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

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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 including malathion, clofibrate, diethylsuccinate, diethylphthalate, and *p*-nitrophenylacetate in liver and kidney of male Swiss–Webster mice and Sprague–Dawley rats. The induction was substrate, tissue, and species dependent. The carboxylesterase activity was induced in mouse from 1.2- to 2.2-fold (liver) and from 1.1- to 1.7-fold (kidney) depending upon substrate used. Analogous values from rat ranged from 1.0- to 1.4-fold (liver) and from 1.1- to 1.8-fold (kidney). Enzyme activities were either decreased or not affected in testes of treated mice and rats. Substituted trifluoroketones ("transition-state" inhibitors of carboxylesterase) were found to be very potent inhibitors of clofibrate-metabolizing carboxylesterase(s) 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; Hosokawa *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, hepatocellular 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 cytosolic epoxide hydrolase (Hammock and Ota, 1983). These responses suggest that microsomal 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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limited to the hydrolysis of acyl-CoA esters (Kawashima *et al,* 1983; Reddy and Lalwani, 1983; Berge and Aarsland, 1985).

Since hepatic carboxylesterases are instrumental in the metabolism and thus influence the efficacy 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 carboxylesterase activities on five substrates of toxicological and pharmacological interest, namely, clofibrate, malathion, diethylsuccinate, 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 carboxylesterase activity were qualitative as well as quantitative, a series of substituted trifluoroketones were tested as inhibitors of clofibrate-metabolizing carboxylesterase(s) in liver microsomes from mice and rats.

METHODS

Chemicals. Clofibrate, ethyl *p*-chlorophenoxyisobutyrate, was provided by Ayerst Laboratories (New York, NY). Malathion (99.2%) 0,0-dimethyl-*S*-1,2-bis(ethoxycarbonyl) ethyl phosphorodithioate, was provided by Dr. M. Mallipudi, American Cyanamid Corp. (Princeton, New Jersey). Diethylsuccinate (DES)[4] and *p*-nitrophenylacetate (*p*-NpAc) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Diethylphthalate (DEP), *p*-iodonitrophenyltetrazolium 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). 3-Nonylthio-1,1,1-trifluoropropan-2-one, 3-dodecylthio-1,1,1-trifluoropropane-2-one, 3-phenylthio-1,1,1-trifluoropropan-2-one, 3-*p*-chlorophenylthio-1,1,1-trifluoropropan-2-one, 1,1,1-trifluorooctan-2-one, 1,1,1-trifluorododecan-2-one, 3-phenyl-1,1,1-trifluoropropan-2-one, and 4-phenyl-1,1,1-trifluorobutan-2-one were available from previous syntheses (Abdel-Aal and Hammock, 1986; Ashour and Hammock, 1987).

[4] Abbreviations used: DES, diethylsuccinate; *p*-NpAc, *p*-nitrophenylacetate; DEP, diethylphthalate; INT, *p*-iodonitrophenyltetrazolium.

Animals. Male Sprague–Dawley rats (180–200 g, CD strain, Charles River, Wilmington, MA) and Swiss–Webster mice (25–30 g, Bantin–Kingman, Fremont, CA) were housed in steel cages with kiln-dried pine shavings as bedding in an environmentally controlled room (23 ± 1.5°C, 14:10 hr light:dark, constant humidity). Food (Purina Rodent Chow) and water were provided *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 amounts of corn oil added (5 ml/100 g). Experimental animals were given clofibrate-contaning 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.

Enzyme preparation. Animals were killed (mice by cervical dislocation, rats by exsanguination under light ether anesthesia) between 9:00 and 10:00 AM. Livers were removed, perfused with cold 1.15% KCl, rinsed, placed in sodium phosphate buffer (76 mM, pH 7.4), minced, and weighed. Kidneys and testes were removed, dissected free of adhering tissues, rinsed in phosphate buffer, minced, and weighed. Tissues were homogenized for 20 sec using a Polytron on setting 6. Homogenates of livers and kidneys were made to 10% (w/w) and those of testes to 5% (w/w) of the organ weight. They were centrifuged at 16,000*g* for 20 min, and the supernatant fractions were centrifuged at 105,000*g* for 60 min. The cytosol (105,000*g* 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 recentrifuged. Washed microsomes were resuspended in phosphate buffer (76 mM, pH 7.4) to give 10 or 5% 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 enzyme rate relative to protein concentration and which gave a linear increase in absorbance values with time. The spectrophotometric method of Talcott (1979) was used to assay carboxylesterase activities on clofibrate, malathion, diethylsuccinate, and diethylphthalate in the crude microsomal fraction of rat and mouse liver, kidney, and testis.

