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Metabolic Changes Induced by Ethanol in Muscle and Liver Tissue of the Rat *in vivo*

ERIK FELLENIUS, ULLA BJÖRKROTH and KARL-HEINZ KIESSLING

Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden

The metabolic changes in the rat liver and skeletal muscle after infusion of ethanol into the rat *in vivo* were studied. Samples of the liver and muscle tissue were taken simultaneously by the freeze-clamp technique and analyzed for content of lactate, pyruvate, α-glycerophosphate, dihydroxyacetonephosphate, malate, adenine nucleotides, inorganic phosphate, creatinephosphate and creatine. The changes in the redox-state in the cytoplasm and mitochondria of the liver were measured as the [lactate]/[pyruvate]-ratio, and the [β-hydroxybutyrate]/[acetoacetate]-ratio, respectively. The ratios in the liver varied with the total amount of ethanol infused. The [lactate]/[pyruvate]-ratio of the muscle was not affected by the oxidation of ethanol. Thus the reduced state of the liver caused by the oxidation of ethanol and which partly can be exported to extrahepatic tissues will be readjusted in the resting muscle cell. The [β-hydroxybutyrate]/[acetoacetate]-ratio of the liver was, however, not restored to its normal value in the muscle. This was probably due to a low activity of the β-hydroxybutyrate dehydrogenase in the muscle.

The changes of the adenine nucleotides were mainly in the level of AMP. These changes occurred both in the liver and in the muscle tissue and were related to the metabolism of acetate, the main product during oxidation of ethanol in the liver.

Most work has been done on the effect of ethanol on liver and brain.[1] Little is known about the effect on skeletal muscle although it has been demonstrated that long-term ethanol intake leads to serious damage in skeletal muscle.[2,3] The aim of the present study was therefore to investigate the simultaneous metabolic changes in the liver and skeletal muscle of the rat during short-term exposure to ethanol.

METHODS

Animal treatment and sampling procedure. Female Wistar rats (150 – 250 g) from the laboratory's stock were used. The animals were starved for 48 h and anaesthetized by intraperitoneal injection of Evipan-Sodium®, 50 mg/200 g rat weight, dissolved in 0.9 %

Table 1. Changes of the concentrations of redox substrates and energy-rich compounds in liver and skeletal muscle tissue after infusion of ethanol and sodium acetate. Two different solutions of ethanol (0.95 M and 2.84 M) were infused into the saphenous vein at a rate of 2.2 ml/h per 200 g rat weight during 30 min to 48 h starved animals. Sodium

Substrate infused	Tissue	Malate	α-GP	Lactate	Pyruvate	DAP	β-OH
None	L	0.277 ± 0.025	0.211 ± 0.019	0.374 ± 0.075	0.045 ± 0.005	0.038 ± 0.003	0.907 ± 0.171
	M	0.188 ± 0.011	0.046 ± 0.011	1.20 ± 0.14	0.078 ± 0.007	0.035 ± 0.007	0.485 ± 0.086
Ethanol (0.95 M)	L	0.996 ± 0.086[a]	0.743 ± 0.083[a]	0.671 ± 0.081	0.063 ± 0.009	0.041 ± 0.003	0.504 ± 0.095
	M	0.155 ± 0.005[a]	0.076 ± 0.008	1.50 ± 0.15	0.105 ± 0.012	0.017 ± 0.003	0.361 ± 0.075
Ethanol (2.84 M)	L	1.20 ± 0.13[a]	0.827 ± 0.051[a]	0.985 ± 0.106[a]	0.052 ± 0.004	0.035 ± 0.004	1.05 ± 0.11
	M	0.146 ± 0.020	0.063 ± 0.010	1.04 ± 0.08	0.075 ± 0.010	0.019 ± 0.003	0.475 ± 0.102
Sodium-acetate (2.84 M)	L	0.221 ± 0.009	0.185 ± 0.011	0.291 ± 0.040	0.065 ± 0.007	0.064 ± 0.004[a]	0.831 ± 0.047
	M	0.076 ± 0.006[a]	0.062 ± 0.005	0.798 ± 0.074	0.048 ± 0.006[a]	0.028 ± 0.002	0.435 ± 0.051

