Conclusion. A method of measuring internal pressures of ampoules has been described. The internal pressure of ampoules can be measured by displacement of water. Internal pressures in ampoules are generally lower than atmospheric pressure and may be one of the reasons for contamination by glass particles when the ampoule is opened.

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Dose-dependent decrease in rat plasma amino acids after acute administration of ethanol

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Abstract—Male rats were given three different doses of ethanol in i.p. injections (0·66, 1·33 and 2·00 g kg^{-1}). A dose-dependent decrease in the concentrations of most plasma amino acids was observed. For the total amino acid concentration this decrease was 5, 16 and 22%, respectively, compared with a saline-treated control group. It has previously been suggested that the oxidation of ethanol plays an important role in the amino acid decreasing effect of ethanol. In this study the lowest dose used (0·66 g kg^{-1}) was calculated to be high enough to keep the enzyme systems involved in ethanol oxidation saturated during the 60 min course of the experiment. The observation that the ethanol-induced decrease in plasma amino acid levels was more pronounced with higher ethanol doses indicates that not only the oxidation of ethanol but also ethanol itself is important in the effect of ethanol on plasma amino acid concentrations.

We have previously reported that ethanol, when given in acute doses, induces a rapid decrease in the concentrations of most plasma amino acids in rat (Eriksson et al 1980) and in man (Eriksson et al 1983). The mechanisms underlying this effect of ethanol is complicated and not well understood. Both effects of ethanol itself (Eriksson et al 1981) and effects of its metabolites (Hagman & Jagenburg 1989) have been proposed.

The ethanol elimination rate in rats is virtually constant down to the rather low saturation level of hepatic alcohol dehydrogenase (Makar & Mannering 1970; Braggins & Crow 1982). Furthermore, this rate is also, within a wide range, independent of the administered dose of ethanol (Braggins & Crow 1981). These observations offer an opportunity to distinguish between effects caused by ethanol itself and effects caused by its metabolites. At ethanol concentrations above the saturation level the rats can only metabolize a specific amount of ethanol per unit time. This means that all further effects of ethanol above this level must be due to ethanol itself, and that they are independent of the metabolism of ethanol.

The present study is an attempt to distinguish between direct and indirect effects of ethanol on plasma amino acid concentrations, using this approach.

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Materials and methods

Male Sprague Dawley rats, about 200 g (ALAB, Sollentuna, Sweden), were used. They were housed for at least one week in a room maintained on a 12 h light/dark cycle and had free access to food and water. During the experiment no food or water was given. Ethanol was diluted in saline and injected intraperitoneally in doses of 0·66, 1·33 or 2·00 g kg^{-1}. Control rats received an equivalent volume of saline (10 mL kg^{-1}). Sixty min after the injections the animals were killed by decapitation. About 5 mL blood was collected in EDTA tubes and immediately centrifuged at 10 000 *g* for 10 min. The plasma samples were stored at −70°C until amino acid analysis.

Amino acids were analyzed by ion-exchange chromatography after deproteinization with sulphosalicylic acid as described elsewhere (Eriksson et al 1980).

Statistical significances were assessed by linear regression analysis.

Results

Ethanol exerts a decreasing effect on the total concentration of amino acids in rat plasma and the magnitude of this effect is dependent on the administered dose of ethanol (Table 1). The lowest ethanol dose used (0·66 g kg^{-1}) induced a decrease of 5%, whereas the highest dose (2·00 g kg^{-1}) induced a decrease of 22%.

The concentrations of most of the individual amino acids also declined in a dose-dependent manner after acute administration of ethanol (Table 1). This correlation was statistically significant for all measured amino acids except glutamine and lysine. Alanine was the amino acid the concentration of which showed the largest decrease (17, 34 and 44%, respectively) after administration of the three ethanol doses.

Table 1. Effects of various doses of ethanol on the concentrations of amino acids in rat plasma.