In brief, the hydrolysis of the four substrates was coupled 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 absorption at 500 nm with time) using an extinction coefficient of 13.8 mM^{-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 interfaced with an Apple IIe computer. The incubation mixture contained 0.5 ml of enzyme solution and 0.5 ml of reagent mixture in 0.1 M Tris–HCl buffer, pH 7.5. A reagent blank was used in the reference cuvette. The reac-

TABLE 1

MICROSOMAL CARBOXYLESTERASE ACTIVITIES IN THREE TISSUES OF CONTROL AND CLOFIBRATE-FED SWISS–WEBSTER MICE[a]

	Specific activity (nmol/min/mg protein)[b]						
	Liver			Kidney		Testis	
Substrate	Control	Clofibrate	Clofibrate withdrawn[c]	Control	Clofibrate	Control	Clofibrate
Clofibrate	113 ± 7.0	170 ± 16* (1.50)[d]	129 ± 16* (1.14)	40.7 ± 13.4	50.7 ± 11 (1.23)	30.8 ± 2.6	21.7 ± 4.6* (0.71)
Malathion	17.8 ± 2.1	27.0 ± 2.7* (1.52)	9.8 ± 2.0* (0.55)	7.6 ± 2.6	13.0 ± 2.8* (1.71)	6.1 ± 1.4	3.8 ± 1.2* (0.62)
DES	146 ± 6	197 ± 11* (1.35)	138 ± 16 (0.95)	89 ± 14	110 ± 11* (1.24)	71.8 ± 10.1	59.2 ± 10.6 (0.83)
DEP	32.4 ± 0.1	38.6 ± 2.1* (1.19)	29.6 ± 0.9 (0.91)	11.4 ± 0.6	11.1 ± 4.2 (0.97)	ND[e]	ND
p-NpAc	810 ± 64	1760 ± 460* (2.17)	736 ± 259 (0.91)	598 ± 110	680 ± 117 (1.14)	260 ± 65	188 ± 42 (0.72)

[a] Male mice were fed diets containing 0.5% (w/w) clofibrate in ground chow or ground chow only for 14 days. Tissues were prepared and assays were conducted as described under Methods.

[b] Values are the mean ± SD of four mice.

[c] Mice were fed 0.5% clofibrate-containing diet for 14 days and then control diet for 5 more days.

[d] Values in parentheses are ratios of treated to control.

[e] ND, not detectable.

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

tion was started by injecting 1.0 μl of an acetone solution of substrate into the cuvette and shaking. Thus, the final substrate concentrations were 3×10^{-4} M for malathion and 1×10^{-4} M for the other three substrates. These concentrations were sufficient to saturate the carboxylesterases assayed in this study.

p-Nitrophenylacetate esterase(s) activity was assayed spectrophotometrically according to the method of Ljungquist and Augustinsson (1971). The rate of *p*-NpAc hydrolysis was monitored at 37°C for 5 min on the Varian–Cary 219 spectrophotometer, and the liberation of *p*-nitrophenol was measured at 4000 nm. The sample cuvette contained enzyme solution in 1.0 ml of Tris–HCl buffer (0.1 M, pH 7.5) and 1.0 μl of an acetone solution of *p*-NpAc was injected into the cuvette yielding a 0.15 mM final substrate concentration. Except for the enzyme, the reference and sample cuvettes contained the same components.

Protein concentrations (μg/ml) were 50, 100, and 37.5 (clofibrate); 100, 25, and 37.5 (malathion); 25, 25, and 37.5 (DES); 50, 100, and 37.5 (DEP); and 2.5, 2.5, and 7.5 (*p*-NpAc) for mouse liver, kidney, and testis, respectively, while in rat tissues they were 0.5 (liver) and 5.0 (kidney and testis) for *p*-NpAc, and 25 (liver) and 50 (kidney and testis) for the rest of the substrates. For the purpose of this study the term "induction" is used to refer to measurable increase in enzyme activity following the *in vivo* treatment of animals with a chemical. It is not intended to indicate a molecular mechanism for this increase.

In vitro inhibition of clofibrate-metabolizing carboxylesterase(s). To evaluate the toxicological significance of the induction of a complex enzyme family such as the hepatic carboxylesterases, it is critical that enzyme activities be monitored in the presence and absence of diagnostic concentrations of inhibitors. Toward this end, the inhibitory potency of eight substituted trifluoroketones on hepatic microsomal carboxylesterase(s) hydrolyzing clofibrate in control and clofibrate-treated mice as well as control rats were tested *in vitro* using a 96-well plate reader (Titertek Multiskan, Flow Laboratories) interfaced to an IBM PC computer.

Inhibitor was added in 3 μl of acetone to wells containing 150 μl of enzyme solution in 0.1 M Tris–HCl buffer (pH 7.5) and mixed and incubated for 10 min in the reader's plate chamber (31 ± 0.5°C), and then 150 μl of reagent mixture (Talcott, 1979) in the same buffer was added followed by injection of 3 μl of 1×10^{-2} M clofibrate in acetone. The absorbance of the 96-well plate could be determined in approximately 1 min and readings were taken repeatedly at 90-sec intervals for 20 min at 492 nm. Reagent blanks and acetone controls were done. Protein concentrations were 50 and 12.5 μg/ml for mouse and rat, respectively.