[a] = Significance of difference ($p < 0.01$) compared with control

sodium chloride. After continuous infusion of the different agents for 30 min, the liver and skeletal muscle were at the same time rapidly frozen between small aluminium clamps cooled in liquid nitrogen.[4] The rate of infusion was 2.2 ml/h. Prior to the sampling of the muscle tissue, which was mainly the posterior-inferior thigh muscles, visible fat pads were removed.

The further treatment of the tissues for the determination of different metabolites has been described by others.[5]

Determination of metabolites. The concentrations of the following compounds were estimated in the neutralized perchloric acid extract: lactate, malate, α-glycerophosphate (α-GP), dihydroxyacetonephosphate (DAP) and pyruvate,[6] acetoacetate (AcAc) and β-hydroxybutyrate (β-OH),[7] adenosinetriphosphate (ATP),[8] adenosinediphosphate (ADP) and adenosinemonophosphate (AMP),[9] inorganic phosphate (P),[10] creatinephosphate (CP),[11] creatine,[12] and ethanol.[13]

Definitions. The term redox-state is used as a description of the ratio free [NADH]/free [NAD^+] in a cell compartment. According to this concept [14] the ratio can be assumed to be in equilibrium with these metabolite pairs which are interconverted by means of sufficiently active NAD-coupled dehydrogenases located in the corresponding compartment. In this paper evidence is presented of the existence of two functional compartments regarding the redox-state within the cytoplasm, one represented by the [lactate]/[pyruvate]-ratio and one by the [α-GP]/[DAP]-ratio. The [β-OH]/[AcAc]-ratio represents that of the mitochondrial compartment.

Statistical analyses. Student's *t*-test was used for statistical analyses, $p < 0.01$ being considered significant.

RESULTS

Ethanol, acetate and "redox substrates". The changes of the concentration of lactate, malate, α-GP, pyruvate, and DAP in the liver and skeletal muscle after a 30 min infusion of various amounts of ethanol are shown in Table 1. At the lower dose of ethanol, giving a final ethanol concentration in the liver of 4.08 ± 0.40 μmol/g wet wt (mean ± S. E. of 5 observations) the amount of

acetate (2.84 M) was infused at the same rate. All the substances were dissolved in 0.9 % sodium chloride. 0.9 % sodium chloride was infused in the control animals. The concentrations are expressed as μmol/g wet weight of the liver (L) or skeletal muscle (M) tissue and are given as mean ± S. E. of 5 observations.

AcAc	ATP	ADP	AMP	P_i	CP	Creatine
.956± 0.050	3.35± 0.09	1.02 ± 0.06	0.149± 0.017	2.81± 0.24		
.119± 0.018	6.70± 0.11	0.860± 0.008	0.029± 0.003	11.8 ± 0.4	21.1± 0.7	8.89± 0.31
.516± 0.093[a]	3.38± 0.17	0.967± 0.022	0.230± 0.013[a]	2.68± 0.26		
.106± 0.025	6.44± 0.09	0.824± 0.015	0.049± 0.004[a]	11.7 ± 1.0	18.1± 0.8[a]	6.91± 0.82
.264± 0.027[a]	3.56± 0.12	0.870± 0.051	0.228± 0.015[a]	2.11± 0.23		
.058± 0.010[a]	7.01± 0.15	0.790± 0.051	0.048± 0.004[a]	11.3 ± 1.0	21.3± 0.8	4.39± 0.41[a]
.03 ± 0.04	2.88± 0.05[a]	1.22 ± 0.035	0.787± 0.027[a]	3.27± 0.99		
.183± 0.034	7.57± 0.12[a]	0.936± 0.026	0.076± 0.008[a]	9.78± 1.1	24.5± 0.7[a]	4.55± 0.18[a]

