The Rapid Induction of Liver Glycerophosphate Acyltransferase in Mice by Clofibrate, a Hypolipidemic Agent*

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Administration of clofibrate to adult mice (20–25 mg of clofibrate/day) increased liver glycerophosphate acyltransferase (EC 2.3.1.15) activity by 2.3-fold within 24 h. This increased glycerophosphate acyltransferase activity was mainly localized in the liver microsomal fraction. Three other hypolipidemic drugs, i.e., bezafibrate (Boehringer Mannheim Co.), gemfibrozil (Warner-Lambert Co.) and Wy-14,645 (Wyeth Laboratories), when fed to mice, also increased the liver glycerophosphate acyltransferase activity by 2–3-fold in 24 h. Simultaneous administration of inhibitors of protein biosynthesis, such as cycloheximide or actinomycin D completely abolished such stimulation of liver glycerophosphate acyltransferase by clofibrate.

Clofibrate, a widely used hypolipidemic drug (1) has been shown to cause the proliferation of mitochondria, peroxisomes, and smooth endoplasmic reticulum in the livers of experimental animals (2–4). Chronic administration of clofibrate and other hypolipidemic drugs also has been shown to increase the activity of a number of other enzymes such as mitochondrial glycerol-3-phosphate dehydrogenase (2), peroxisomal fatty acid oxidation enzymes (5), and carnitine acyltransferases (6). We have previously shown that rat liver glycerol-3-phosphate acyltransferase and dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42) are increased 2–3-fold after chronic administration of clofibrate to the animals for 8–10 days (7). This has been recently confirmed by Pollard and Brindley (8). Further investigation showed that mouse and rat liver mitochondrial glycerol-3-phosphate, palmitoyl-CoA:bovine serum albumin ratio in the incubation mixture.

MATERIALS AND METHODS

Lithium salt of glycerophosphate phosphatidylcholine, acetyl-CoA, and acetyl-CoA synthetase were from Sigma Chemical Co. (St. Louis, Mo.). Clarithromycin and chloroquine were used as gifts from Astra Inc., and chloroquine was used as gifts from Astra Inc., Ciba-Geigy AG, and Kaken Pharmaceutical Co. Ltd. Tissue culture reagents were obtained from Flow Laboratories, Division of American Type Culture Collection. [3H]-glycerol-3-phosphate (specific activity 65 mCi/mmol) was purchased from New England Nuclear. [14C]-glycerol-3-phosphate (specific activity 65 mCi/mmol) was purchased from Amersham. [14C]-glycerol-3-phosphate (specific activity 65 mCi/mmol) was purchased from Amersham. [14C]-glycerol-3-phosphate (specific activity 65 mCi/mmol) was purchased from Amersham.

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Glycerophosphate acyltransferase activity is very sensitive to palmitoyl-CoA/histidine serum albumin ratio in the incubation mixture. We found the optimum ratio to be 2.0 mol/mol.
Clofibrate Induces Liver Glycerophosphate Acyltransferase

Table 3

Effect of Clofibrate Administration on Mice Liver Glycerophosphate Acyltransferase and Glycerol-3-Phosphate Phosphate Acyltransferase

Groups of mice (3 in each group, age 6-30 g) were fed powdered food either mixed with clofibrate (0.5% w/w) or without clofibrate (control) for 4 h. The liver glycerophosphate acyltransferase activity total homogenate and subcellular fractions was determined as described in the text. Results were calculated as the activity of Clofibrate-induced homogenate or total homogenate which was determined for only one group. The average change in weight of individual mice 1.15 ± 0.03 g, and that of the clofibrate-fed group was 1.15 ± 0.02 g. The average total protein per g of liver in 5:4 = 1.5 ± 0.5 (Table 3) controls and clofibrate and in total homogenate were 1.4 ± 0.5 mg (Such in controls and clofibrate-fed group).

