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INHIBITION OF LIPOGENESIS IN RAT BROWN ADIPOSE TISSUE BY CLOFIBRATE

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Abstract—The effect of clofibrate (Atromid S, ethyl-2-(4-chlorophenoxy)-2-methylpropionate) administration for 7 days to rats on lipogenesis and on some lipogenic enzyme activities in brown adipose tissue (BAT), liver and white adipose tissue (WAT) was examined. As compared to control rats the rate of lipogenesis in BAT in the clofibrate-treated animals was significantly decreased. The rate of liver lipogenesis increased slightly, whereas lipogenesis in the WAT was not affected by clofibrate. In BAT, the drug treatment resulted in depression of fatty acid synthase, ATP-citrate lyase, malic enzyme, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities. The activity of liver fatty acid synthase did not change, ATP-citrate lyase activity slightly decreased, whereas the activity of malic enzyme significantly increased in this organ after clofibrate feeding. The ATP-citrate lyase activity in WAT decreased, while fatty acid synthase and other lipogenic enzymes were not changed after clofibrate feeding. Clofibrate treatment did not influence the activity of NADP-linked isocitrate dehydrogenase and malate dehydrogenase (enzymes not linked directly to lipogenesis), either in BAT, liver or WAT. The data presented suggest that the hypolipidaemic effect of clofibrate in the rat may be due (possibly among other mechanisms) to reduction of the rate of fatty acid synthesis in BAT but not in the liver and WAT.

Clofibrate (Atromid S, ethyl-2-(4-chlorophenoxy)-2-methylpropionate) is used to treat all forms of hyperlipidaemia and is the drug of choice for type III hyperlipidaemia [1]. The mechanism of action of this drug is still unknown despite extensive studies having been performed on animals and isolated tissues. Numerous reports indicate that clofibrate causes a significant decrease in serum triacylglycerol, total cholesterol and low density lipoprotein-cholesterol concentrations [2–9]. A variety of mechanisms have been proposed for the antilipidaemic action of this drug, i.e. inhibition of cholesterol biosynthesis [10–12], accelerated catabolism of cholesterol [13], inhibition of fatty acid transport and biosynthesis [10, 14], and increased mitochondrial and/or peroxisomal oxidation of fatty acids [15–19]. However, the relative importance of these (and possibly other) mechanisms to the antilipidaemic action of clofibrate has yet to be determined. Depression of fatty acid or triacylglycerol biosynthesis may be caused by an inhibitory effect of clofibrate on acetyl-CoA carboxylase [20] or acylation of glycerol-3-phosphate [21]. Direct effects of clofibrate on lipid metabolism in isolated hepatocytes [22] and in subcellular liver fractions [21, 23] have been published. However, some investigators reported conflicting results. For instance Laker and Mayes [2] showed that lipogenesis in the liver was depressed upon addition of the drug to the perfusate, but was increased significantly in the livers of rats treated with clofibrate for 1 week. Miyazawa *et al.* [3] have also reported an increase of liver lipogenesis after clofibrate treatment of rats. In virtually all previous investigations concerning the action of clofibrate on lipogenesis, only the liver [2, 3] and white adipose tissue (WAT†) [3, 24] have been considered. It is well documented, however, that the capacity of brown adipose tissue (BAT) for fatty acid biosynthesis is very high [25–32]. Some data suggest that this tissue could play an important role in controlling the serum triacylglycerol level [33]. Thus, the effect of clofibrate on BAT lipogenesis and lipogenic enzyme activities is the major concern of this communication. The effect of clofibrate treatment on the activities of lipogenic enzymes and fatty acid synthesis in the rat liver and WAT is also reported.

MATERIALS AND METHODS

All substrates for enzyme activity measurements were purchased from the Sigma Chemical Co. (St Louis, U.S.A.). Clofibrate was obtained from Serva (Heidelberg, Germany), Triton X-100 from Koch-Light Laboratories (Colnbrook-Buchs, U.K.), Folin-Ciocalteu phenol reagent from E. Merck (Darmstadt, Germany) and sodium dodecyl sulphate from LKB-Producter AB (Bromma, Sweden). Tritiated water was obtained from Amersham International (Aylesbury, U.K.). All other chemicals were of the highest purity available from POCh (Gliwice, Poland).

Male Wistar rats weighing approximately 250 g were housed in wire mesh cages at 20° with alternating 12 hr light/12 hr dark and were fed *ad lib.* on a commercially available standard laboratory diet. The experimental group of rats were given clofibrate (as a water suspension prepared as described previously [34]) by stomach tube at a daily dose of 250 mg/kg of body weight for 7 successive days. On the 8th day rats were used for *in vivo* fatty acid synthesis estimation or *in vitro* enzyme activity studies.

