Apparent Induction of Microsomal Carboxylesterase Activities in Tissues of Clofibrate-Fed Mice and Rats

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Received December 29, 1986; accepted March 17, 1987

Apparent Induction of Microsomal Carboxylesterase Activities in Tissues of Clofibrate-Fed Mice and Rats. ASHOUR, M.-B. A.; MOODY, D. E.; AND HAMMOCK, B. D. (1987). Toxicol. Appl. Pharmacol. 89, 361-369. Treatment with 0.5% (w/w) dietary clofibrate, a peroxisome proliferator, for 14 days induced microsomal carboxylesterase activities for five substrates in- cluding malathion, clofibrate, diethylcarbamyl, dieldrin, and 4-nitrophenylacetate in liver and kidney of male Swiss-Wtner mice and Sprague-Dawley rats. The induction was greatest, tissue, and species dependent. The carboxylesterase activity was induced in mouse from 1.5- to 2.2-fold (liver) and from 1.1- to 1.4-fold (kidney) depending upon substrate used. Analogue values from rat ranged from 1.6- to 1.4-fold (liver) and from 1.1- to 1.8-fold (kidney). Enzyme activities were either decreased or not affected in tests of treated mice and rats. Substituted trifluoroketones ("transition-state" analogues of carboxylesterase) were found to be very potent inhibitors of clofibrate-metabolizing carboxylesterases and to be potentially useful in distinguishing among isozymes. The inhibition data suggested that changes in carboxylesterase activity following clofibrate treatment were both qualitative and quantitative. © 1987 Academic Press, Inc.

Carboxylesterases (EC 3.1.1.1) are a number of distinct enzymes which hydrolyze xenobiotics containing an ester, thioester, or amide group (Heymann, 1982). In mammalian liver microsomes there are multiple forms of carboxylesterases having different substrate specificities (Fonnum et al., 1985; Kao et al., 1985). It has been shown that carboxylesterases can be induced by treatment with a variety of chemicals known to induce other xenobiotic-metabolizing enzymes (Kaur and Ali, 1983; Hasekawa et al., 1984; Nousiainen et al., 1984). However, the extent of compounds which have this effect is not known. Clofibrate is the prototype of a group of compounds known as peroxisome proliferators which cause an increase in hepatic and renal peroxisomes, hypolipidemia, and, with some compounds tested in rodents, hepato- cellular carcinomas after long-term treatment. Along with peroxisomes, the content of liver smooth endoplasmic reticulum is also increased after treatment with these compounds (Svoboda and Azarnoff, 1966; Reddy and Lalwani, 1983). The induction of these organelles is accompanied by unique increases in peroxisome-associated enzymes (Moody and Reddy, 1978), microsomal mixed function oxidase activities (Orton and Parker, 1982), and microsomal and cyto- solic epoxide hydrolase (Hammock and Ota, 1983). These responses suggest that micro- somal carboxylesterase activities may also be altered by peroxisome proliferators. At this time, studies on the response of these enzymes to peroxisome proliferators have been

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0041-00XX/87 $3.00
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limized to the hydrolysis of acyl-CoA esters (Kawashima et al., 1983; Reddy and Lalwani, 1983; Berge and Aarestad, 1985).

Since hepatic carboxylsterases are instrumental in the metabolism and thus influence the toxicity of a variety of known drugs, toxins, and other xenobiotics, the influence of peroxisome proliferators on these enzymes may be of pharmacological or toxicological significance. In this study the response of carboxylsterases to five substrates of toxicological and pharmacological interest, namely, clofibrate, malathion, dichlorodicyc- nate, diethylphthalate, and p-nitrophenylacetate, has been investigated in liver, kidney, and testis microsomes from clofibrate-treated mice and rats. In order to test the hypothesis that changes in carboxylsterase activity are qualitative as well as quantitative, a series of substituted trifluoroketones were tested as inhibitors of clofibrate-metabolizing carboxylsterase(s) in liver microsomes from mice and rats.