Table 3

<table>
<thead>
<tr>
<th>Enzyme Assayed</th>
<th>Specific Activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Glycerophosphate acyltransferase</td>
<td></td>
</tr>
<tr>
<td>In s_1</td>
<td>0.61 ± 0.01</td>
</tr>
<tr>
<td>In homogenate</td>
<td>0.80</td>
</tr>
<tr>
<td>Glycerol-3-phosphate acyltransferase</td>
<td></td>
</tr>
<tr>
<td>In s_1</td>
<td>3.7 ± 0.19</td>
</tr>
<tr>
<td>In homogenate</td>
<td>2.90 ± 0.09</td>
</tr>
</tbody>
</table>

* Figures in parenthesis are increase over control.

Table 4

Subcellular Distribution of Liver Glycerophosphate Acyltransferase

Control, Clofibrate, and Clofibrate-fed (24 h) mice.

Table 4

<table>
<thead>
<tr>
<th>Subcellular Fraction **</th>
<th>Specific Activity in Homogenate</th>
<th>Relative Specific Activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Clofibrate</td>
<td>Clofibrate</td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>2.3 (0.06)</td>
<td>3.6 (0.26)</td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>7.5 (0.31)</td>
<td>6.2 (0.31)</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>5.6 (0.01)</td>
<td>26.9 (0.01)</td>
</tr>
<tr>
<td>Nissl body</td>
<td>1.0 (0.81)</td>
<td>6.1 (0.81)</td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate relative specific activity (i.e., enrichment) of the endoplasmic reticulum

** Abbreviations used: N: nuclear fraction, Mit: mitochondrial fraction, Micro: microsomal fraction, Endo: endoplasmic reticulum, Nissl: Nissl body, and No: none detected.

Fig. 1 Relative change in glycerophosphate acyltransferase activity in post-nuclear supernatant (PNS) or liver of mice which were either fed clofibrate (0.5% w/w) or control diet for only 2 days and then treated with clofibrate or the next five days lasted 5 days. All clofibrate-fed mice showed increased activity of glycerophosphate acyltransferase in the post-nuclear fraction (PNF) but not in the cytosol fraction (CF). A significant increase in the activity of glycerophosphate acyltransferase in the PNF and CF fractions was observed in the clofibrate-fed group. The average change in weight of individual mice 1.15 ± 0.03 g, and that of the clofibrate-fed group was 1.15 ± 0.02 g. The average total protein per g of liver in 5:4 = 1.5 ± 0.5 (Table 3) controls and clofibrate and in total homogenate were 1.4 ± 0.5 mg (Such in controls and clofibrate-fed group).

Fig. 2 Comparative effects of different hypolipidemic drugs on the glycer-ophosphate acyltransferase activity of mice liver total homogenate. Different groups of mice (3 in each group) were fed diet either containing clofibrate (0.5% w/w), or bezafibrate (0.5% w/w), or wy-14,464 (0.05% w/w), or gemfibrozil (0.25% w/w) for 24 h and the liver glycerophosphate acyltransferase activity (U/mg protein) in total homogenate was assayed as described in the text. Control experiments were also performed. The results presented above are the average values of two such experiments.

Fig. 3 Effect of administration of clofibrate or with or without actinomycin D or actinomycin O on Mice Liver Glycerophosphate Acyltransferase activity. The experimental animals were injected intraperitoneally either with 0.2 ml saline (control) or 0.1 ml saline (clofibrate). In 0.2 ml saline (clofibrate), in 0.2 ml saline (clofibrate + actinomycin D) or 0.0 ml saline (actinomycin D). After 24 h the liver glycerophosphate acyltransferase activity in total homogenate was measured as described in the text. The results given were the average (standard deviation) of four such experiments. (a) The experimental control was the same as in A, except that instead of saline, 0.2 ml saline (clofibrate). (b) The control was the same as in A, except that instead of saline, 0.2 ml saline (clofibrate + actinomycin D) was used. Only one experiment using cycloheximide was done.
Clofibrate Induces Liver Glycerophosphate Acyltransferase

As mentioned previously, clofibrate has been shown to induce several mitochondrial and peroxisomal catabolic enzymes such as glycerophosphate dehydrogenase (2), carnitine acyltransferases (3), and fatty acid β-oxidation enzymes (5). Recently, cytosolic acyl-CoA hydrolase activity has been shown to be increased after chronic clofibrate feeding (25).