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† Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue.

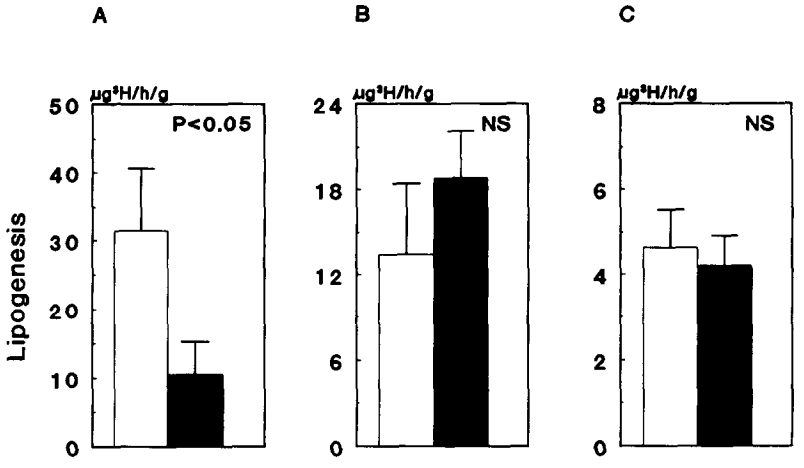

Fig. 1. The effect of clofibrate treatment on *in vivo* lipogenesis in interscapular BAT (A), liver (B) and WAT (C). The vertical lines indicate standard deviation. The significance level of difference in the rate of ^3H from ^3H_2O incorporation into lipids between clofibrate-treated (black) and control (white) rats was tested by Student's *t*-test and is presented on the figure (NS means that $P > 0.05$).

The fatty acid synthesis *in vivo* was measured by incorporation of tritium from tritiated water into fatty acids [35, 36]. Rats were injected intraperitoneally with 5 mCi ^3H_2O. One hour after injection, animals were killed by decapitation, blood was collected, the total interscapular BAT and approximately 1 g of liver and WAT were removed. Tissues were weighed, saponified with ethanolic KOH, acidified and fatty acids were extracted with petroleum ether [35]. The incorporation of tritium into fatty acids was assayed using a LS-6000 liquid scintillation spectrometer (Beckman). Rate of fatty acid synthesis was calculated as micrograms ^3H incorporated per hour per gram of wet tissue weight by dividing the radioactivity in the lipid fraction by the specific radioactivity of plasma water measured in a blood sample taken at the same time as the tissue sample.

For enzyme activity determination animals were killed by decapitation, the total interscapular BAT and approximately 1 g of liver and WAT were removed, and weighed. Tissues were homogenized in 8 mL of ice-cold 20 mM Tris–HCl buffer (pH 7.8) containing 0.2% Triton X-100. The homogenates were centrifuged at 20,000 *g* for 30 min. The resulting supernatants were decanted and pellets were rehomogenized in 5 mL of the isolation medium and centrifuged again. The combined supernatants were used for the enzyme assay. The fatty acid synthase (EC 2.3.1.85), ATP-citrate lyase,* malic enzyme, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP-linked isocitrate dehydrogenase activities were assayed as described recently [32]; lactate dehydrogenase and malate dehydrogenase as described in Ref. 37. All assays were performed using a Specord M40 spectrophotometer (Carl Zeiss Jena) at 37°.

Protein assays were performed according to Petersen [38].

RESULTS

The food consumption and the changes in total body weight of control and clofibrate-treated rats were the same. Thus, all the changes presented below may be assumed to be caused by the drug administration itself and not by dietary factors. Although the ratio of liver weight to whole body weight was higher in the clofibrate-treated than in the control rats, the amount of soluble protein per gram of liver was essentially the same in both groups (not shown). this is in accordance with previously reported data [34] in which prolonged clofibrate feeding induced hepatomegaly but did not change the amount of soluble protein. Administration of clofibrate for 7 days to rats led to a significant decrease in total fatty acid biosynthesis in BAT (Fig. 1A). Under the same conditions the rate of fatty acid synthesis in WAT was similar in control and clofibrate-treated rats (Fig. 1C). In contrast, the rate of hepatic lipogenesis was higher after clofibrate feeding, but the difference was not statistically significant (Fig. 1B). Thus, the effect of clofibrate on hepatic and WAT lipogenesis was found to be essentially similar to that reported by Laker and Mayes [2] and Miyazawa *et al.* [3]. To gain an insight into the mechanism of clofibrate action on lipogenesis in BAT, we have examined the activity of some lipogenic enzymes. The activity of fatty acid synthase (expressed as micromoles per minute per gram of wet tissue) in BAT was significantly depressed in

* Enzymes: malic enzyme [L-malate: $NADP^+$ oxidoreductase (oxaloacetate decarboxylating), EC 1.1.1.40]; NADP-linked isocitrate dehydrogenase [threo-D-isocitrate: $NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.42]; glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate: $NADP^+$ 1 oxidoreductase, EC 1.1.1.49); 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate: $NADP^+$ 2 oxidoreductase (decarboxylating), EC 1.1.1.44]; ATP-citrate lyase [ATP-citrate(pro-3S)-lyase, EC 4.1.3.8]; lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27); malate dehydrogenase (L-malate: NAD^+ oxidoreductase, EC 1.1.1.37).

