J. Pharm. Pharmacol. 1991, 43: 625–629
Received February 8, 1991

Development of Tolerance to the Plasma Amino Acid-Decreasing Effect of Ethanol in the Rat

MATS HAGMAN AND TOMAS ERIKSSON*

*Department of Clinical Chemistry, Sahlgren's Hospital, Gothenburg University, S-413 45 Gothenburg and *Department of Pharmacology, Gothenburg University, S-400 33 Gothenburg, Sweden*

Abstract—Male Sprague-Dawley rats were treated for one month with daily intraperitoneal injections of ethanol (2 g kg^{-1}), or saline. After this pretreatment, animals from each group were given acute doses of ethanol (2 g kg^{-1}) or saline. Plasma amino acid concentrations and brain tyrosine, tryptophan, dopamine, 5-HT and 5-HIAA concentrations were measured in samples collected 1 h after the injections. Acute administration of ethanol induced a dramatic fall in the concentrations of 18 out of 20 plasma amino acids in animals pretreated with saline. In animals chronically pretreated with ethanol this decrease was much smaller. Furthermore, the decrease was significantly lower for 6 of the measured amino acids in the chronic ethanol group compared with the saline-treated control group. Tolerance to the plasma amino acid decreasing effect of ethanol had thus developed. This acquired tolerance might be explained by both pharmacokinetic and pharmacodynamic mechanisms. Chronic administration of ethanol induced increased concentrations of tyrosine and dopamine in the brain, probably due to increased transport of tyrosine into the brain caused by an increase in the ratio of tyrosine to large neutral amino acids in plasma.

Acute administration of ethanol decreases the concentrations of most amino acids in plasma in both man (Siegel et al 1964; Kreisberg et al 1972; Eriksson et al 1983) and rat (Eriksson et al 1980; Milakofsky et al 1986). Two different mechanisms underlying this effect of ethanol have been identified. One mechanism is dependent on the oxidation of ethanol and is probably related to the hepatic redox-shift that is induced when ethanol is oxidized (Hagman & Jagenburg 1989). The other mechanism depends on ethanol itself (Hagman & Eriksson 1990) and is mediated via β-adrenergic receptors (Eriksson et al 1981).

Siegel et al (1964) noted that the plasma amino acid pattern in alcoholics differs from that in control subjects. In control subjects ethanol loading induced decreased concentrations of many plasma amino acids, whereas in alcoholics this decrease was less pronounced or even reversed.

Tolerance to many of the acute effects of ethanol can develop after chronic administration of the compound (Kalant et al 1971; Tabakoff & Hoffman 1987). This acquired tolerance can involve an increased rate of ethanol clearance (pharmacokinetic tolerance) or it can involve an adaptation to the effects of ethanol at a particular ethanol concentration in the body (pharmacodynamic tolerance).

The effects of ethanol on brain amino acids are also of interest. The two amino acids tyrosine and tryptophan serve as precursors to monoaminergic neurotransmitters in the central nervous system (CNS). Dopamine, noradrenaline and adrenaline are synthesized from tyrosine and 5-hydroxytryptamine (5-HT) from tryptophan (Fernstrom 1983; Milner & Wurtman 1986).

Tyrosine and tryptophan are transported from the blood into the brain by a specific carrier system. The carrier is saturable and common to all the large neutral amino acids (LNAAs: valine, isoleucine, leucine, tyrosine, phenylalanine and tryptophan). LNAAs compete with each other for transport into the brain and the relation between them rather than their actual concentration seems to determine how much of the different LNAAs will be transported into the brain. Changes in the relative concentrations of amino acids in plasma may thus influence the availability of various amino acids in the brain as well as the synthesis of monoamines (Wurtman 1982).

To investigate the possibility of a development of tolerance to the plasma amino acid-decreasing effect of ethanol we have administered acute doses of ethanol to rats, chronically pretreated with ethanol and 0·9% NaCl (saline), respectively. Concomitantly, we have investigated the effects of these treatments on brain concentrations of tyrosine, tryptophan, dopamine, 5-HT and 5-hydroxyindolacetic acid (5-HIAA).

Materials and Methods

Male Sprague-Dawley rats, ~150 g, were purchased from ALAB, Sweden. Before use they were housed for at least one week in a room maintained on a 14/10 h light/dark cycle. They were allowed free access to food and water throughout the experiment.

One group of rats was given intraperitoneal (i.p.) injections of ethanol (2·0 g kg^{-1}, 20% w/v in saline) once a day for more than one month. A control group received equivalent doses of saline. Individual body weights were measured once a week.

After the chronic treatment period each group was divided into two groups for different acute treatments. One group from each of the previous groups was given i.p. injections of ethanol (2·0 g kg^{-1}). Corresponding groups received equivalent doses of saline.

