INFLUENCE OF THE ACTIVITY OF MITOCHONDRIAL α-GLYCEROPHOSPHATE OXIDASE ON THE α-GLYCEROPHOSPHATE SHUTTLE DURING ETHANOL OXIDATION

A. R. PÖSÖ

Research Laboratories of State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10 and *Department of Physiology, College of Veterinary Medicine, Helsinki, Finland*

Received 21 September 1977

1. Introduction

Cytoplasmic α-glycerophosphate dehydrogenase (L-glycerol 3-phosphate: NAD oxidoreductase, EC 1.1.1.8) and the mitochondrial α-glycerophosphate oxidase (L-glycerol 3-phosphate: (acceptor) oxidoreductase, EC 1.1.99.5) form a shuttle for NADH transport from cytoplasm to mitochondria. Such a shuttle, first proposed by Bücher and Klingenberg [1], functions in insect flight muscle, but its role in mammalian tissues is not yet clear. The shuttle has been claimed to be regulated either by the activity of mitochondrial glycerophosphate oxidase or by the cell concentration of α-glycerophosphate [2,3].

The present study was planned to clarify the effects of mitochondrial glycerophosphate oxidase activity on the functioning of the glycerophosphate shuttle during ethanol oxidation. The activity of glycerophosphate oxidase was modified with clofibrate and triiodothyronine [2,4] and the rate of ethanol elimination was assumed to characterize NADH translocation through the mitochondrial membrane. It was found as previously in vitro [5] that NADH translocation is not the rate limiting factor in ethanol elimination in fed rats. When the malate–aspartate shuttle was inhibited to decrease NADH translocation through the mitochondrial membrane by this route, the activity of glycerophosphate oxidase was reflected in the rate of ethanol elimination, suggesting that the activity of glycerophosphate oxidase regulates the functioning of the glycerophosphate shuttle in vivo.

2. Experimental

Male Sprague-Dawley rats, weighing about 300 g, were used in the experiment. Rats had free access to pelleted food (Orion, Helsinki) and tap water. One group of ten rats was given clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate, Medica, Pharmaceutical Co., Helsinki) 200 mg/kg/day, s.c., for two successive days. The second group of ten rats received triiodothyronine (3,3′,5-triiodo-L-thyronine, Sigma Chemical Co., St Louis, MI) in a slightly alkaline solution, 0.20 mg/kg/day, i.p., for two days. The third group of ten rats served as control and received injections of saline. Neither clofibrate nor triiodothyronine (T_3) had any influence on the body weight of the rats. As found previously [4] clofibrate treatment increased the liver-to-body weight ratio (controls 2.43 ± 0.18%, clofibrate-treated 2.82 ± 0.22%, statistical difference $p < 0.001$). In T_3-treated rats the liver-to-body weight ratio was decreased (2.27 ± 0.14%, statistical difference to controls $p < 0.05$).

On the third day each rat was given ethanol as an intraperitoneal injection (1.5 g/kg, 15 (w/v)% solution). Half of the rats in each group were also given amino-oxyacetate (0.3 mmol/kg, i.p.), an inhibitor of aspartate aminotransferase [6], 10 min before ethanol administration. The rate of ethanol elimination was calculated from blood ethanol concentrations measured from samples taken from the tip of the tail every half hour for 2.5 h. Ethanol was determined gas chromatographically by the head space technique [7].

Rats were decapitated and the livers were quickly removed, chilled and weighed. Livers were homogenized in 0.25 M sucrose and the mitochondrial fraction was centrifuged down, and washed. α-Glycerophosphate dehydrogenase activity from the cytoplasmic fraction and glycerophosphate oxidase activity from the mitochondrial fraction were determined by methods described previously [8,9]. Protein was determined according to Lowry et al. [10].

3. Results and discussion

Relative to saline-treated controls, the activity of cytoplasmic glycerophosphate dehydrogenase was not changed after treatment with either clofibrate or triiodothyronine (T_3) (table 1). The activity of glycerophosphate oxidase was significantly increased after both clofibrate and T_3 treatments (table 1).

The rates of ethanol elimination are presented in table 2. Examination of the rate of ethanol elimination and the activity of mitochondrial glycerophosphate oxidase reveals that there is no change in the rate of ethanol elimination in the clofibrate-treated group even though the enzyme activity is increased. In T_3-treated rats both the rate of ethanol elimination and the enzyme activity are increased and the increase in the enzyme activity is markedly higher than the increase in the rate of ethanol removal. Accordingly, it is suggested that the capacity of NADH translocation shuttles is higher than the amount of NADH formed during ethanol oxidation.

