of the total plasma amino acid pool is shown in Table 2. For most of the amino acids the relative part was unchanged by ethanol but for glutamate, glutamine, lysine and ornithine there was an increase and for alanine, asparagine, phenylalanine and threonine, a decrease in the relative part of the total plasma amino acid pool.

The amounts of single amino acids in whole brain were measured. Only the concentration of alanine in the brain was decreased significantly in the ethanol-treated group (control 0·68 ± 0·015 μmol g^{-1} whole brain;

Table 1. Effect of ethanol on rat plasma amino acids. Ethanol 2 g kg^{-1} (20% w/v) or an equivalent volume of saline to the control rats was given intraperitoneally. Sixty min after the ethanol injection the animals were decapitated and blood samples collected. Plasma amino acids are given as means ± s.e.m., μmol $litre^{-1}$. Statistical significances were calculated by Student's *t*-test.

Amino acid	Controls (n = 6)	Ethanol treated (n = 6)	Decrease %	$P <$
Alanine	512 ± 14·9	241 ± 6·4	53	0·001
Arginine	227 ± 5·2	166 ± 7·6	27	0·001
Asparagine	86 ± 4·0	54 ± 2·8	37	0·001
Aspartate	<50	<50	—	—
Citrulline	114 ± 3·8	86 ± 2·6	25	0·001
Cystathione	<6	<6	—	—
Cystine	<25	<25	—	—
Glutamate	148 ± 5·7	139 ± 9·9	6	NS
Glutamine	724 ± 12·5	718 ± 21·9	1	NS
Glycine	387 ± 6·0	309 ± 9·8	20	0·001
Histidine	76 ± 2·8	62 ± 3·8	18	0·025
Isoleucine	96 ± 2·0	68 ± 2·9	29	0·001
Leucine	155 ± 2·8	108 ± 4·5	30	0·001
Lysine	504 ± 10·6	428 ± 13·2	15	0·005
Methionine	68 ± 3·3	46 ± 1·6	32	0·001
Ornithine	73 ± 1·9	64 ± 2·2	12	0·01
Phenylalanine	73 ± 2·2	50 ± 2·4	32	0·001
Serine	267 ± 3·9	195 ± 6·2	27	0·001
Threonine	274 ± 4·2	196 ± 2·7	28	0·001
Tryptophan	79 ± 2·3	55 ± 3·5	30	0·001
Tyrosine	115 ± 7·5	84 ± 1·0	27	0·005
Valine	217 ± 4·0	166 ± 9·2	24	0·001

* Correspondence.

Table 2. Effect of ethanol on the relative amount of individual amino acids in per cent of the total amino acid pool. Calculations based on the data of Table 1. In the table the values of the individual amino acids are expressed in per cent of the total amino acid pool (means ± s.e.m.). Statistical significances were calculated by Student's *t*-test.

Amino acid	Controls (n = 6)	Ethanol treated (n = 6)	Change in %	P <
Alanine	12·2 ± 0·26	7·4 ± 0·11	−39	0·001
Arginine	5·4 ± 0·10	5·1 ± 0·17	− 6	NS
Asparagine	2·1 ± 0·09	1·7 ± 0·07	−19	0·025
Citrulline	2·7 ± 0·08	2·7 ± 0·07	0	NS
Glutamate	3·5 ± 0·13	4·3 ± 0·27	+23	0·025
Glutamine	17·3 ± 0·27	22·2 ± 0·39	+28	0·001
Glycine	9·2 ± 0·19	9·6 ± 0·24	+ 4	NS
Histidine	1·8 ± 0·08	1·9 ± 0·08	+ 6	NS
Isoleucine	2·3 ± 0·06	2·1 ± 0·09	− 9	NS
Leucine	3·7 ± 0·08	3·4 ± 0·15	− 8	NS
Lysine	12·0 ± 0·23	13·2 ± 0·22	+10	0·005
Methionine	1·6 ± 0·07	1·4 ± 0·05	−13	NS
Ornithine	1·8 ± 0·03	2·0 ± 0·06	+11	0·025
Phenylalanine	1·8 ± 0·06	1·6 ± 0·04	−11	0·025
Serine	6·4 ± 0·11	6·0 ± 0·27	− 6	NS
Threonine	6·6 ± 0·09	6·0 ± 0·07	− 9	0·001
Tryptophan	1·9 ± 0·07	1·7 ± 0·13	−11	NS
Tyrosine	2·7 ± 0·18	2·6 ± 0·07	− 4	NS
Valine	5·2 ± 0·09	51· ± 0·25	− 2	NS

