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THE EFFECT OF ETHANOL ON THE CONCENTRATIONS OF ADENINE NUCLEOTIDES IN RAT LIVER

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Received 15 November 1968

1. Introduction

In an investigation with rat liver slices it was reported that ethanol caused an increase in the AMP level, whereas the concentrations of ATP and of ADP did not change significantly [1]. Increased AMP concentration caused by ethanol has also been reported in perfused rat livers [2] and in the livers of intact animals [3].

Other workers find, however, that ethanol either has no effect on the concentration of AMP or even causes a slight reduction [4]. In the present work some experimental observations concerning the effect of ethanol on the adenine nucleotide level are reported and an explanation for the discrepancy between the results of different experiments is suggested. A preliminary account of this work has been published [5].

2. Materials and methods

Female Wistar rats fed ad libitum weighing about 200 g were used. In a part of the experiments the rats were anesthetized with 20 mg penthothal-Na (Abbott, USA), tracheotomized and artificially respired. The external jugular vein was cannulated and used for the infusions. 0.5 ml of isotonic sodium chloride was infused at the beginning of the experiment. 5 min later a sample of the liver was removed by the freezing clamp technique. Immediately thereafter 4 μmoles of ethanol or 2 μmoles of acetate per g body weight were infused. 5 min later a second sample of the liver was taken using the same technique as before. In control experiments only sodium chloride was infused both times.

In the second part of the experiments ethanol was administered intraperitoneally by giving 6 or 60 μmoles of ethanol per g body weight in 8 ml isotonic sodium chloride. 30 min later the rats were anesthetized with pentothal-Na, the abdomen opened and a sample of the liver removed by the freezing clamp technique. No artificial respiration was used in these experiments.

The following determinations were made by the methods indicated: ATP (6), ADP (7), AMP (7) and ethanol (8). The ratios ATP/ADP and $\frac{ATP\ AMP}{ADP^2}$ are calculated for each experiment. The means and S.E. values are then obtained in the usual way.

3. Results

Intravenous infusion of 4 μmoles ethanol per g body weight resulted in nearly a doubling of the AMP concentration (table 1). In control experiments, when only saline was infused no increase in AMP concentration was observed.

A substantial increase in AMP concentration occurred also after infusion of 2 μmoles of neutralized acetate solution (table 1).

The effect of different amounts of ethanol was investigated by the administration of ethanol intraperitoneally. 6 μmoles per g body weight caused a definite increase in AMP, whereas no increase was observed when a 10 times greater quantity (60 μmoles per g body weight) was injected (table 2).

Table 1

The effects of intravenous administration of ethanol and acetate on the concentrations of adenine nucleotides in the liver. Numbers 1 and 2 indicate that the sample of the liver for the determination of adenine nucleotides is taken 5 min after the first or second infusion in the same rat of the substrates indicated. The amount of ethanol given was 4 μmoles per g body weight, acetate 2 μmoles per g body weight. The results are means ± S.E., the number of experiments is given in parentheses.

Treatment	Concentrations (μmoles/g)			
	ATP	ADP	AMP	ATP+ADP+AMP
1. NaCl	3.56 ± 0.12 (7)	0.75 ± 0.05 (7)	0.122 ± 0.009 (7)	4.43 ± 0.11 (7)
2. NaCl	3.60 ± 0.06 (7)	0.75 ± 0.05 (7)	0.130 ± 0.012 (7)	4.48 ± 0.10 (7)
P	n.s.	n.s.	n.s.	n.s.
1. NaCl	3.42 ± 0.08 (7)	0.77 ± 0.03 (7)	0.128 ± 0.007 (7)	4.32 ± 0.09 (7)
2. NaCl + ethanol	3.07 ± 0.19 (7)	0.86 ± 0.06 (7)	0.273 ± 0.029 (7)	4.20 ± 0.20 (7)
P	n.s.	n.s.	< 0.01	n.s.
1. NaCl	3.64 ± 0.13 (6)	0.70 ± 0.03 (6)	0.116 ± 0.006 (6)	4.45 ± 0.14 (6)
2. NaCl + acetate	2.45 ± 0.26 (6)	1.12 ± 0.11 (6)	0.390 ± 0.068 (6)	3.96 ± 0.15 (6)
P	< 0.01	< 0.01	< 0.01	n.s.