Table 1. The effect of clofibrate feeding on the activity of lipogenic and some non-lipogenic enzymes in BAT

Enzyme	Enzyme activity (μmol/min/g wet tissue)	
	Control	Clofibrate-treated
Fatty acid synthase	1.93 ± 0.83	0.95 ± 0.29*
Malic enzyme	29.6 ± 5.01	14.07 ± 4.88‡
ATP-citrate lyase	5.74 ± 1.4	1.01 ± 0.60†
Glucose 6-phosphate dehydrogenase	11.7 ± 3.2	7.07 ± 1.9*
6-Phosphogluconate dehydrogenase	15.5 ± 3.49	10.01 ± 3.0*
Isocitrate dehydrogenase	11.8 ± 1.4	11.6 ± 1.5
Lactate dehydrogenase	70.2 ± 10	71.9 ± 21
Malate dehydrogenase	122 ± 15	104 ± 18

The values are means ± SD of determinations from six rats.
Significant differences (Student's *t*-test) between control and clofibrate-treated rats are indicated by *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

Table 2. The effect of clofibrate feeding on the activity of lipogenic and some non-lipogenic enzymes in the liver

Enzyme	Enzyme activity (μmol/min/g wet tissue)	
	Control	Clofibrate-treated
Fatty acid synthase	0.52 ± 0.16	0.46 ± 0.12
Malic enzyme	2.83 ± 1.25	13.2 ± 2.91†
ATP-citrate lyase	1.63 ± 0.37	1.01 ± 0.12*
Glucose 6-phosphate dehydrogenase	1.85 ± 1.14	1.03 ± 0.25
6-Phosphogluconate dehydrogenase	8.06 ± 1.82	6.4 ± 0.81
Isocitrate dehydrogenase	51.3 ± 3.4	53.6 ± 4.5
Lactate dehydrogenase	235 ± 36	332 ± 34†
Malate dehydrogenase	113 ± 13	99.5 ± 15

The values are means ± SD of determinations from six rats.
Significant differences (Student's *t*-test) between control and clofibrate-treated rats are indicated by *$P < 0.05$, †$P < 0.001$.

the clofibrate-treated rats (Table 1). A similar pattern of fatty acid synthase suppression by clofibrate was observed if the results were expressed as micromoles per minute per milligram of protein (not shown). These results correspond very well with the data presented in Fig. 1. It is unlikely that this was a direct clofibrate effect on BAT fatty acid synthase activity, as the drug was not only without effect on the liver (Table 2) and WAT (Table 3) enzyme, but it also showed no *in vitro* effect on BAT fatty acid synthase activity up to a concentration of 2 mM. Malic enzyme and ATP-citrate lyase activities in BAT were decreased to about 50% and 20% of control activity, respectively, by clofibrate feeding (Table 1). Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the enzymes participating (like malic enzyme) in NADPH production required for fatty acid synthesis, were also substantially decreased in the BAT of the clofibrate-treated rats (Table 1). Thus, the rate of lipogenesis and the activities of fatty acid synthase, ATP-citrate lyase, malic enzyme and hexose monophosphate shunt dehydrogenases are coordinately depressed by clofibrate in BAT (Fig. 1A and Table 1). In contrast, clofibrate feeding caused a several-fold increase in malic enzyme activity in the liver (Table 2) and was without effect in WAT (Table 3). The effect of clofibrate treatment on ATP-citrate lyase activity in the liver and WAT is also presented in Tables 2 and 3. The activity of this enzyme was depressed in these tissues. The activity of hexose monophosphate shunt dehydrogenases was not changed by clofibrate treatment either in the liver (Table 2) or WAT (Table 3). We also studied the effect of clofibrate treatment on lactate dehydrogenase, isocitrate dehydrogenase and malate dehydrogenase in BAT (Table 1), the liver (Table 2) and WAT (Table 3). These enzymes are not involved directly in fatty acid biosynthesis. Clofibrate feeding caused a slight increase in lactate dehydrogenase activity in the liver but was without effect on this enzyme in BAT and WAT. Isocitrate dehydrogenase and malate dehydrogenase activities were not changed by clofibrate feeding in either the