METHODS

Chemicals. Clofibrate, ethyl phenylphenoxybutyrate, was provided by Ayerst Laboratories (New York, NY). Malathion (5% in 0.05%-demineralized 0.1%-bitchloro- methanethyl) phosphorothioic acid, was provided by Dr. M. Mallipudi, American Cyanamid Corp. (Princeton, New Jersey). Diethylstilbestrol (DES) and p-nitrophenylacetate (p-NPA) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Diethylphthalate (DEP), pen- donitrophenyltetratrazole violet (INT), alcohol dehydrogenase, NAD, NAD diaphorase, and bovine serum albumin (fraction V) were obtained from Sigma Chemical Co. (St. Louis, MO). Bio-Rad protein dye reagent was purchased from Bio-Rad Laboratories (Richmond, CA). 2-Nitrophenol-1,1,1-trifluoropropan-2-ol, 2-de- clythio-1,1,1-trifluoropropan-2-ol, 2-phenoxyethanol, 1,1,1-trifluoropropan-2-ol, 2-p-chlorophenethyl-1,1,1-trifluoropropan-2-ol, 1,1,1-trifluorocyclooctan-2-ol, 1,1,1-trifluoro-10-cyclooctan-2-ol, 1,1,1-trifluorodecan-2-ol, 3-phenoxy-1,1,1-trifluoropro- 2-ol, and 4-phenoxy-1,1,1-trifluoropropan-2-ol were available from previous syntheses (Adelstein and Hammock, 1986; Adams and Hammock, 1987).

Animals. Male Sprague-Dawley rats (180-200 g, CD strain, Charles River, Wilmington, MA) and Swiss-Web- ster mice (25-30 g, BioZ-Kingman, Fremont, CA) were housed in steel cages with kiln-dried pine shavings as bedding in an environmentally controlled (22 ± 1°C, 14:10 h light/dark, constant humidity). Food and water were available ad libitum. The animals were divided into two groups: one group (control) was fed a standard laboratory diet of commercial pellets and water ad libitum. Clofibrate was dissolved in corn oil and mixed into ground Chow at a concentration of 0.5% (w/w). Control animals received ground chow with similar amount of corn oil added (5 ml/100g). Experimental animals were given clofibrate-containing diet for 14 days. One group of mice was given clofibrate-containing diet for 14 days and then received control diet for another 5 days.

Extraneous preparations. All animals were killed (mouse by cervical dislocation, rats by exsanguination under light ether anesthesia) between 9:00 and 11:00 a.m. Animals were removed, refluxed with cold 1.15% KCl, mixed, placed in sodium phosphate buffer (76 mm, pH 7.4), mixed, and weighed. Kidneys and testes were removed, dissected free of adhering tissues, rinsed in phosphate buffer, mixed, and weighed. Tissues were homogenized for 20 sec using a Polytron on setting 6. Homogenates of livers and kidneys were made to 10% (w/v) and those of testes to 5% (w/v) of the organ weight. They were centrifuged at 16,000g for 20 min, and the supernatant frac- tions were centrifuged at 105,000g for 60 min. The cyto- sol (105,000g supernatant) was collected after drawing off the fatty layer. Pellets were resuspended in 50 mM Tris-HCl buffer (pH 7.4), 125 mM KCl and recenteri- fuge. Washed microsomes were resuspended in phos- phate buffer (76 mm, pH 7.4) to give 10% five homogenate based on the initial homogenization and stored at 70°C.

Enzyme assays. All enzyme assays were carried out under conditions which gave linear dependence of en- zyme rate relative to protein concentration and which gave a linear increase in absorbance values with time. The spectrophotometric method of Talbot (1979) was used to assay carboxylsterase activities on clofibrate, malathion, diethylphthalate, and diethylphthalate in the crude microsomal fraction of rat and mouse liver, kid- neys, and testis.

In brief, the hydrolysis of the four substrates was cou- pled to the reduction of INT with alcohol dehydrogenase and NADH diaphorase. The enzyme activity, which is equivalent to the reduction of the tetrazolium dye, was calculated from the linear portion of the recording (change in absorbance at 500 nm with time) using an ex- tinction coefficient of 13.8 mmol-1 cm-1. Assays were monitored for 5 min at 37°C in cuvettes containing 1.0 ml of incubation mixture using a Varian Cary 219 UV/ visible spectrophotometer equipped with time drive, temperature-controlled sample compartment, and inter- faced with an Apple IIe computer. The absorbance mix- ture contained 0.5 ml of enzyme solution and 0.5 ml of reagent mixture in 0.1 ml Tris-HCl buffer, pH 7.5. A re- agent blank was used in the reference cuvette. The mea-
## EFFECT OF CLOFIBRATE ON CARBOXYLASES