Results presented here show that clofibrate also causes an induction of a microsomal enzyme, glycerophosphate acyltransferase, which is supposedly a constitutive anabolic enzyme (20, 21). In contrast to the other clofibrate-induced enzymes which reached the maximum activity only after chronic administration of the drug for 1 to 3 weeks (4), the glycerophosphate acyltransferase activity is rapidly increased to its maximum value within 24 h (Fig. 1). The abolition of this increase of glycerophosphate acyltransferase by actinomycin D or cycloheximide indicates that, directly or indirectly, clofibrate and other hypolipidemic agents cause an induction of liver microsomal glycerophosphate acyltransferase. Moody and Reddy (6) found similar inhibition of the induction of carnitine acyltransferase in the clofibrate-fed mice (30 h) by these agents. This type of action of these hypolipidemic drugs on the nuclear control of protein gene synthesis is not surprising as these agents have been shown to have both mitogenic and carcinogenic properties (26, 27). The rapid decrease in the induced glycerophosphate acyltransferase activity in mouse liver after the drug is withdrawn (Fig. 1) indicates that continuous administration of clofibrate is necessary to maintain the high liver glycerophosphate acyltransferase activity.

The induction of a key enzyme for glycerolipid biosynthesis by hypolipidemic agents is somewhat paradoxical. These drugs have been shown to inhibit dihydroxyacetone phosphate acyltransferase in vitro at very low concentrations (28). It may be that the inhibition of the acyl dihydroxyacetone phosphate acyltransferase pathway is compensated for by the stimulation of the alternate glycerophosphate pathway for glycerolipid biosynthesis. Also, clofibrate and other hypolipidemic agents have been shown to increase the liver weight (hepatomegaly effect) (2, 4), induce a proliferation of peroxisomes and smooth endoplasm reticulum (4), and increase the amount of lecithin in bile (25). All these effects would seem to require an increase in the rate of synthesis of glycerolipids in liver, hence an increase in glycerophosphate acyltransferase activity. Compared to the other microsomal enzymes of the glycerolipid biosynthesis pathway, glycerophosphate acyltransferase activity is low (20, 21) and probably is the rate-limiting step. Although under different physiological and nutritional conditions, small and slow changes of glycerophosphate acyltransferase activity (20-70%) have been observed, (30-32), such large (200-300%) and rapid in vivo changes in the liver glycerophosphate acyltransferase activity as detailed here have not been previously reported. In the livers of clofibrate-treated animals, we did not find any appreciable change in the specific activities (nanomoles/min/mg of protein) of other glycerolipid-metabolizing enzyme such as phosphatidic acid phosphatase (7.7 in normal and 8.3 in clofibrate-treated liver when the substrate used was phosphatidate and 7.1 and 7.0, respectively, when 1-alkyl glycerophosphate was used as the substrate), diglyceride acyltransferase (0.58 in normal and 0.51 in clofibrate-treated animals), cholinephosphotransferase (3.0 in normal and 3.3 in clofibrate-treated animals) and ethanolamine phosphotransferase (1.06 in normal and 1.35 in clofibrate-treated animals) within 24 h.

Although clofibrate has been clinically used for a number of years to treat hyperlipidemia, the mechanism of action of this drug is not known (1). Further studies are needed to establish whether the hypolipidemic action of clofibrate is related to this induction of glycerophosphate acyltransferase in liver. However, this drug-induced rapid increase (up to 3-fold) of glycerophosphate acyltransferase activity may provide a useful system to study the biosynthesis and physiological control of this key membrane-bound enzyme catalyzing glycerolipid biosynthesis.

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