Sixty min after the respective injections the animals were killed by decapitation. Five mL blood was collected in EDTA tubes and immediately centrifuged. The brains were

Correspondence: M. Hagman, Department of Clinical Chemistry, Östra Sjukhuset, S-416 85 Gothenburg, Sweden.

rapidly taken out and frozen on dry ice, homogenized and deproteinized according to procedures previously described (Bertler et al 1958). Plasma samples and brain preparations were stored at −70°C until analysis.

Plasma amino acids were determined by ion-exchange chromatography on an automatic amino acid analyser (Kontron Liquimat III) using the ninhydrin reaction for detection. Before analysis 0·1 mL sulphosalicylic acid (50% w/v) was added to 1·0 mL plasma. The mixture was centrifuged at 9000 *g* for 2 min and the clear supernatant was used for analysis.

Brain concentrations of tyrosine, tryptophan, dopamine, 5-HT and 5-HIAA were determined according to Atack & Magnusson (1978).

Statistical significances were assessed by one-way analysis of variance followed by a two-tailed *t*-test. Significance of differences between decreases was assessed by a one-tailed *t*-test, based on absolute decrease values and pooled variances.

Results

Animals chronically pretreated with daily injections of ethanol gained body weight more slowly than their saline-treated controls during the first two weeks, and thereafter the weight gain was almost identical in the two groups (Fig. 1).

Acute administration of ethanol induced statistically significant decreases in the concentrations of all measured plasma amino acids, except glutamine and cystine, in the group pretreated with saline (Table 1). The total concentration of amino acids decreased by 29%. Alanine showed the largest response to ethanol with a decrease of 53%.

In the group chronically pretreated with ethanol, the effect of acute ethanol administration on plasma amino acids was less pronounced, but ethanol still induced statistically significant decreases in 11 of the 20 measured amino acids (Table 1). The decreases were, however, significantly reduced for 6 of the amino acids in the chronic ethanol group (alanine, methionine, leucine, lysine, tryptophan and arginine). The total concentration of amino acids decreased by 15% and the concentration of alanine by 29%. The levels after acute ethanol administration were also significantly higher for 13 amino acids (aspartate, threonine, serine, glutamine, glycine, alanine, citrulline, methionine, tyrosine, ornithine, lysine and arginine) in the chronic ethanol group than in the saline-treated control group.

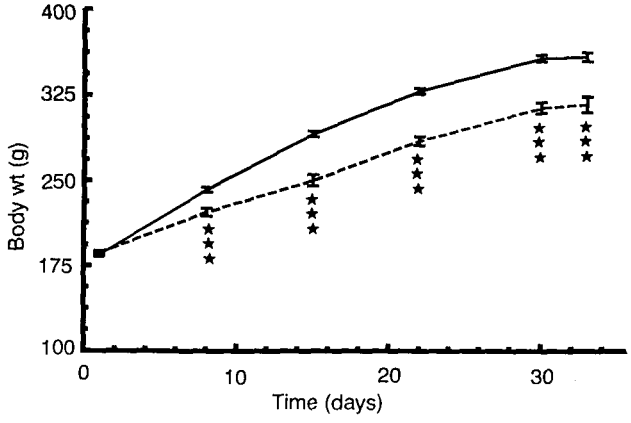

FIG. 1. Body weight of rats treated with daily i.p. injections of ethanol (2 g kg^{-1}) for more than one month (————). Controls received equivalent doses of saline (- - - -). Curves are the mean ± s.e.m. of 14 rats. *** $P < 0{\cdot}001$.

Chronic administration of ethanol had no significant effect on the total concentration of amino acids in rat plasma (Table 1). Among the individual amino acids the concentrations of valine and tryptophan showed statistically significant decreases ($P = 0{\cdot}040$ and 0·025, respectively).

The ratios of the concentrations of tyrosine and tryptophan to the total concentration of the LNAAs are presented in Table 2. No significant changes in these ratios were seen after acute administration of ethanol. Chronic administration of ethanol induced, however, a significant increase in the tyrosine/LNAA ratio, whereas the tryptophan/LNAA ratio was essentially unaffected.

Chronic administration of ethanol induced an increase in the concentrations of tyrosine, tryptophan and dopamine in rat brain (Table 3). The concentrations of 5-HT and 5-HIAA remained essentially unaffected. There were no significant effects of acute ethanol administration on the concentrations of any of the measured compounds in the brain, either in the control group or in the chronic ethanol group.

Discussion

This study shows that chronic ethanol administration to rats leads to the development of tolerance to the plasma amino acid decreasing effect of ethanol. There are two types of mechanisms that might be responsible for this type of acquired tolerance.