malate and α-GP increased significantly in the liver, while lactate and β-OH were less affected. This is also seen in the changes of the [α-GP]/[DAP]-[lactate]/[pyruvate]-, and [β-OH]/[AcAc]-ratios (Table 2). Thus, the [α-GP]/[DAP]-ratio increased significantly while the [lactate]/[pyruvate]- and the [β-OH]/[AcAc]-ratios were only slightly affected. At the higher concentration of ethanol, giving a final concentration in the liver tissue of 14.7 ± 0.9

'able 2. Changes of the redox-state and the "energy charge" in liver and skeletal muscle after infusion of ethanol nd sodium acetate. From the concentrations presented in Table 1 different ratios have been calculated. The ratios are given as mean ± S. E. of 5 observations. L = liver, M = skeletal muscle.

ubstrate infused	Tissue	$\frac{[Lactate]}{[Pyruvate]}$	$\frac{[\alpha\text{-GP}]}{[DAP]}$	$\frac{[\beta\text{-OH}]}{[AcAc]}$	$\frac{[ATP]}{[ADP][P_i]}$	$\frac{[ADP]^2}{[ATP][AMP]}$	$\frac{[ATP][Creatine]}{[ADP][CP]}$
one	L	8.5± 1.7	5.4± 0.5	1.2± 0.2	1.2± 0.1	3.0± 0.5	
	M	13.7± 1.8	3.5± 0.6	4.2± 0.6	0.8± 0.1	4.0± 0.4	3.3± 0.2
thanol	L	12.3± 1.2	21.5± 1.6[a]	1.4± 0.3	1.2± 0.2	1.3± 0.6[a]	
).95 M)	M	13.4± 0.8	4.5± 0.6	3.6± 0.4	0.7± 0.1	2.3± 0.2[a]	3.0± 0.4
thanol	L	20.3± 1.5[a]	29.8± 2.0[a]	4.0± 0.3[a]	1.8± 0.2[a]	1.2± 0.2[a]	
?.84 M)	M	14.8± 2.2	2.9± 0.4	8.1± 0.7[a]	1.0± 0.2	2.2± 0.1[a]	1.9± 0.3[a]
odium cetate	L	4.8± 1.0	2.8± 0.2[a]	0.8± 0.6	0.8± 0.1	0.7± 0.03[a]	
?.84 M)	M	17.5± 2.5	2.1± 0.3	3.2± 0.4	0.9± 0.1	1.6± 0.1[a]	1.5± 0.1[a]

[a] = Significance of difference ($p < 0.01$) compared to control

μmol/g wet wt. (mean $\pm$ S. E. of 5 observations) a significant increase of all the redox-ratios was obtained. This shows that the changes of the [lactate]/[pyruvate]-ratio and the [β-OH]/[AcAc]-ratio, representing the redox-state in the cytosol and the mitochondria, respectively, are dependent upon the amount of ethanol infused.

With the exception of the [β-OH]/[AcAc]-ratio the redox-ratios in the skeletal muscle were not much influenced by infusion of the two doses of ethanol. The increased ratio was mainly due to a decrease in the level of AcAc.

Infusion of acetate did not change the content of the various redox substrates in the liver significantly except for DAP (Table 1). This caused the [α-GP]/[DAP]-ratio to decrease.

In the skeletal muscle a significant decrease of malate and pyruvate was found. The changes did not, however, affect the [lactate]/[pyruvate]-ratio.

Ethanol, acetate and the "energy charge". The content of ATP and ADP was practically unchanged in the liver and skeletal muscle 30 min after the infusion of ethanol (Table 1). The concentration of AMP was increased in both tissues by ethanol infusion, while the amount of CP was practically unchanged.