Amino acid	Saline μmol L^{-1}	Ethanol (0·66 g kg^{-1}) μmol L^{-1}	% change	Ethanol (1·33 g kg^{-1}) μmol L^{-1}	% change	Ethanol (2·00 g kg^{-1}) μmol L^{-1}	% change	$P<$
Aspartate	35 (1·5)	34 (1·6)	−4	29 (0·8)	−19	27 (0·8)	−25	0·001
Threonine	264 (6·5)	238 (14·5)	−10	207 (8·1)	−22	185 (9·9)	−30	0·001
Serine	245 (10·9)	238 (14·5)	−3	200 (7·6)	−18	191 (6·5)	−22	0·001
Glutamate	148 (4·3)	163 (6·3)	+10	143 (4·8)	−3	133 (8·6)	−10	0·05
Glutamine	545 (22·2)	598 (11·6)	+10	524 (24·9)	−4	509 (14·5)	−7	NS
Proline	197 (19·8)	177 (13·9)	−10	144 (7·8)	−27	120 (16·6)	−39	0·001
Glycine	329 (6·2)	317 (19·6)	−4	256 (6·3)	−22	257 (10·1)	−22	0·001
Alanine	357 (7·1)	296 (11·0)	−17	234 (21·2)	−34	199 (8·2)	−44	0·001
Citrulline	119 (7·5)	97 (6·6)	−18	92 (6·8)	−23	85 (3·8)	−29	0·001
Valine	208 (8·8)	173 (8·4)	−17	168 (10·1)	−19	151 (5·4)	−27	0·001
Methionine	52 (3·9)	50 (2·2)	−5	47 (2·9)	−10	38 (2·5)	−26	0·01
Isoleucine	89 (2·4)	79 (3·2)	−11	79 (2·6)	−11	75 (2·5)	−16	0·001
Leucine	135 (5·3)	117 (4·5)	−13	115 (5·1)	−15	105 (3·2)	−22	0·001
Tyrosine	81 (2·8)	76 (2·8)	−6	67 (2·7)	−17	66 (2·6)	−18	0·001
Phenylalanine	68 (3·2)	59 (2·6)	−13	50 (2·4)	−26	46 (1·6)	−32	0·001
Ornithine	62 (3·7)	61 (1·7)	−2	52 (3·2)	−15	55 (1·4)	−10	0·05
Lysine	450 (21·8)	461 (14·7)	+2	452 (24·1)	0	409 (10·4)	−10	NS
Histidine	68 (1·6)	66 (2·4)	−4	59 (2·2)	−14	61 (1·4)	−10	0·001
Tryptophan	111 (6·6)	82 (8·5)	−26	83 (2·2)	−26	67 (3·1)	−40	0·001
Arginine	196 (7·2)	172 (6·2)	−5	168 (5·8)	−14	143 (5·1)	−27	0·001
Total	3775 (45·7)	3571 (81·3)	−5	3187 (113)	−16	2932 (45·3)	−22	0·001

Concentrations and variations are expressed as mean values and s.e.m., respectively. For ethanol-treated groups the change in percent of control values is also given. *P* values were obtained by linear regression analysis. n = 7 in each group. NS = not significant.

Discussion

Our results show that the amino acid decreasing effect of ethanol is dose-dependent, at least up to doses of 2 g ethanol per kg body mass.

In a previous investigation we have shown that male Sprague Dawley rats, under conditions similar to those in this study, eliminate ethanol from the body water at a rate of about 15 mmol L^{-1} h^{-1} (Hagman & Jagenburg 1989). This rate is believed to be essentially constant down to ethanol levels of about 5 mmol L^{-1} (Makar & Mannering 1970). Assuming a distribution volume of 70%, it can be calculated that the injection of 0·66 g ethanol per kg body mass gives rise to an initial ethanol concentration of 20 mmol L^{-1}. This dose should thus be sufficient to keep the ethanol elimination at a maximal rate during one hour.

In this study only a minor part of the decrease in the plasma amino acid concentrations was seen when 0·66 g kg^{-1} ethanol was administered, while the major part was seen after administration of higher doses. This indicates that ethanol itself indeed is an important factor in the amino acid decreasing effect of ethanol and that the metabolism of ethanol is of minor importance.

The way in which ethanol exerts its effect on plasma amino acids is not clear. It might, however, involve β-adrenergic mechanisms. This idea is supported by the findings that ethanol stimulates the release of catecholamines from the adrenal medulla (Perman 1960; Thiagarajan et al 1989), that adrenaline causes hypoaminoacidaemia (Shamoon et al 1980) and that the β-adrenergic antagonist propranolol partly inhibits the ethanol-induced decrease in plasma amino acid concentrations (Eriksson et al 1981).

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