### TABLE I

**MICROSOMAL CARBOXYLASE ACTIVITIES IN THREE TYPES OF CONTROL AND CLOFIBRATE-RESISTANT WEBSTER MICE**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Clofibrate</th>
<th>Clofibrate withdrawal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>113 ± 7.0</td>
<td>170 ± 16a</td>
<td>125 ± 16a (0.90)</td>
</tr>
<tr>
<td>Malate</td>
<td>7.8 ± 0.6a</td>
<td>21.0 ± 2.2*</td>
<td>9.8 ± 2.0* (1.23)</td>
</tr>
<tr>
<td>DES</td>
<td>146 ± 6</td>
<td>193 ± 11b</td>
<td>138 ± 16 (0.93)</td>
</tr>
<tr>
<td>ND Fucose</td>
<td>32.4 ± 0.1</td>
<td>36.6 ± 2.1</td>
<td>29.6 ± 0.3 (0.91)</td>
</tr>
<tr>
<td>p-Nitropeh</td>
<td>810 ± 64</td>
<td>1700 ± 60a</td>
<td>736 ± 239 (0.71)</td>
</tr>
</tbody>
</table>

* Measured as % of control.

### Notes

- Malate and DES were assayed by using 10% of a 10 mM solution in microsomes and 30% of a 10 mM solution in liver homogenate, respectively.
- p-Nitropeh was assayed by using 10% of a 10 mM solution in microsomes and 30% of a 10 mM solution in liver homogenate, respectively.
- ND Fucose was assayed by using 10% of a 10 mM solution in microsomes and 30% of a 10 mM solution in liver homogenate, respectively.
- p-Nitropeh was assayed by using 10% of a 10 mM solution in microsomes and 30% of a 10 mM solution in liver homogenate, respectively.

### References

minimum of two inhibitor concentrations showing greater than and two showing less than 50% inhibition on the linear region of the curve were used to determine the IC₅₀ values from weighing plots. Only those points on the linear portion of the curve were used to calculate the IC₅₀ and slope values. When IC₅₀ were determined by selected compounds using the method of Tietze and Vartian-Cary 219 spectrophotometric, no significant differences were noted in the resulting data. We took advantage of the smaller sample size and greater linearity reproducibility, and sample throughput of the Tietz's system.

Data were collected using a software program known as IC₅₀/A (Dorum software) and then transfered to an in-house program known as TRANSFER into a format suitable for Lotus 1-2-3 (Lotus Development, Inc.). The Lotus software was used for all data management and to determine rates of enzyme hydrolysis by regression analysis of the linear portion of the curve.

Protein assay. Protein was measured by a dye-binding method according to Bicinchoninic acid modificted by Bio-Rad, using bovine serum albumin as standard protein. To a cuvette, 100 µl of protein was added followed by the addition of 400 µl of 5% Pd BiocilRad reagent (20% in distilled water), and the measure was incubated for 10 min at room temperature. Absorbance at 620 nm was then measured using a Gilford EHS manual EUISA reader interfaced with an Astar 400 microcomputer programmed by R. Wixom, this laboratory.

Calculations and statistics. Therese's activities were routinely calculated using specific activity (nmol/hr/mg protein). The significance of difference between 14-day clonitrate-treated samples and controls were determined using Student's t test. A p < 0.05 considered significant. IC₅₀ data were calculated using Finney's probit analysis in an Apple IIe computer (Lieberman, 1983). Values were considered significantly different if there was no overlap of their 95% confidence limits calculated with 95% confidence. The IC₅₀ values determined with this procedure were identical to those obtained by least squares regression of the lines.

RESULTS

In vivo effects of clonitrate on microsomal carboxyesterase activities in Swiss-Webster mouse tissues. Treatment with 0.5% clonitrate for 14 days resulted in significant increases in hepatic carboxyesterase activities toward all substrates used (Table 1). Compared to control, the highest induction was observed with p-NPAc activity (117%) followed by malathion (53.7%), chloride (50.4%), DES (34.9%), and DEP (18.8%). In mice fed on clonitrate-treated diet for 14 days and then er control diet for another 5 days, it was interesting to find a dramatic decrease in hepatic carboxyesterase activities when compared with those where activity was measured after 14 days of treatment. The decreases were significant in the case of clonitrate, malathion, and DEP carboxyesterases.

The induction of microsomal carboxyesterase activities observed in kidney was significant on malathion and DES, and there was no marked effect on clonitrate, p-NPAc, and DEP activities. Except for malathion activity, this induction was weaker than that observed in liver but more selective in clonitrate, p-NPAc, and DEP activities were affected less with clonitrate treatment.

