EFFECT OF CLOFIBRATE TREATMENT ON THE ACTIVITY OF CARNITINE ACETYLTRANSFERASE IN RAT TISSUES

MATTI T. KÄHÖNEN and REINO H. YLIKAHRI

Department of Medical Chemistry and Third Department of Medicine, University of Helsinki, Finland

Received 25 April 1974

1. Introduction

Carnitine acetyltransferase (CAT) (EC 2.3.1.7) is an enzyme catalyzing the reversible transfer of an acetyl group from acetyl-CoA to carnitine. This enzyme is primarily located in the membranous part of the mitochondria [1]. The activity of the enzyme has been found to be high in heart, testis, brown adipose tissue and skeletal muscle but low in liver [2]. Fasting increases the activity of the liver enzyme [3].

In spite of this substantial information about the behaviour and location of CAT, its physiological function is uncertain. Some investigators have proposed a role as an acetyl carrier throughout the cell [4] but this has been disputed by many authors who have suggested an acetyl group buffering function for CAT [5]. In either case, through acetyl group and carnitine metabolism, it could influence both lipid and carbohydrate metabolism [6].

Clofibrate (ethyl-α-p-chlorophenoxyisobutyrate) is a well-known drug decreasing the concentrations of plasma lipids, especially that of triglycerides [7]. It affects the hepatic metabolism of free fatty acids and triglycerides [8] and changes the activity of several enzymes [9,10]. Because CAT may have a central role in lipid metabolism, we have now studied the effect of clofibrate treatment on CAT activity in some rat tissues. Two different doses of clofibrate were administered, and the resulting changes in CAT activity were compared with the effect of thyroxine.

2. Materials and methods

Two series of experiments were conducted: in the first, 30 or 200 mg clofibrate/day/kg body weight was administered subcutaneously to male Wistar rats weighing 180–250 g for 14 successive days. Body weights were recorded and the doses of the drug were corrected twice during treatment. In the second series 50 mg clofibrate or 0.1 mg Na-L-thyroxine/day/animal was given subcutaneously to rats weighing 270–310 g for 9 successive days. Control animals were subjected to the same treatment except that clofibrate and thyroxine were omitted. The rats in both the experimental and control groups showed a steady increase in body weight during treatment. No fasting preceded the experiments.

CAT was extracted from heart and skeletal muscle three times with 0.1 M phosphate buffer, pH 8.0, containing 0.1% deoxycholate and 10 mM EDTA [2]. In the liver the activity of CAT was so low that it could be accurately measured only from isolated mitochondria. The mitochondria were isolated in 0.25 M sucrose with 1 mM EDTA and 5 mM Tris, pH 7.4, and CAT was extracted from them as described above. The activity of CAT was measured with a coupled assay using acetyl-D,L-carnitine as substrate [2]. The reaction temperature was 25°C. The activity of mitochondrial α-glycerophosphate dehydrogenase was measured polarographically [11]. Protein was determined by the biuret method [11].

The results are given as means ± SEM. Statistical significance was calculated with Student's *t*-test.

Clofibrate was a generous gift from Orion Pharmaceutical Co., Helsinki, Finland. Na-L-thyroxine was from Fluka AG, Buchs, Switzerland. Acetyl-D,L-carnitine, coenzymes and enzymes needed were obtained from Boehringer und Soehne GmbH, Mann-

heim, Germany.

3. Results and discussion

Two doses of clofibrate were used in the first series of experiments. The larger dose (200 mg/kg body wt. daily s.c.) has previously been found to have a near maximal effect on the hepatic enzyme activities which we have studied (Kähönen, to be published) and the smaller dose (30 mg/kg body wt. daily) has a maximal effect on plasma triglyceride level in rats when administered daily subcutaneously (Kähönen, to be published). In addition, the smaller dose is about the same as the therapeutic dose in human subjects calculated on weight basis.

The larger dose of clofibrate induced an almost ten fold increase in the activity of CAT in rat liver mitochondria (table 1). The smaller dose had no significant effect. Thus there was a discrepancy between the lipid lowering effect of clofibrate and the induction of enzyme activity.

Daae and Aas found in rat liver a rise in the activity of carnitine palmityltransferase to 260% of the activity of control livers as a result of clofibrate treatment [12]. Their dose of clofibrate (0.30% in the diet) is not easily convertible to our own doses. However, assuming an average consumption of 10 g food/day, the dose they used corresponds to 150–200 mg clofibrate/kg daily, which is close to the larger dose we used. Thus, the clofibrate-induced increase in the CAT activity seems to be much greater than the rise in the activity of carnitine palmityltransferase.

Table 1
Effect of clofibrate treatment on the activity of carnitine acetyltransferase of rat liver mitochondria, heart and muscle tissue

	Liver mitochondria (μmoles/min/g protein)	Heart (μmoles/min/g wet. wt.)	Muscle
Control	10.83 ± 2.41	13.67 ± 0.91	3.39 ± 0.44
Clofibrate 30 mg/kg/day	13.87 ± 1.81	15.46 ± 1.56	3.14 ± 0.20
Clofibrate 200 mg/kg/day	98.68 ± 9.59*	19.91 ± 1.10**	3.14 ± 0.40

Six rats in each group. Figures are means ± SEM. Significance of the values in comparison to the control group.
* $p = < 0.001$.
** $p = < 0.01$.