Table 3. The effect of clofibrate feeding on the activity of lipogenic and some non-lipogenic enzymes in WAT

Enzyme	Enzyme activity (μmol/min/g wet tissue)	
	Control	Clofibrate-treated
Fatty acid synthase	0.023 ± 0.002	0.019 ± 0.005
Malic enzyme	0.735 ± 0.300	0.676 ± 0.230
ATP-citrate lyase	0.093 ± 0.003	0.059 ± 0.007*
Glucose 6-phosphate dehydrogenase	0.36 ± 0.11	0.30 ± 0.01
6-Phosphogluconate dehydrogenase	15.5 ± 0.5	15.0 ± 0.7
Isocitrate dehydrogenase	0.061 ± 0.002	0.053 ± 0.003
Lactate dehydrogenase	3.3 ± 0.8	3.6 ± 0.7
Malate dehydrogenase	11.6 ± 3.2	10.5 ± 1.3

The values are means ± SD of determinations from six rats.
Significant difference (Student's *t*-test) between control and clofibrate-treated rats is indicated by $^*P < 0.001$.

liver, BAT or WAT. The effect of clofibrate treatment on liver lactate dehydrogenase, malate dehydrogenase and NADP-linked isocitrate dehydrogenase was essentially similar to that reported previously by Platt and Cockrill [39].

DISCUSSION

The data presented in this paper indicate that total fatty acid biosynthesis, estimated by the incorporation of tritium from ^3H_2O into lipid, substantially decreased after clofibrate treatment. Parallel decrease in enzymatic activities related to this process (i.e. fatty acid synthase, ATP-citrate lyase, NADP-linked malic enzyme and hexose monophosphate shunt dehydrogenases, Table 1) suggests that the lower BAT lipogenesis is a consequence of diminished lipogenic enzyme activities. The effect of this drug on hepatic lipogenesis has been extensively examined; however, contradictory results have been reported, namely: inhibition [2, 20, 21, 40]; stimulation [2, 3, 24, 41, 42] and no effect [43]. Different results from various laboratories may be due to diverse assay systems and varying experimental conditions. For instance, clofibrate (as either ester or sodium salt) has been administered to animals under a variety of conditions at different doses. Our results (Fig. 1) suggest that clofibrate treatment causes a slight increase in ^3H incorporation into liver fatty acids, whereas under identical conditions BAT lipogenesis decreases significantly. In the liver no correlation between the rate of fatty acid synthesis and some lipogenic enzyme activities has been observed. The activity of NADP-linked malic enzyme was increased 5-fold after administering the drug (Table 2) as was anticipated from previous work [34, 39, 44–47]. In contrast, clofibrate feeding led to a reduction in ATP-citrate lyase (Table 2). Similar results have been reported previously [45]. Unexpectedly (considering the increase in ^3H from ^3H_2O incorporation into fatty acid), the activity of a key lipogenic enzyme, fatty acid synthase, was not changed in the liver after clofibrate feeding (Table 2). In contrast to BAT (depression) and to the liver (a slight increase), the administration of clofibrate did not affect incorporation of ^3H from tritiated water in the total fatty acids in the epididymal fat pad. This is in a good agreement with data reported by Miyazawa *et al.* [3] and contradictory to the data reported by Giocoli *et al.* [24]. Giocoli *et al.* [24] showed that the addition of clofibrate to a glycerol–lard diet fed to rats resulted in increased incorporation of ^{14}C from [^{14}C]glucose and ^3H from ^3H_2O into long chain fatty acids by fat pads *in vitro*. The drug, however, had only a small or no effect when added to a fat-free sucrose diet [24]. The unchanged rate of lipogenesis in WAT after clofibrate feeding (Fig. 1C) correlates with the unchanged fatty acid synthase, malic enzyme and hexose monophosphate dehydrogenase activities but not with ATP-citrate lyase activity (Table 3).

In conclusion, the results presented in this paper clearly indicate that the selective depression of fatty acid synthesis in rat BAT by clofibrate as a result of decreased key and auxiliary lipogenic enzyme activities does occur. Considering that BAT lipogenesis is contributing to the overall fatty acid production in rat [33, 48], one can assume that the hypolipidaemic effect of clofibrate is due in part to the reduction of the rate of BAT lipogenesis. Moreover, the present study confirms the previous suggestion that the hypolipidaemic effect of this drug is not directly linked to fatty acid biosynthesis in rat liver and WAT [2, 3, 24]. Further investigation will be necessary to establish the importance of the results presented in this paper for the hypolipidaemic effect of clofibrate in man.

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