 Unlike liver and kidney, carboxyesterase activity on clonitrate and malathion in testis decreased significantly following treatment with clonitrate, while the decrease in DES and p-NPAc activities were not significant with this protocol. Under the assay condition used here, DEP activity in testis was not detected. Generally, mouse liver microsomal esterase showed the highest specific activity toward all substrates used followed by kidney.

In vivo effects of clonitrate on microsomal carboxyesterase activities in tissues from Sprague-Dawley rats. In rats treated with diet clonitrate a significant increase in malathion carboxyesterase activity was observed in the liver, while changes in clonitrate, DEP, p-NPAc, and DEP activities were not significant (Table 2). In kidney tissues, significant increases in carboxyesterase activities were observed with clonitrate, DEP, and p-NPAc as substrates. In testis, carboxyesterase activities toward clonitrate and malathion were significantly decreased as a result of clonitrate treatment.

Regarding specific activities of the enzymes the lowest, clonitrate, DEP, DES, and p-NPAc were hydrolyzed by rat liver microsomes at a much faster rate than by either kidney or testis microsomes. In the case of p-NPAc esterase, testis had the highest specific activity followed by kidney and liver microsomes. Except for p-NPAc, the rate of hydrolysis of the rest of the substrates was...
TABLE 2
MICROSOMAL CARBOXYLTRANSFERASE ACTIVITIES IN THREE TISSUES OF CONTROL AND CLOFIBRATE-FOED SPAGUS-DAWLEY RATS

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Clofibrate</th>
<th>Control</th>
<th>Clofibrate</th>
<th>Control</th>
<th>Clofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clofibrate</td>
<td>381 ± 16</td>
<td>400 ± 29</td>
<td>45.8 ± 1.8</td>
<td>52.7 ± 3.8*</td>
<td>92.4 ± 12.3</td>
<td>73.5 ± 12.6*</td>
</tr>
<tr>
<td>Malathion</td>
<td>304 ± 3</td>
<td>427 ± 56*</td>
<td>49.0 ± 8.6</td>
<td>52.9 ± 1.8</td>
<td>41.4 ± 1.9</td>
<td>37.9 ± 1.8*</td>
</tr>
<tr>
<td>DES</td>
<td>323 ± 44</td>
<td>368 ± 22</td>
<td>14.4 ± 2.6</td>
<td>16.2 ± 1.8</td>
<td>62.2 ± 5.7</td>
<td>53.8 ± 11.8</td>
</tr>
<tr>
<td>DEP</td>
<td>199 ± 7</td>
<td>198 ± 42</td>
<td>4.6 ± 0.1</td>
<td>6.23 ± 0.2*</td>
<td>19.2 ± 3.1</td>
<td>16.0 ± 7.3</td>
</tr>
<tr>
<td>p-Nitro</td>
<td>18.2 ± 1</td>
<td>18.4 ± 1.8</td>
<td>156 ± 52</td>
<td>287 ± 108*</td>
<td>1040 ± 210</td>
<td>860 ± 130</td>
</tr>
<tr>
<td>Testis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Substrates: Clofibrate, Malathion, DES, DEP, 4-Nitroacetanilide.

Values are the mean ± SD of four rats.

* Values in parentheses are ratios of treated to control.

** Significantly different from the respective control, p < 0.05.

was much higher by rat hepatic microsomes (Table 2) than by mouse hepatic microsomes (Table 1).

In vitro inhibition of clofibrate-metabolizing carboxyltransferase(s). I_{50} values presented in Table 3 clearly indicate the inhibitory potency of the tested substituted trifluoroacetones on the hydroxylation of the drug and peroxisome proliferator, clofibrate, by crude mouse and rat liver microsomes. Compared with nontreated mice, data indicate that, in the treated mice, compounds 1, 5, and 8 had significantly higher I_{50} values while compound 4 had a significantly lower value. Under these assay conditions, the compounds having a sulfide bond beta to the carbonyl (compounds 1–4) were less potent than those lacking the sulfide bond (compounds 5–8). On the basis of I_{50} and slope values, it is interesting to notice a species specificity toward the inhibitory potency of these compounds on the hepatic microsomal clofibrate-metabolizing carboxyltransferase(s) in normal mouse and rat. In mice, compound 7 was the most potent one followed by 5 and then 6, while in rats compound 5 was superior followed by 6 and then 8. On the other hand, the enzyme(s) were more sensitive to the clofibrate mimic (4) in rat than in mice, while the opposite was true for the rest of the inhibitors.

