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EFFECT OF 4-METHYLPYRAZOLE ON ETHANOL-INDUCED DECREASE IN RAT PLASMA AMINO ACIDS

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Abstract—It has been shown earlier that an acute dose of ethanol causes an immediate decrease in the concentration of most plasma amino acids, and that this decrease involves both β-adrenergic and adrenocortical mechanisms. In this work is shown that the oxidation of ethanol also plays an important role in the amino acid decreasing effect. Male rats were pretreated with 4-methylpyrazole, an inhibitor of liver alcohol dehydrogenase, in doses causing an 85% inhibition of the ethanol elimination. In this group the ethanol-induced decrease in amino acids was much less pronounced than in a control group pretreated with saline.

Ethanol, when given in acute doses, induces a rapid decrease in the concentrations of most plasma amino acids, in rat [1–3], rabbit [4] and man [5–8]. This effect of ethanol can be partly inhibited by the β-adrenergic antagonist propranolol or by adrenalectomy or hypophysectomy [9], indicating that the decrease depends on both β-adrenergic and adrenocortical mechanisms. However, it is possible that non-hormonal mechanisms might also be involved. Alanine is the amino acid which concentration decreases most obviously after ethanol administration [2–8]. This amino acid has also a key role in the gluconeogenesis [10], and is readily deaminated to pyruvate in the liver. The oxidation of ethanol results in an increased lactate/pyruvate ratio [11] as a consequence to the massive formation of NADH from NAD^+ [12, 13]. Thus, it seems likely that the ethanol-induced redox-shift in the liver might increase the deamination of alanine to pyruvate.

4-Methylpyrazole and some other pyrazole derivatives, are known to be potent inhibitors of ethanol oxidation [14, 15], and to diminish the ethanol-induced increase in the NADH/NAD^+ and lactate/pyruvate ratios [16]. By using this drug, we have studied if the oxidation of ethanol is of importance for the ethanol-induced decrease in the concentrations of alanine and other plasma amino acids.

MATERIALS AND METHODS

Male Sprague–Dawley rats (130–150 g) were purchased from Anticimex (Sollentuna, Sweden). Before use, the animals were housed for at least one week in a room maintained on a 14/10 hr light/dark cycle. They were allowed free access to food and water.

The animals were pretreated with an intraperitoneal injection of 4-methylpyrazole (3.37 mmol/kg b.w. as a neutralized, 4% w/v, solution of the hydrochloride) at about 4 p.m. the first day of the experiment. A control group received an equivalent volume of saline.

Starting at about 9 a.m. the following day, ethanol (2 g/kg b.w., 20% w/v), or saline, was injected intraperitoneally. After these injections no food or water was given. Sixty minutes after the last injection the animals were killed by decapitation. Blood (5 ml) was collected in EDTA tubes and immediately centrifuged. The plasma samples were stored at −70° until analysis of amino acids.

The plasma amino acids were determined by ion-exchange chromatography after deproteinization with sulphosalicylic acid as described elsewhere [2].

In order to determine the elimination rate of ethanol, tailtip samples were taken at regular intervals from animals receiving the same treatments as described above. The samples were immediately centrifuged and stored at −70°. Ethanol was analyzed on a gas-chromatograph (Perkin Elmer F 11) with methanol as internal standard [17].

Ethanol elimination rates were calculated by means of linear regression, assuming zero-order kinetics throughout the observation periods.

Statistical significances were assessed by Students *t*-test, one-tailed or two-tailed where appropriate. P-values were corrected according to Bonferroni.

RESULTS

The elimination rate of ethanol was 14.4 $mmol \cdot l^{-1} \cdot hr^{-1}$ in the control group, and 2.1 $mmol \cdot l^{-1} \cdot hr^{-1}$ in animals pretreated with 4-methylpyrazole (Fig. 1), i.e. the elimination rate was reduced by 85% after administration of 4-methylpyrazole.