Table 3. Effect of ethanol on rat brain amino acids. The same experiment as in Table 1. Means ± s.e.m. for individual amino acids are given as μ mol g^{-1} whole brain. Statistical significances were calculated by Student's *t*-test.

Amino acid	Controls (n = 6)	Ethanol-treated (n = 6)	P<
Alanine	0·68 ± 0·015	0·61 ± 0·016	0·025
Arginine	0·24 ± 0·005	0·23 ± 0·005	NS
Aspartate	4·76 ± 0·173	4·62 ± 0·085	NS
Citrulline	0·05 ± 0·002	0·05 ± 0·002	NS
Cystathionine	0·07 ± 0·002	0·08 ± 0·004	NS
Cystine	0·02 ± 0·003	0·02 ± 0·004	NS
GABA	3·03 ± 0·087	3·15 ± 0·098	NS
Glutamate	13·09 ± 0·214	12·73 ± 0·147	NS
Glutamine	4·66 ± 0·190	4·94 ± 0·101	NS
Glycine	1·13 ± 0·022	1·11 ± 0·018	NS
Histidine	0·08 ± 0·003	0·08 ± 0·003	NS
Isoleucine	0·04 ± 0·005	0·04 ± 0·005	NS
Leucine	0·08 ± 0·007	0·08 ± 0·005	NS
Lysine	0·36 ± 0·007	0·35 ± 0·007	NS
Methionine	0·06 ± 0·004	0·06 ± 0·002	NS
Ornithine	0·02 ± 0·001	0·02 ± 0·000	NS
Phenylalanine	0·05 ± 0·002	0·05 ± 0·002	NS
Serine	1·21 ± 0·033	1·14 ± 0·024	NS
Threonine	0·71 ± 0·012	0·69 ± 0·022	NS
Tyrosine	0·12 ± 0·004	0·13 ± 0·008	NS
Valine	0·11 ± 0·007	0·15 ± 0·031	NS

ethanol-treated 0·61 ± 0·016 μmol g^{-1} n = 6). All other analysed amino acids remained unchanged.

The ethanol-induced decrease in most plasma amino acids, including the large neutral amino acids competing with L-dopa for the same carrier in the blood-brain barrier (Oldendorf 1975), provides a possible explanation for the increased passage through the blood-brain barrier of exogenously administered L-dopa after ethanol pretreatment. If this explanation is correct, the same phenomenon should obtain for other exogenously administered amino acids. In fact we have found that the passage through the blood-brain barrier of exogenously administered tyrosine, tryptophan, 5-hydroxytryptophan and α-methyldopa, too, is increased after ethanol treatment (to be published).

The proportion between different plasma amino acids transported by the same carrier, rather than their absolute concentrations, appears to determine their availability for uptake by the brain. This could be the explanation of our finding that only alanine, which showed the most marked decrease in plasma (Table 1), decreased in the brain after ethanol loading.

One possibility that might explain how ethanol exerts its amino acid-decreasing effect could be that the effect is mediated via some stress mechanism. We have found that intraperitoneal injections of saline alone give rise to a decrease in some plasma amino acids but to a much lower extent than does ethanol (unpublished data). This could be due to a stress reaction which in some way might be accentuated by ethanol.

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