Treatment	Concentrations (μmoles/g)			
	ATP/ADP	$\frac{ATP\ AMP}{ADP^2}$	Ethanol	
			blood	liver
1. NaCl	4.89 ± 0.38 (7)	0.82 ± 0.10 (7)		
2. NaCl	4.89 ± 0.32 (7)	0.83 ± 0.03 (7)		
P	n.s.	n.s.		
1. NaCl	4.50 ± 0.21 (7)	0.75 ± 0.04 (7)		
2. NaCl + ethanol	3.68 ± 0.32 (7)	1.18 ± 0.12 (7)	7.3 ± 1.0 (7)	5.4 ± 1.6 (6)
P	< 0.05	< 0.01		
1. NaCl	5.24 ± 0.24 (6)	0.85 ± 0.11 (6)		
2. NaCl + acetate	2.45 ± 0.56 (6)	0.93 ± 0.23 (6)		
P	< 0.001	n.s.		

The ATP and ADP concentrations did not change significantly when ethanol was administered in the 2 kinds of experiments (tables 1 and 2). Acetate caused, however, a definite decrease in ATP ($P < 0.01$, table 1). The concentrations of all the adenine nucleotides varied much more after infusion of acetate than after infusion of NaCl or ethanol.

The concentrations of AMP, ADP and ATP are governed by the equilibrium constant K of the adenylate kinase reaction if we assume that the activity of this enzyme is sufficiently high to maintain the concentrations of all 3 reactants near the equilibrium. In the present investigation this quotient varied between 0.8 and 1.5. Ethanol caused a striking increase in this quotient, at least when administered in small concentrations.

In experiments when ethanol was given, the concentrations of ethanol in the liver sample and in blood from heart puncture was determined (tables 1 and 2).

4. Discussion

Small amounts of ethanol increase the AMP concentration. An increase is observed also after infusion of acetate. As ethanol in the liver is converted to

Table 2

Adenosine nucleotide levels in the liver and the concentrations of ethanol in blood and liver after intraperitoneal administration of ethanol. The samples of the liver are taken 30 min after intraperitoneal injection of the compound tested. The results are expressed as the means ± S.E. with the number of determinations in parentheses.

Treatment	Concentrations (μmoles/g)			
	ATP	ADP	AMP	ATP+ADP+AMP
1. NaCl	3.28 ± 0.05 (6)	0.71 ± 0.03 (6)	0.134 ± 0.006 (6)	4.12 ± 0.03 (6)
2. NaCl + 6 μmoles ethanol per g body wt.	3.19 ± 0.17 (6)	0.69 ± 0.05 (6)	0.209 ± 0.012 (6)	4.09 ± 0.23 (6)
3. NaCl + 60 μmoles ethanol per g body wt.	3.25 ± 0.11 (7)	0.69 ± 0.03 (7)	0.139 ± 0.006 (7)	4.07 ± 0.12 (7)
P values: 1 versus 2	n.s.	n.s.	<0.001	n.s.
1 versus 3	n.s.	n.s.	n.s.	n.s.

Treatment	Concentrations (μmoles/g)			
	ATP/ADP	$\frac{ATP\ AMP}{AMP^2}$	Ethanol	
			blood	liver
1. NaCl	4.68 ± 0.23 (6)	0.91 ± 0.11 (6)		
2. NaCl + 6 μmoles ethanol per g body wt.	4.70 ± 0.15 (6)	1.46 ± 0.12 (6)	2.0 ± 0.3 (6)	0.5 ± 0.2 (6)
3. NaCl + 60 μmoles ethanol per g body wt.	4.76 ± 0.29 (7)	0.91 ± 0.08 (7)	77.2 ± 6.4 (7)	55.4 ± 4.0 (7)
P values: 1 versus 2	n.s.	<0.01		
1 versus 3	n.s.	n.s.		

acetate it may be assumed that at least a part of the increased AMP formed results from the activation of acetate to acetyl-CoA. This activation will cause an increased AMP formation. An immediate reversion to the initial conditions and a fall in AMP to the initial levels caused by the phosphorylating processes inside the mitochondria might then be impeded by the inhibitory effect of AMP on the exchange of ATP and of ADP through the mitochondrial membrane [9,10]. The result will be a more or less persistent increase in AMP.

High concentrations of ethanol do not increase the AMP. Other workers even report a slight decrease [4]. Higher alcohols, not metabolizable narcotics such as ethyl ether and even some hypnotics have the same effect [4]. It could be explained by the toxic effect of high concentrations of ethanol on the mitochondrial membrane, although such an effect has not been directly observed.

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