Ethanol alone caused a significant decrease in the concentrations of most amino acids (Table 1). The concentration of alanine decreased by 53% and the sum of the concentrations of all other amino acids

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Table 1. Effect of ethanol on individual plasma amino acids in rats with and without 4-methylpyrazole pretreatment

Amino acid	Treatment	Control	4-Methylpyrazole
Aspartate	saline	35.4 (0.8)	29.9 (0.9)
	ethanol	26.6 (0.8)***	24.7 (1.2)*
Threonine	saline	327 (12.3)	308 (15.1)
	ethanol	237 (13.9)***	262 (10.6)ns
Serine	saline	281 (5.9)	315 (15.1)
	ethanol	177 (9.2)***	260 (4.3)*
Glutamate	saline	144 (8.6)	121 (5.9)
	ethanol	112 (5.7)*	94 (10.5)ns
Glutamine	saline	598 (19.5)	602 (24.0)
	ethanol	589 (30.7)ns	564 (20.4)ns
Proline	saline	221 (13.8)	228 (16.2)
	ethanol	147 (4.5)**	205 (13.7)ns
Glycine	saline	458 (10.5)	459 (12.2)
	ethanol	332 (7.6)***	358 (16.2)**
Alanine	saline	453 (16.6)	463 (26.2)
	ethanol	211 (12.2)***	357 (15.1)*
Citrulline	saline	105 (3.8)	93.9 (5.9)
	ethanol	76.4 (5.0)**	94.1 (4.5)ns
Valine	saline	201 (12.5)	159 (6.7)
	ethanol	134 (3.3)***	170 (10.5)ns
Cystine	saline	18.1 (1.5)	18.7 (0.9)
	ethanol	16.8 (1.7)ns	20.7 (1.8)ns
Methionine	saline	64.8 (2.9)	61.6 (1.8)
	ethanol	46.6 (2.2)**	54.8 (2.4)ns
Isoleucine	saline	93.0 (6.0)	80.9 (3.8)
	ethanol	66.5 (1.7)**	90.6 (6.1)ns
Leucine	saline	145 (10.9)	121 (5.5)
	ethanol	90.8 (2.1)**	117 (6.7)ns
Tyrosine	saline	83.4 (3.0)	75.3 (2.5)
	ethanol	59.0 (3.2)***	71.6 (5.6)ns
Phenylalanine	saline	68.3 (3.6)	69.9 (2.5)
	ethanol	43.5 (1.1)***	59.2 (2.4)*
Ornithine	saline	60.9 (2.2)	59.7 (3.1)
	ethanol	54.2 (2.2)ns	67.0 (4.9)ns
Lysine	saline	403 (6.2)	400 (27.8)
	ethanol	347 (8.7)***	368 (11.7)ns
Histidine	saline	68.4 (3.0)	63.5 (1.5)
	ethanol	53.4 (2.0)**	59.9 (3.6)ns
Tryptophane	saline	105 (4.0)	77.9 (5.9)
	ethanol	60.3 (3.1)***	66.6 (5.8)ns
Arginine	saline	174 (4.5)	169 (7.9)
	ethanol	127 (3.6)***	146 (5.0)ns

Mean values in μmol/l (and SEM) are given, N = 6. * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$) indicate significance of difference in amino acid concentration between animals given ethanol and saline respectively.

by 24% (Fig. 2). In animals pretreated with 4-methylpyrazole this effect of ethanol was less pronounced. The alanine concentration decreased by 23% and the sum of the concentrations of all other amino acids by 12%. The alanine concentration and the sum of the concentrations of all other amino acids were significantly higher ($P < 0.001$ and $P < 0.05$, respectively) in animals treated with ethanol in combination with 4-methylpyrazole than in control animals receiving saline instead of 4-methylpyrazole (Fig. 2). Pretreatment with 4-methylpyrazole *per se* had no significant effect either on the alanine concentration or on the sum of the concentrations of other amino acids.