DISCUSSION

Clofibrate is a hypolipidemic drug which causes an increase in hepatic and renal peroxisomes and in the content of liver smooth endoplasmic reticulum as well. The induction of these organelles is accompanied by increases in peroxisome and microsome-associated enzymes (Hammond and Ota, 1983; Reddy and Lalwani, 1983; Bergs and Aarsland, 1985). Induction of smooth endoplasmic reticulum suggested that microsomal carboxytransferase activities may also be affected by peroxisome proliferators.

Five substrates were selected to study the response of microsomal carboxytransferase activities in liver, kidney, and testes to the peroxisome proliferator, clofibrate, in mouse and rat.
<table>
<thead>
<tr>
<th>Structure no.</th>
<th>Normal</th>
<th>Cholate-fed</th>
<th>Normal rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CH(CH₃)₂CH₂CH₂CP₂</td>
<td>2.5 × 10⁻⁷</td>
<td>3.7 × 10⁻⁷</td>
<td>1.9 × 10⁻⁷</td>
</tr>
<tr>
<td>(1.7)²</td>
<td>(2.0)</td>
<td>(0.8)</td>
<td></td>
</tr>
<tr>
<td>2. CH₂(CH₃)CH₂CH₂CP₂</td>
<td>3.8 × 10⁻⁸</td>
<td>5.6 × 10⁻⁸</td>
<td>1.6 × 10⁻⁷</td>
</tr>
<tr>
<td>(0.6)</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>3.5 × 10⁻⁸</td>
<td>2.0 × 10⁻⁷</td>
<td>8.8 × 10⁻⁷</td>
</tr>
<tr>
<td>(0.7)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td></td>
</tr>
<tr>
<td>4. Cl⁻</td>
<td>5.7 × 10⁻⁸</td>
<td>2.4 × 10⁻⁸</td>
<td>2.3 × 10⁻⁷</td>
</tr>
<tr>
<td>(1.2)</td>
<td>(0.6)</td>
<td>(0.4)</td>
<td></td>
</tr>
<tr>
<td>5. CH₂(CH₃)CH₂CP₂</td>
<td>4.7 × 10⁻⁹</td>
<td>1.0 × 10⁻⁸</td>
<td>1.1 × 10⁻⁷</td>
</tr>
<tr>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td></td>
</tr>
<tr>
<td>6. CH₂(CH₃)CP₂</td>
<td>1.4 × 10⁻⁹</td>
<td>3.1 × 10⁻⁹</td>
<td>9.6 × 10⁻¹⁰</td>
</tr>
<tr>
<td>(1.3)</td>
<td>(0.9)</td>
<td>(0.4)</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>3.0 × 10⁻¹⁰</td>
<td>3.9 × 10⁻¹⁰</td>
<td>3.7 × 10⁻¹⁰</td>
</tr>
<tr>
<td>(0.4)</td>
<td>(0.5)</td>
<td>(1.1)</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>9.6 × 10⁻¹⁰</td>
<td>1.1 × 10⁻⁹</td>
<td>6.2 × 10⁻¹⁰</td>
</tr>
<tr>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Is values were obtained from at least three separate determinations of two assays per treatment. The substrate concentration was 1 × 10⁻⁷ M, and protein concentrations were 50 and 12.5 mg/ml for Swiss-Webster mice and Sprague-Dawley rats, respectively.

#Values in parentheses are the slopes.

Malathion is an effective insecticide, and its selectivity, low mammalian toxicity, and propensity to the development of resistance are related to its metabolism by carboxylesterases (Kao *et al.* 1985). In addition, Talcott *et al.* (1982) suggested that hepato-cellular damage and malathion carboxylesterase solubilization are related, and they explored the use of
diethyl succinate as a substrate to assay serum ethyl esterases as a prospective liver function test. Phthalate esters are extensively used as industrial solvents and plasticizers in the manufacture of a wide variety of plastics, including food-packaging material and many biomedical devices. Phthalates with branched chain esters are known to be hypolipidemic peroxisome proliferators, with hydrolysis of at least one ester essential for this activity. Hydrolysis of phthalate esters has been compared in several tissues and species, and DEP hydrolysis was suggested to be representative of the phthalates which lead to peroxisome proliferation (Lake et al., 1976; Gollan et al., 1985). N-P-Nitrophenylacetate is a model ester rapidly metabolized by esterases from a variety of sources (Lungquist and Augustinus, 1971; Kao et al., 1985).

