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 liver enzymes such as mitochondrial glycerophosphate dehydrogenase (2), peroxisomal fatty acid, & oxidase enzymes (5), and carnitine acyltransferases (6). We have previously shown that rat liver glycerol-3-phosphate acyltransferase and dihydroxyacetone phosphate acyltransferase activity after chronic administration of clofibrate to the animals for 8-10 days (7).

We have shown that mouse and rat liver glyceraldehyde 3-phosphate dehydrogenase (2), peroxisomal fatty acid & oxidase enzymes, and carnitine acyltransferases (6) are reported here. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

MATERIALS AND METHODS

Lithium salt of glycerolphosphate phosphatase, sodium dodecyl sulfate, acyl-CoA, and carnitine-acyltransferase were from Sigma Chemical Co. (St. Louis, Mo.). Clofibrate and chloroform were either gifts from Ayerst Laboratories or were purchased from Sigma Chemical Co. The other hypolipidemic agents used were gifts from different drug companies as indicated. Bezafibrate (1-4-[2-4-chlorophenyl-ethyl]phenoxy-2-naphthylpropionic acid) was from Ayerst Laboratories, Inc., whereas clofibrate (Boehringer-Mannheim) was purchased from Sigma Chemical Co. Liver, muscle, and brain tissue from Sprague-Dawley rats and Fischer 344 rats were supplied by the Division of Laboratory Animals of The Johns Hopkins Medical Institutions. These drugs were mixed with powdered mouse food (standard formula) in proportions as indicated in the text and fed ad libitum to adult Swiss male mice (Harlan Sprague Dawley Inc., Madison, WI). The control mice were fed powdered food only. Each mouse 20-35 g was weighed and dosed in 1 mg/ml saline. The mice were killed 24 h after dosing, and the liver was excised and homogenized in 10 vol of 10% trichloracetic acid in water (150 mg). Aliquots of 1 ml, and/or acyl-CoA (0.5-1 ml) or carnitine-acyltransferase (2.5-3 ml) were placed into prechilled test tubes (1 ml). The tubes were incubated at 37 °C for 45 min, and the reaction terminated by the addition of 0.5 ml of 10% trichloracetic acid. The reaction mixture was then centrifuged at 1,000 

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** Portions of this paper (including "Materials and Methods," "Results," Tables I and II, and Figs. 1-3) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9500 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-5433, cite authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

3 Glycerophosphate acyltransferase activity is very sensitive to palmitoyl-CoA/bovine serum albumin ratio in the incubation mixture. We found the optimum ratio to be 2.0 mol/mol.
**TABLE 1**

Effect of Clofibrate or Atherogenic Diet on Mammal Glycerophosphate Acyltransferase

<table>
<thead>
<tr>
<th>Enzyme Assayed</th>
<th>Specific Activity (millimoles per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Clolfibrate-fed*</td>
</tr>
<tr>
<td>Glyceroxyoctanoate-free glycerolphosphate acyltransferase</td>
<td>0.61 ± 0.00</td>
</tr>
<tr>
<td>Glycerophosphate</td>
<td>3.72 ± 0.00</td>
</tr>
</tbody>
</table>

* Figures in parenthesis are 1 increase over control.

**TABLE 2**

Subcellular Distribution of Liver Glycerophosphate Acyltransferase

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>Specific Activity (millimoles per hour)</th>
<th>Relative Specific Activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>32.5 (0.26)</td>
<td>100</td>
</tr>
<tr>
<td>Microsomes</td>
<td>17.0 (0.18)</td>
<td>53</td>
</tr>
<tr>
<td>Rough Endoplasmic</td>
<td>17.0 (0.18)</td>
<td>53</td>
</tr>
<tr>
<td>Smooth Endoplasmic</td>
<td>17.0 (0.18)</td>
<td>53</td>
</tr>
<tr>
<td>Nuclear</td>
<td>17.0 (0.18)</td>
<td>53</td>
</tr>
</tbody>
</table>

† Figures in parenthesis indicate the relative specific activity (i.e., enrichment) of the enzyme in each fraction.

**Figures 2**

Comparison of different hypolipidemic drugs on the glycerophosphate acyltransferase activity of mice liver total homogenate. Different groups of mice (6 mice in each group) were fed a diet containing clofibrate (0.5% w/w), or bezafibrate (0.5% w/w), or Wy-44143 (0.5% w/w) or Wy-146-443 (0.5% w/w), or a control diet for 6 weeks and the glyceroxyoctanoate-free glycerolphosphate acyltransferase activity (millimoles/min per mg protein) in total homogenate was measured as described in the text. The results presented above are the average values of 2 observations.
Clofibrate Induces Liver Glycerophosphate Acyltransferase

As mentioned previously, clofibrate has been shown to induce several mitochondrial and peroxisomal catalytic 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 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 endoplasmic reticulum (4), and increase the amount of lecithin in bile (29). 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 ethanolaminedephasphotransferase (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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REFERENCES

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