The clofibrate-induced rise in CAT activity is probably responsible for the great increase in the oxygen consumption after addition of carnitine to mitochondrial suspension using octanoate as substrate [13]. A possible change in the activity of the third enzyme known to use carnitine as a substrate, carnitine octanoyltransferase [14], is probably not involved in this effect, because the oxidation of short chain fatty acids in liver preparations of normal rats is not dependent on the presence of carnitine [15].

In skeletal muscle we found no increase in CAT activity after clofibrate treatment (table 1). The activity in heart muscle was increased 45% by the larger dose. As with liver mitchondria the smaller dose did not cause any significant rise in heart muscle CAT activity.

Some effects of clofibrate have been suggested to be mediated by thyroxine [10], which is displaced from its binding sites in plasma proteins by clofibrate [16]. For example the effects of thyroxine and clofibrate on the activity of mitochondrial α-glycerophosphate dehydrogenase activity in rat liver are similar and very large [10]. Therefore, it was interesting to know, whether thyroxine and clofibrate treatments had similar effect on mitochondrial CAT activity, too. Both treatments markedly increased the mitchondrial α-glycerophosphate dehydrogenase activity, but only clofibrate had an effect on the activity of CAT (table 2). This result confirms the view that all effects of clofibrate are not mediated by thyroxine.

The mechanism by which clofibrate increases CAT activity is unsolved. Most probably it induces de novo synthesis of the enzyme protein, but this remains to be elucidated. The activity of the enzyme was previously believed to be located almost exclusively in the membranous part of mitochondria, but a more recent report indicates that in the rat liver a considerable part of the activity is found in peroxisomes and in a lipid-rich membranous fraction [17]. Clofibrate treatment has been found to increase the content of peroxisomes in the liver [18]. It also increases the activity of the peroxisomal marker enzyme, catalase [19]. The method which we used to isolate mitochondria does not separate peroxisomes from the mitochon-

Table 2
Effect of clofibrate and thyroxine treatment on the activity of carnitine acetyltransferase and α-glycerophosphate dehydrogenase in rat liver mitochondria

	Carnitine acetyl-transferase (μmoles/min/g protein)	α-Glycerophosphate dehydrogenase (μatoms O/min/g protein)
Control	7.11 ± 1.40	2.60 ± 0.16
Thyroxine		
0.1 mg/day	11.47 ± 3.24	27.44 ± 3.92**
Clofibrate		
50 mg/day	102.74 ± 20.00*	15.99 ± 1.65**

Five rats in each group. Figures are means ± SEM. Significance of the values in comparison to the control group.
* $p = < 0.01$.
** $p = < 0.001$.

drial fraction. Thus it is possible that at least part of the increase in CAT activity occurs in peroxisomes.

The physiological significance of CAT is open. Thus it is too early to speculate about the meaning of the clofibrate-induced increase in its activity. It is hardly important in the hypolipidaemic action of clofibrate, because therapeutic doses which lower lipid concentrations have no effect on the CAT activity. However, the change produced by larger doses of clofibrate must have a profound effect on the metabolism of acetyl groups and carnitine in the liver. Therefore the clofibrate-treated rat offers a model in which the metabolic role of hepatic CAT can be tested.

Acknowledgements

Technical assistance of Miss Pirjo Värttö is gratefully acknowledged. This work was supported by a grant from The Finnish Cultural Foundation.

References

[1] Norum, K. R. and Bremer, J. (1967) J. Biol. Chem. 242, 407–411.
[2] Marquis, N. R. and Fritz, I. B. (1965) J. Biol. Chem. 240, 2193–2196.
[3] Norum, K. R. (1965) Biochim. Biophys. Acta 98, 652–654.
[4] Bressler, R. and Brendel, K. (1966) J. Biol. Chem. 241, 4092–4097.
[5] Pearson, D. J. and Tubbs, P. K. (1967) Biochem. J. 105, 953–963.
[6] Fritz, I. B. (1967) Perspect. Biol. Med. 10, 643–677.
[7] Oliver, M. F. (1963) J. Atheroscler. Res. 3, 427–439.
[8] Nikkilä, E. A. (1972) in: Pharmacological Control of Lipid Metabolism (Holmes, W. L., Paoletti, R. and Kritchevsky, D., eds), pp. 113–133, Plenum, New York.
[9] Zakim, D., Paradini, R. S. and Herman, R. H. (1970) Biochem. Pharmacol. 19, 305–310.
[10] Westerfeld, W. W., Richert, D. A. and Ruegamer, W. R. (1968) Biochem. Pharmacol. 17, 1003–1016.
[11] Kadenbach, B. (1966) Biochem. Z. 344, 49–75.
[12] Daae, L. N. W. and Aas, M. (1973) Atherosclerosis 17, 389–400.
[13] Hassinen, I. E. and Kähönen, M. T. (in press) in: First International Symposium on Alcohol and Acetaldehyde Metabolizing Systems, Academic Press.
[14] Solberg, H. E. (1971) FEBS Letters 12, 134–136.
[15] Fritz, I. B. (1959) Amer. J. Physiol. 197, 297–304.
[16] Platt, D. S. and Thorp, J. M. (1966) Biochem. Pharmacol. 15, 915–925.
[17] Markwell, M. A. K., McGroarty, E. J., Bieber, L. L. and Tolbert, N. E. (1973) J. Biol. Chem. 248, 3426–3432.
[18] Svoboda, D. J. and Azarnoff, D. L. (1966) J. Cell Biol. 30, 442–450.
[19] Hess, R., Stäubli, W. and Riess, W. (1965) Nature 208, 856–858.