The response of xenobiotic-metabolizing carboxylesterases to peroxisome proliferators has been limited to a very recent study by Mentlen et al. (1986) who reported that the specific activities of acetaldehyde carboxylesterase and deacetyl-DL-carnitine hydrolyase increased more than three-fold in clofibrate-treated rat liver microsomes, while the specific clofibrate hydrolyase activity remained unchanged. Our results reveal that carboxylesterase activities, on the substrates used, in liver, kidney, and testis microsomes from both mouse and rat are significantly affected by clofibrate treatment; however, these effects are species, tissue, and substrate dependant. The induction is higher in mice than in rat. It is interesting that clofibrate-metabolizing carboxylesterase(s) is induced significantly in liver of mouse as well as in kidney of rat and not significantly in rat liver microsomes as a result of clofibrate treatment. Enhancement in carboxylesterase activities on the substrates used can be arranged in mouse as follows: P-NPAc > malathion > clofibrate > DES > DEP (liver), and malathion > DES > clofibrate > P-NPAc > DEP (kidney). In rat tissues, hepatic microsomal carboxylesterase activity on malathion shows the highest increase whereas in kidney the highest induction is obtained with the enzyme activities on P-NPAc followed by DEP. Hosokawa et al. (1984) have reported that administration of testosterone propionate to castrated male rats increases the activities of P-NPAc and malathion hydrolyses in liver microsomes. Hepatic and extrahepatic carboxylesterase activities on P-NPAc were studied after the exposure of rats to polycyclic aromatic hydrocarbons (Nousiainen et al., 1984). They have reported that the carcinogens benz[a]anthracene, benzo[k]pyrene, and 3-methylcholanthrene moderately induced the hepatic cytosolic and kidney microsomal carboxylesterase activities.

In our study enzyme activities are either decreased or not affected in microsomes from testes of treated mice and rats. Since the induction of the enzymes studied appears to be coupled with peroxisome proliferation in the liver and kidney, the lack of carboxylesterase induction in testis may reflect the absence of proliferation following clofibrate treatment in testis. This absence may, in turn, be associated with a lack of receptor (Laiwani et al., 1985).

The value of inhibitors in classifying and distinguishing among families of carboxylesterases well documented; however, such inhibitors have been limited to irreversible inhibitors such as organophosphates and carbamates (Hymann, 1982). Trifluoromethylketones are thought to be potent, reversible inhibitors of esterases due to their resemblance to a tetrahedral transition state or transient intermediate on the reaction coordinate to the acylated enzyme (Abdel-Aal and Hammock, 1986). In a previous study (Ashour and Hammock, 1987), it was reported that substituted trifluoroketones are potent and selective inhibitors of hepatic carboxylesterase(s) hydrolyzing malathion, DES, and P-NPAc and the compounds are useful for testing the toxicological role of carboxylesterases in xenobiotic metabolism. The use of multiple diagnostic substrates and inhibitors is likely to be important in many approaches designed to elucidate the toxicological significance of multiple forms of hepatic carboxylesterases. The use of a 96-well
plate reader interfaced with a microcomputer offers a cost-effective approach to the collection and reduction of the large data sets generated by the above approaches.

The present study shows that trifluoroketones are powerful inhibitors of hepatic microsomal carboxylesterase metabolizing clodbrate. On the other hand the significantly different I₅₀ may suggest the hypothesis that the changes in clofibrate metabolizing carboxylesterase activity following clofibrate treatment are not only qualitative but quantitative as well. These compounds also have species specificity as they are more potent inhibitors of the mouse hepatic esterases compared with rat; however, the potency and selectivity of these inhibitors depend on the chemical structure of the substituted moiety that is attached to the carbonyl group. Preliminary work using very low concentrations of these compounds indicated that the hydrolysis of clofibrate by mouse and rat hepatic microsomes was activated with some compounds whereas there was inhibition rather than activation with others. The differential effects of these compounds on esterases from control vs treated animals were more marked at low concentrations. These observations suggest that hepatic microsomes contain isozymes that differ in their sensitivity to inhibition by the compounds tested. These differences hopefully can be exploited to characterize the induction of toxicologically significant esterases and to purify them by affinity chromatography (Abdel-Aal and Hammock, 1986).

ACKNOWLEDGMENTS

This work was supported by NIH Grant ES027-01. We are grateful to Mrs. Shirley Gee for her technical assistance and Mrs. Pegge Kaplan for typing the manuscript. Bruce D. Hammock is a recipient of the Burroughs Wellcome Toxicology Award.

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terases, palmitoyl-CoA hydrolase and acetyl-aminooxy-
hydrolase in rat liver after treatment with clofibrate. 
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