The concentrations of the individual amino acids are shown in Table 1. Administration of 4-methylpyrazole caused *per se* a significant decrease in the concentrations of aspartate ($P < 0.01$) and tryptophane ($P < 0.05$) whereas the other amino acids were essentially unaffected.

Ethanol given alone induced a significant decrease in the concentration of all amino acids except glutamine, cystine and ornithine.

After pretreatment with 4-methylpyrazole, ethanol still induced a significant decrease in the concentrations of 5 of the 21 measured amino acids (aspartate, serine, glycine, alanine and phenylalanine). The amino acid decreasing effect of ethanol, however, was less pronounced after pretreatment with 4-methylpyrazole. The decrease was significantly higher for 10 amino acids when ethanol was given to control animals than when ethanol was given in combination with 4-methylpyrazole (Table 2).

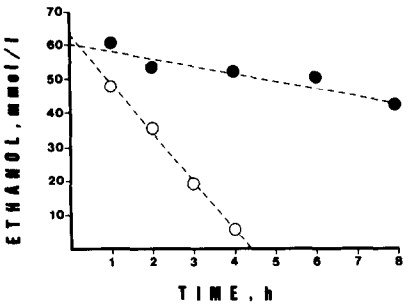

Fig. 1. Ethanol elimination in rats given 2 g ethanol/kg b.w. as an intraperitoneal injection. ●, Animals pretreated with 4-methylpyrazole 3.37 mmol/kg b.w. as an intraperitoneal injection 17 hr prior to administration of ethanol (N = 3); ○, controls, pretreated with saline only (N = 4). Mean values are given.

Table 2. Effect of 4-methylpyrazole on ethanol-induced decrease in rat plasma amino acids

Amino acid	Control	4-Methylpyrazole	P-value
Aspartate	24.9 (3.1)	17.4 (5.0)	NS
Threonine	27.5 (5.7)	14.9 (6.0)	NS
Serine	37.0 (3.9)	17.5 (5.0)	<0.05
Glutamate	22.2 (7.2)	22.3 (9.9)	NS
Glutamine	0.5 (6.1)	6.3 (5.2)	NS
Proline	33.5 (6.6)	10.1 (9.3)	NS
Glycine	27.5 (2.8)	22.0 (4.4)	NS
Alanine	53.4 (4.4)	22.9 (6.5)	<0.001
Citrulline	27.2 (6.0)	−0.2 (7.9)	<0.05
Valine	33.3 (6.4)	−6.9 (7.9)	<0.001
Cystine	7.2 (12.7)	−10.7 (10.7)	NS
Methionine	28.1 (5.6)	11.0 (4.9)	<0.05
Isoleucine	28.4 (6.7)	−12.0 (8.9)	<0.01
Leucine	37.2 (7.7)	3.3 (7.2)	<0.01
Tyrosine	29.3 (5.3)	4.9 (8.4)	<0.05
Phenylalanine	36.3 (5.6)	15.3 (5.0)	<0.05
Ornithine	11.0 (5.1)	−12.2 (9.7)	NS
Lysine	13.9 (2.7)	8.0 (7.6)	NS
Histidine	21.9 (5.3)	5.7 (6.1)	NS
Tryptophane	42.6 (4.9)	14.5 (10.7)	<0.01
Arginine	27.0 (3.3)	13.6 (5.0)	NS

The values given are the mean (and SEM) decrease in percent induced by ethanol, P-values indicate significance of difference between the two groups, N = 6. One-tailed *t*-test was used on the absolute values calculated from Table 1.

DISCUSSION

Ethanol is eliminated from the body by several different pathways [18]. The relative contribution of each pathway has been discussed for many years. It seems now to be generally accepted that the alcohol dehydrogenase pathway is the main route of ethanol oxidation and that the microsomal ethanol-oxidizing system also plays a significant role but that the catalase pathway is of minor importance [18, 19]. The inhibitor 4-methylpyrazole has been shown not only to be a potent inhibitor of alcohol dehydrogenase [15], but also to inhibit the microsomal ethanol-oxidizing system [19–21] and catalase [21], resulting in an overall inhibition of the ethanol elimination by about 85–90% [22, 23]. The non-oxidative elimination of ethanol, i.e. the elimination through lungs and kidneys, seems to account for about 10% of the total elimination when the blood ethanol level is around 40–60 mmol/l [18, 23, 24].