Amino-oxyacetate (AOA) inhibited ethanol elimination by 34% in untreated rats (table 2). The amount of AOA used in the present study has been reported to inhibit aspartate aminotransferase activity by 90% in vitro [11]. However, in vivo the inhibition of ethanol elimination with AOA is found to be about 50%, giving support to suggestions that also other transport mechanisms are functioning in the liver, at least when the malate–aspartate shuttle is inhibited [4,11,12]. In both clofibrate and T_3-treated groups the rates of ethanol elimination were higher and the inhibition with AOA was smaller than in control rats (table 2). If the effects of clofibrate and T_3 on the rate of ethanol elimination are compared (table 2), it is found that both clofibrate and T_3 increase ethanol elimination about 20% more in the presence than in the absence of AOA. This difference of 20% could mark the increase in the capacity of the glycerophosphate shuttle when the malate–aspartate shuttle is inhibited.

If the rate of ethanol elimination is taken as a measure of NADH transport through the mitochondrial membrane in rats treated with a malate–aspartate shuttle inhibitor, it is found that the more active the mitochondrial glycerophosphate oxidase, the higher the rate of ethanol elimination (calculating the correlation as $r = 0.83, p < 0.01, df = 14$). The present findings thus differ from those of Cederbaum et al. [13]. In their studies with isolated mitochondria the activity of glycerophosphate oxidase was without effect on the rate of ethanol elimination, giving support to the suggestion that the α-glycerophosphate content is the main regulator of the glycerophosphate shuttle. However, the rather high concentrations of dihydroxyacetonephosphate and α-glycerophosphate (1 mM and 10 mM, respectively) which were used in their study

Table 1
Activities of glycerophosphate dehydrogenase and glycerophosphate oxidase

Treatment	α-Glycerophosphate dehydrogenase (nmol/mg protein/min)	α-Glycerophosphate oxidase (ng-atom O_2/mg protein/min)	Comparison with control (%)
–	118 ± 46	6.68 ± 1.1	100
Clofibrate	110 ± 15	10.61 ± 3.2[a]	161
T_3	113 ± 38	21.44 ± 4.2[b]	321

[a] $p < 0.05$
[b] $p < 0.001$

The results are the mean ± SD of 8 rats – The animals were treated as described in the Experimental section

Table 2
The rate of ethanol elimination

Treatment	Rate of ethanol elimination (μmol/g liver wt/min)		With AOA	Comparison with control (%)	Inhibition with AOA (%)
	Without AOA	Comparison with control (%)			
–	4.17 ± 0.60	100	2.81 ± 0.37	100	34
Clofibrate	4.23 ± 0.26	101	3.28 ± 0.33	117	22
T_3	5.79 ± 0.38[b]	139	4.45 ± 0.41[c]	158	23

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

The results are the mean ± SD of 5 animals – Rats were treated as described in the Experimental section

might have affected the results obtained. In insect flight muscle where the glycerophosphate shuttle evidently transfers NADH, a low concentration of α-glycerophosphate indicates high activity of the shuttle [1]. Moreover, according to the literature, the increase in α-glycerophosphate concentration during ethanol oxidation is actually greater in control rats than in either T_3- or clofibrate-treated rats [14,15]. It is therefore suggested that in vivo the glycerophosphate shuttle is mainly regulated by the activity of α-glycerophosphate oxidase.

Acknowledgements

I would like to thank Dr O. Forsander for helpful discussions and Mrs Pirkko Johansson for excellent technical assistance. Clofibrate was a generous gift from Medica, Pharmaceutical Co., Helsinki.

References

[1] Bücher, Th. and Klingenberg, M. (1958) Angew. Chem. 70, 552–570.
[2] Lee, Y. P. and Lardy, H. A. (1965) J. Biol. Chem. 240, 1427–1436.
[3] Williamson, J. R., Jacob, A. and Refino, C. (1971) J. Biol. Chem. 246, 7632–7641.
[4] Kähönen, M. Y., Ylikahri, R. H. and Hassinen, I. E. (1971) Life Sci. 10, 661–670.
[5] Meijer, A. J., Van Woerkom, G. M., Williamson, J. R. and Tager, J. M. (1975) Biochem. J. 150, 205–209.
[6] Rognstad, R. and Clark, D. G. (1974) Arch. Biochem. Biophys. 161, 638–646.
[7] Lindros, K. O., Vihma, R. and Forsander, O. A. (1972) Biochem. J. 126, 945–952.
[8] Hohorst, H. J. (1963) in: Methods of Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 215–219, Verlag Chemie, Weinheim.
[9] Ruegamer, W. R., Westerfeld, W. W. and Richert, D. A. (1964) Endocrinology 75, 908–916.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Nordmann, R., Petit, M.-A. and Nordmann, J. (1975) Biochem. Pharmacol. 24, 139–143.
[12] Safer, B., Smith, C. M. and Williamson, J. R. (1971) J. Mol. Cell. Cardiol. 2, 111–124.
[13] Cederbaum, A. I., Lieber, C. S., Beattie, D. S. and Rubin, E. (1973) Arch. Biochem. Biophys. 158, 763–781.
[14] Hawkins, R. A., Nielsen, R. C. and Veech, R. L. (1974) Biochem. J. 140, 117–120.
[15] Ylikahri, R. H. (1970) Metab. Clin. Exp. 19, 1036–1045.