Subcellular distributions of lipid-metabolizing enzymes in rat liver after treatment with tiadenol, clofibrate and phenobarbital

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Clofibrate and an alternative hypolipidaemic drug, tiadenol, cause hepatomegaly with marked proliferation of peroxisomes and mitochondria (Hess et al., 1965; Kurup et al., 1970; Roze et al., 1977). Subcellular fractionation studies have shown that the palmitoyl-CoA hydrolase activity is present both in the microsomal and mitochondrial fractions (Berge & Farstad, 1979; Berge & Døssland, 1979). As recently reported (Berge et al., 1981; Berge & Bakke, 1981), the activity of acyl-CoA hydrolase as well as the activity of other lipid-metabolizing enzymes increase after clofibrate and tiadenol treatment. Furthermore, the specific activity of palmitoyl-CoA hydrolase increases in the particle-free supernatant with no increase in the microsomal fraction.

The aim of the present study was to assess whether palmitoyl-CoA hydrolase and other lipid-metabolizing enzymes are altered or distributed differently in rat liver after treatment with inducers of peroxisomes (tiadenol and clofibrate) and of smooth endoplasmic reticulum (phenobarbital). Phenobarbital was included in the experiments because, according to current theory, the peroxisomes are believed to originate from this latter organelle (Staubli, 1963).

Rats were fed on diets containing clofibrate, tiadenol (0.3%, w/w) or phenobarbital (0.1%, w/w) for 10 days. The livers were homogenized in ice-cold sucrose solution, 0.25 M-sucrose and 10mM-hepes buffer, pH 7.4, using a Potter–Elvehjem homogenizer at 720 rev./min and with two strokes of a loose-fitting Teflon pestle.

The liver weights increased in all groups. The increase induced by tiadenol was much greater than that observed in the animals treated with clofibrate. The total palmitoyl-CoA hydrolase activity was enhanced after administration of the three agents. Tiadenol administration produced a 7–9-fold increase of the total activity of this enzyme, whereas only a 2–3-fold enhancement was obtained with clofibrate or phenobarbital. A large increase in the total activity of peroxisomal palmitoyl-CoA oxidation was obtained after tiadenol treatment, more than that obtained with clofibrate.

The specific activity (Table 1) as well as the amount of the palmitoyl-CoA hydrolase increased in the microsomal (P) fraction after treatment with phenobarbital. The subcellular localization was similar to that observed in the normal liver. As also found in a previous study (Berge et al., 1981; Berge & Bakke, 1981), clofibrate administration resulted in little change of palmitoyl-CoA hydrolase activity in the P fraction, whereas a considerable increase occurred in the cytosol. The altered distribution of palmitoyl-CoA hydrolase activity was more marked with tiadenol than with clofibrate. Tiadenol administration resulted in decreased specific activity in the P-fraction (Table 1).

The increased palmitoyl-CoA hydrolase activity in the P-fraction after phenobarbital treatment suggests that this enzyme is localized in the smooth endoplasmic reticulum. The study of enzyme activities in different subcellular fractions after treatment with hypolipidaemic drugs may shed light on the biogenesis of the peroxisomes. Thus the increased specific activity of the palmitoyl-CoA hydrolase activity in the particle-free supernatant may be derived from the newly induced peroxisomes budding off from the endoplasmic reticulum, possibly due to labilization of the membrane. Since there was also a shift in the distribution of peroxisomal palmitoyl-CoA oxidase and catalase, but not of urate oxidase, after treatment with both clofibrate and tiadenol (results not shown), it is possible that the hypolipidaemic drugs may induce heterogeneous populations of peroxisomes as suggested by Flatmark et al. (1980).

Table 1. Hepatic palmitoyl-CoA hydrolase activity (nmol/min per mg of protein) of rats fed diets containing clofibrate, tiadenol or phenobarbital

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Control</th>
<th>Phenobarbital</th>
<th>Clofibrate</th>
<th>Tiadenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular fraction</td>
<td>Mitochondrial</td>
<td>16.7</td>
<td>17.8</td>
<td>58.9</td>
</tr>
<tr>
<td>Light mitochondridal</td>
<td>21.9</td>
<td>17.2</td>
<td>35.2</td>
<td>42.3</td>
</tr>
<tr>
<td>Microsomal</td>
<td>109.0</td>
<td>130.0</td>
<td>117.2</td>
<td>106.1</td>
</tr>
<tr>
<td>Particle-free supernatant</td>
<td>8.1</td>
<td>7.9</td>
<td>56.0</td>
<td>63.8</td>
</tr>
</tbody>
</table>

Results are for two animals in each group and are means.

Flatmark, T., Christiansen, E. N. & Kryvi, H. (1980) Biokjemiske Kontrikmate, Gassdal Hengellshoell 16, abstr. no. 46

1981