The ratio $[ADP]^2/[ATP]\,[AMP]$ (Table 2), which represent the mass action ratio of the adenylate kinase reaction, was slightly decreased. The ratio should be compared with the ratio 1.5 – 3.5 obtained *in vitro*.[15]

In spite of the fact that the amount of ATP, ADP, and P_i was not significantly changed after ethanol infusion, the changes were large enough to give an elevated "phosphate potential" ($[ATP]/[ADP]\,[P_i]$)[16] at the higher concentration of ethanol.

Acetate influenced the amount of ATP and AMP significantly (Table 1) both in liver and muscle tissue, causing the equilibrium ratio of the adenylate kinase reaction to decrease (Table 2). The "phosphate potential" was not much changed.

DISCUSSION

It is clearly shown in the present investigation that the influence of ethanol on the different redox ratios in the liver depends on the amount of ethanol infused. This is evidently not due to the alcohol dehydrogenase system not being saturated with ethanol. The amount of ethanol found in the liver at the lower dose of ethanol was about twice as high as the reported K_m-value for alcohol dehydrogenase with ethanol as substrate.[17] Moreover, with the two doses of ethanol infused there was a steady increase with time of this compound in the blood. Thus, at the lower dose the following concentrations of ethanol were found in the blood after 10, 30, 60, 120, and 180 min infusion: 5.0, 11.0, 17.7, 20.6, and 20.3 mM, respectively.

A possible explanation for the difference in effect of the different doses of ethanol would be that no immediate equilibrium is established between the α-glycerophosphate and the lactate dehydrogenase systems. This is a consequence of a functional compartmentation. Such disequilibria between the two enzymes has been reported to occur during ethanol oxidation.[18]

The preliminary regulator of the redox state in the cytoplasm is suggested to be the state of phosphorylation,[19] which in turn depends on the concentration of the components of the glyceraldehyde-3-phosphate dehydrogenase and the 3-phosphoglycerokinase reactions. The ratio [ATP]/[ADP] [P_i], the "phosphate potential", is one part of the cited reactions and can be expected to move parallel with any change in the redox state. Our results indicate that the redox state shown in the changes in the [lactate]/[pyruvate]-ratio is regulated by the phosphorylation state, while the redox state of the α-glycerophosphate dehydrogenase system is not primarily regulated by this factor.

This conclusion presupposes that the ratio [3-phosphoglycerate]/[glyceraldehyde-3-phosphate], which can also influence the redox state,[19] is constant.

The compounds malate, lactate, pyruvate, β-OH, and AcAc penetrate the membranes of the mitochondria and the liver cell more or less freely. In this way the reduced state, governed by the oxidation of ethanol, can be exported to extrahepatic tissues. As no changes in the [lactate]/[pyruvate]-ratio occur in the skeletal muscle after infusion of ethanol it follows that the muscle tissue has a great capacity even during rest to readjust the reduced state carried in the circulation from the sites of ethanol metabolism. The situation is somewhat different for the [β-OH]/[AcAc]-ratio. A significant increase in the ratio was found in the muscle tissue after infusion of the higher dose of ethanol. This should not be interpreted as a limited capacity of the muscle to adjust the redox state, since the [β-OH]/[AcAc]-ratio of the muscle sample is not necessary a good indicator of the redox state of this tissue. The activity of the β-hydroxybutyrate dehydrogenase in skeletal muscle is quite low [20] compared to that of the liver. This might be the rate-limiting factor in the readjustment of the [β-OH]/[AcAc]-ratio.

To summarize: There appears to be no dramatic change in the metabolic pattern of compounds related to the redox state in the skeletal muscle during oxidation of ethanol in the liver.