First, it is possible that the tolerance in this study is a type of pharmacokinetic tolerance. Ethanol loading leads to a rapid (Milakofsky et al 1986) and dose-dependent (Hagman & Eriksson 1990) decrease in most rat plasma amino acids. In a previous report it has been shown that part of this decrease is dependent on the oxidation of ethanol (Hagman & Jagenburg 1989). The hypothesis has been raised that the increase in the hepatic NADH/NAD ratio during ethanol oxidation turns the metabolism of amino acids to more reductive pathways, e.g. deamination to ketoacids, and leads to a decline in the amino acid concentrations (Milakofsky et al 1986; Hagman & Jagenburg 1989). Ethanol is mainly eliminated from the body via the hepatic alcohol dehydrogenase pathway, with the formation of large amounts of NADH (Crabb et al 1987). A smaller quantity is also oxidized via the microsomal ethanol oxidizing system in a reaction that is associated with the conversion of NADPH to NADP. The activity of this system is induced by chronic ethanol administration (Coon & Koop 1987; Lieber et al 1987) and this leads to an attenuation of the hepatic redox shift that is seen after acute ethanol loading (Domschke et al 1974). It is possible that the development of tolerance to the amino acid decreasing effect of ethanol reported in this study is a pharmacokinetic type of tolerance due to the induction of the microsomal ethanol oxidizing system.

Secondly, it is possible that the results reflect a pharmacodynamic tolerance due to an adaption inside or outside the CNS. It is known that ethanol loading leads to an increased secretion of adrenaline and noradrenaline by the adrenal gland (Perman 1960; Shamoon et al 1980), and that adrena-

Table 1. Effect of acute administration of ethanol on plasma amino acid concentrations in rats chronically pretreated with ethanol or saline.

Amino acid	Chronic treatment	Acute treatment: Saline	Acute treatment: Ethanol	Decrease %	Statistics (P)
Aspartate	Saline	33±1·7	23±0·9	28±5·9	<0·001
	Ethanol	35±2·3	27±0·5	24±6·3	<0·001
Threonine	Saline	222±9·3	135±5·2	39±4·8	<0·001
	Ethanol	234±31·6	193±9·4	25±13·2	NS
Serine	Saline	223±5·3	139±3·3	38±2·8	<0·001
	Ethanol	241±21·8	165±6·9	31±8·9	<0·001
Glutamate	Saline	188±11·3	154±8·0	18±7·4	<0·05
	Ethanol	182±11·8	169±8·5	7±7·7	NS
Glutamine	Saline	516±38·9	475±31·1	8±9·6	NS
	Ethanol	579±41·6	568±35·6	2±9·4	NS
Glycine	Saline	346±9·5	256±5·8	26±3·2	<0·001
	Ethanol	384±28·9	315±14·9	18±8·1	<0·01
Alanine	Saline	413±13·8	194±6·7	53±3·7	<0·001
	Ethanol	439±30·7	313±8·2	29±6·7	<0·001
Citrulline	Saline	77±3·0	59±2·5	23±5·0	<0·001
	Ethanol	86±5·7	71±3·1	18±7·2	<0·01
Valine	Saline	175±7·3	120±3·0	31±4·5	<0·001
	Ethanol	151±10·6	115±4·0	24±7·1	<0·001
Cystine	Saline	10±1·1	9±1·3	8±13·3	NS
	Ethanol	9±1·2	7±1·4	24±21·3	NS
Methionine	Saline	49±3·3	31±1·8	36±7·6	<0·001
	Ethanol	48±5·6	42±3·2	12±12·9	NS
Isoleucine	Saline	78±4·9	58±2·8	26±7·2	<0·01
	Ethanol	68±6·8	61±4·3	11±11·4	NS
Leucine	Saline	125±5·7	83±3·0	34±13·1	<0·001
	Ethanol	110±6·8	87±4·0	21±6·9	<0·01
Tyrosine	Saline	80±3·3	53±2·7	34±5·3	<0·001
	Ethanol	82±5·2	67±4·2	18±8·0	<0·05
Phenylalanine	Saline	63±4·4	40±2·3	37±7·9	<0·001
	Ethanol	64±3·6	41±2·0	37±6·2	<0·001
Ornithine	Saline	54±4·0	43±1·4	20±7·8	<0·01
	Ethanol	55±1·6	50±1·9	9±4·6	<0·05
Lysine	Saline	438±13·6	339±11·6	23±4·1	<0·001
	Ethanol	402±20·0	397±9·7	1±5·3	NS
Histidine	Saline	64±1·7	46±4·5	29±2·8	<0·001
	Ethanol	68±3·2	57±1·7	17±5·0	<0·001
Tryptophan	Saline	89±3·6	54±2·0	39±4·6	<0·001
	Ethanol	69±9·0	55±4·9	20±14·9	NS
Arginine	Saline	189±9·5	126±3·5	33±5·4	<0·001
	Ethanol	181±9·9	161±8·0	11±7·0	NS
Total	Saline	3428±86·9	2450±53·3	29±3·0	<0·001
	Ethanol	3502±153·1	2975±54·4	15±4·3	<0·01

Mean concentrations in μmol L^{-1} ± s.e.m. of 6 to 7 animals are given. The decrease in percent is calculated from the absolute concentration values. NS = not significant.