We have treated the animals with 4-methylpyrazole in a dose that inhibits the ethanol elimination by about 85%, i.e. the ethanol oxidation was almost totally inhibited [23, 24]. One can therefore presume that in the animals treated with 4-methylpyrazole in combination with ethanol the redox state

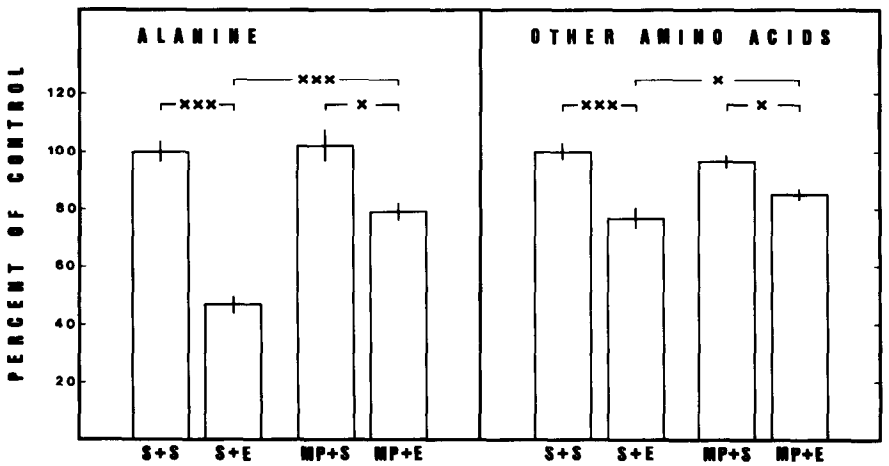

Fig. 2. Effect of 4-methylpyrazole, ethanol and a combination of these on the concentration of alanine and on the sum of concentrations of all other amino acids in rat plasma. Animals were pretreated with 4-methylpyrazole (3.37 mmol/kg b.w.) or saline. Seventeen hours later they were given ethanol (2 g/kg b.w.) or saline as intraperitoneal injections. Blood samples were collected 60 min after the last injection. The figure illustrates mean value (N = 6) for each group in per cent of the controls (S + S) and SEM. S = saline; E = ethanol; MP = 4-methylpyrazole; x = $P < 0.05$; xxx = $P < 0.001$.

in the liver and the lactate/pyruvate ratio was essentially normal in spite of the high ethanol concentration. This is in agreement with the findings of Blomstrand and Theorell [16], that 4-methylpyrazole inhibits the ethanol-induced rise in the lactate/pyruvate ratio in blood, even when given in small doses.

The present results show that the ethanol-induced decrease in the concentrations of alanine and in the other plasma amino acids can be partly abolished by administration of 4-methylpyrazole. This supports the theory that the formation of NADH during ethanol oxidation, by increasing the lactate/pyruvate ratio, leads to an increased deamination of alanine to pyruvate. The metabolism of other amino acids would correspondingly be turned to more reductive pathways. It is possible that this would lead to an increased deamination of amino acids to ketoacids.

It cannot be excluded that other ethanol metabolites, e.g. acetaldehyde and acetate, that also affects the liver metabolism [18] might be involved in the amino acid decreasing effect. 4-Methylpyrazole alone seems to have only minor effects on the concentration of plasma amino acids.

The part of the ethanol-induced decrease in plasma amino acids that is not abolished by treatment with 4-methylpyrazole might involve β-adrenergic mechanisms. This idea is supported by the findings that propranolol, a β-adrenergic antagonist, partly inhibits the decrease in plasma amino acids [9], and that adrenaline causes hypoaminoacidemi [25–27].

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