The changes of the level of AMP in the muscle tissue should be considered as one effect that might have consequences for the muscle metabolism in the chronic state. This compound is a powerful modifier of the flow through important metabolic steps.[21] The changes of the AMP level is due to the activation of acetate in the muscle tissue. This acetate is formed in the liver during oxidation of ethanol and carried in the circulation to the muscle. This was clearly demonstrated when only acetate was infused. Similar changes of the level of AMP occur when ethanol alone in infused. The changes in the liver tissue seem to be dependent upon the amount of ethanol adminstrated.[22–25] In the present investigation the doses of alcohol were low enough to prevent any toxic effect of ethanol.

In conclusion it may be said that any alterations in the metabolic pattern of skeletal muscle after administration of ethanol result from an indirect effect *via* the metabolites carried in the circulation from the sites of ethanol metabolism. At this point it is impossible to decide the chronic effects of the metabolism of acetate. However, in evaluating the reason for the muscle derangements occurring in alcoholism, it may be fruitful to consider the consequences of unilateral combustion of acetate.

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REFERENCES

1. Wallgren, H. and Barry, H. III, *Actions of Alcohol*, Elsevier, Amsterdam 1970.
2. Hed, R., Lundmark, C., Fahlgren, H. and Orell, S. *Acta Med. Scand.* **171** (1962) 585.
3. Kiessling, K.-H. and Pilström, L. *Clin. Science. In press.*
4. Wollenberger, A., Ristau, O. and Schoffa, G. *Pflügers Arch. ges. Physiol.* **270** (1960) 399.
5. Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. and Schulz, D. W. *J. Biol. Chem.* **239** (1964) 18.
6. Hohorst, H. J., Kreutz, F. H. and Bücher, Th. *Biochem. Z.* **332** (1959) 18.
7. Williamson, D. H., Mellanby, J. and Krebs, H. A. *Biochem. J.* **82** (1962) 90.
8. Lamprecht, W. and Trautschold, I. In Bergmeyer, H.-U., Ed., *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim 1962, p. 543.
9. Adam, H. *Ibid.* p. 573.
10. Harper, A. E. *Ibid.* p. 788.
11. Lamprecht, W. and Stein, P. *Ibid.* p. 610.
12. Bernt, E., Bergmeyer, H.-U. and Möllering, H. *Ibid.* p. 407.
13. Dickinson, F. M. and Dalziel, K. *Biochem. J.* **104** (1967) 165.
14. Krebs, H. A. *Advan. Enzyme Regul.* **5** (1967) 409.
15. Egglestone, L. V. and Hems, R. *Biochem. J.* **52** (1952) 156.
16. Atkinson, D. E. In Goodwin, T. W., Ed., *Metabolic Roles of Citrate*, Academic, London 1969, p. 23.
17. Marshall, E. K. and Fritz, W. F. *J. Pharmacol. Exptl. Therap.* **109** (1953) 431.
18. Schimassek, H., Walli, A. K. and Höfer, G. In Martini, G. A. and Bode, Ch., Eds., *Metabolic Changes Induced by Alcohol*, Springer, Berlin-Heidelberg-New York 1971, p. 157.
19. Stubbs, M., Veech, R. L. and Krebs, H. A. *Biochem. J.* **126** (1972) 59.
20. Lehninger, A. L., Sudduth, H. C. and Wise, J. B. *J. Biol. Chem.* **235** (1960) 2450.
21. Newsholme, E. A. In Bartley, W., Kornberg, H. L. and Quayle, J. R., Eds., *Essays in Cell Metabolism*, Wiley-Interscience, London 1970, p. 189.
22. Oura, E., Räihä, N. C. R. and Suomalainen, H. *Ann. Med. Exp. Fenn.* **45** (1967) 57.
23. Lindros, K. and Aro, H. *Abstr. 5th Meeting Fed. Europ. Biochem. Soc.*, Prague 1968, p. 461.
24. Scholz, R. In Staib, W. and Scholz, R., Eds., *Stoffwechsel der isolierten perfundierten Leber*, Springer, Berlin-Heidelberg-New York 1968, p. 225.
25. Thieden, H. I. D. *FEBS Lett.* **2** (1968) 121.

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