Table 2. Effect of acute administration of ethanol on plasma ratios of tyrosine/LNAA and tryptophan/LNAA in rats chronically pretreated with ethanol or saline.

Ratio	Chronic treatment	Acute treatment: Saline	Acute treatment: Ethanol	Statistics
Tyrosine/LNAA	Saline	0·131±0·003	0·129±0·007	NS
	Ethanol	0·151±0·004*	0·157±0·006**	NS
Tryptophan/LNAA	Saline	0·146±0·005	0·133±0·003	NS
	Ethanol	0·124±0·012*	0·130±0·007	NS

Values given are the mean ratios ± s.e.m. of 6–7 animals. Calculations are based on values presented in Table 1. The statistics indicated with stars give the effect of chronic ethanol treatment vs chronic treatment with saline. *P < 0·05; **P < 0·01; NS = not significant.

Table 3. Effect of acute administration of ethanol on brain concentrations of tyrosine, tryptophan, dopamine, 5-HT and 5-HIAA in rats chronically pretreated with ethanol or saline.

		Acute treatment		
Substance	Chronic treatment	Saline	Ethanol	Statistics
Tyrosine	Saline	13·2±0·29	13·0±0·83	NS
	Ethanol	16·0±0·90*	16·3±1·26*	NS
Tryptophan	Saline	3·6±0·10	3·4±0·14	NS
	Ethanol	3·9±0·24	3·7±0·05	NS
Dopamine	Saline	0·63±0·013	0·65±0·013	NS
	Ethanol	0·68±0·025*	0·65±0·013	NS
5-HT	Saline	0·26±0·010	0·25±0·007	NS
	Ethanol	0·25±0·006	0·26±0·006	NS
5-HIAA	Saline	0·17±0·004	0·16±0·003	NS
	Ethanol	0·17±0·005	0·17±0·005	NS

Mean values of 6–7 animals in nmol g^{-1} ± s.e.m. are given. The statistics indicated with asterisks give the effect of chronic ethanol treatment vs chronic treatment with saline. * $P < 0.05$; NS = not significant.

line exerts an amino acid decreasing effect (Eriksson & Carlsson 1982; Strombeck et al 1984; Thiagarajan et al 1989). Since the secretion by the adrenal medulla is controlled by sympathetic nerves from the CNS and tolerance to many of the effects of ethanol on the CNS are known, the results of the present study might also be explained by this kind of pharmacodynamic mechanism.

The effect of ethanol administration on the concentrations of brain monoamines has received much attention during the last decades (Pohorecky & Brick 1988), although there are discrepancies in the early studies, probably due to methodological differences. We report here that daily injections of ethanol (2·0 g kg^{-1}), for about one month, elevates the concentrations of dopamine and its precursor tyrosine in rat brain. The elevation in brain tyrosine is probably due to the increase in the tyrosine/LNAA ratio that is observed in the plasma. This increased ratio would stimulate the transport of tyrosine into the brain, since all the LNAAs are considered to compete with each other for the carrier mediated-transport mechanism (Wurtman 1982). Increased availability might possibly stimulate the synthesis of dopamine (Fernstrom 1983). The results of the present study thus underline the role of the plasma amino acid concentrations in the control of monoamine synthesis in the brain.

In the present study only minor differences in plasma amino acid concentrations were observed between the group that was chronically pretreated with ethanol and the saline treated control group. There were significant decreases in the concentrations of tryptophan and valine but the other branched-chain amino acids also tended to fall. Similar results have been obtained by Farbiszewski et al (1986), who noted decreases in valine, isoleucine and leucine together with increases in phenylalanine, tyrosine and methionine after four weeks of intraventricular administration of ethanol (6·0 g kg^{-1} per day) to rats. These results are, however, contrary to those of several other studies (Siegel et al 1964; Shaw & Lieber 1979; Stanko et al 1979) in which valine, isoleucine and leucine are elevated after chronic ethanol consumption. The reason for the discrepancy is not clear, but may be due to differences in the procedures for ethanol administration.

Acknowledgements

This work was supported by the Faculty of Medicine, Gothenburg University and the Swedish Medical Research Council (project no. 8665). The expert technical assistance of Mrs Birgitta Holmgren is gratefully acknowledged.

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