A series of inhibitor concentrations were tested and at least three replicates of each concentration were used. A

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 I_{50} values from semilog plots. Only those points on the linear portion of the curve were used to calculate the I_{50} and slope values. When I_{50}s were determined on selected compounds using both Titertek and Varian–Cary 219 spectrophotometers, no significant differences were noted in the resulting data, whereas we took advantage of the smaller sample size and greater linearity, reproducibility, and sample throughput of the Titertek system.

Data were collected using a software program known as PC-EIA (Dorian software) and then translated by 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 rate 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 Bradford (1976) as modified 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× diluted Bio-Rad reagent (20% in distilled water), and the mixture was incubated for 10 min at room temperature. Absorbance at 620 nm was then measured using a Gilford EIA manual ELISA reader interfaced with an Atari 400 microcomputer (programmed by R. Wixtrom, this laboratory).

Calculations and statistics. The enzyme activities were routinely calculated as specific activity (nmol/min/mg protein). The significance of differences between 14-day clofibrate-treated samples and controls were determined using Student's t test, with $p < 0.05$ considered significant. I_{50} data were calculated using Finney's probit analysis on an Apple IIe computer (Lieberman, 1983). Values were considered significantly different if there were no overlap of their fiducial limits calculated with 95% confidence. The I_{50} values determined with this procedure were identical to those obtained by least-squares regression of the lines.

RESULTS

In vivo effect of clofibrate on microsomal carboxylesterase activities in Swiss–Webster mouse tissues. Treatment with 0.5% clofibrate for 14 days resulted in significant increases in hepatic carboxylesterase activities toward all substrates used (Table 1). Compared to control, the highest induction was observed with *p*-NpAc activity (117%) followed by malathion (51.7%), clofibrate (50.4%), DES (34.9%), and DEP (18.8%). In mice fed on clofibrate-treated diet for 14 days and then on control diet for another 5 days, it was interesting to find a dramatic decrease in hepatic carboxylesterase activities when compared with those where activity was measured after 14 days of treatment. The decreases were significant in the case of clofibrate, malathion, and DEP carboxylesterases.

The induction of microsomal carboxylesterase activities observed in kidney was significant on malathion and DES. There was no marked effect on clofibrate, *p*-NpAc, and DEP activities. Except for malathion activity, this induction was weaker than that observed in liver but more selective since clofibrate, *p*-NpAc, and DEP activities were affected less with clofibrate treatment.

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

In vivo effect of clofibrate on microsomal carboxylesterase activities in tissues from Sprague–Dawley rats. In rats treated with dietary clofibrate a significant increase in malathion carboxylesterase(s) activity was observed in the liver, while changes in clofibrate, DES, *p*-NpAc, and DEP activities were not significant (Table 2). In kidney tissues, significant increases in carboxylesterase activities were obtained with clofibrate, DEP, and *p*-NpAc as substrates. In testis, carboxylesterase activities toward clofibrate and malathion were significantly decreased as a result of clofibrate treatment.

Regarding specific activities of the enzymes studied in the control tissues, clofibrate, DES, malathion, and DEP 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(s), 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

TABLE 2

MICROSOMAL CARBOXYLESTERASE ACTIVITIES IN THREE TISSUES OF CONTROL AND CLOFIBRATE-FED SPRAGUE–DAWLEY RATS[a]

	Specific activity (nmol/min/mg protein)[b]					
	Liver		Kidney		Testis	
Substrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Clofibrate	381 ± 16	400 ± 29 (1.05)[c]	45.8 ± 1.8	52.7 ± 3.8* (1.15)	92.4 ± 12.3	73.5 ± 12.6* (0.80)
Malathion	304 ± 31	427 ± 36* (1.41)	49.0 ± 8.6	52.9 ± 1.8 (1.08)	41.4 ± 1.9	37.9 ± 1.8* (0.92)
DES	323 ± 44	368 ± 22 (1.14)	14.4 ± 2.6	16.2 ± 1.8 (1.13)	62.2 ± 5.7	53.8 ± 11.8 (0.87)
DEP	199 ± 7	199 ± 42 (1.0)	4.6 ± 0.1	6.23 ± 0.2* (1.35)	19.2 ± 3.1	16.0 ± 7.3 (0.83)
p-NpAc	18.2 ± 1.1	19.4 ± 1.8 (1.07)	156 ± 52	287 ± 108* (1.84)	1040 ± 210	860 ± 330 (0.83)

[a] Male rats were fed diets containing 0.5% (w/w) clofibrate in ground chow or ground chow only for 14 days. Tissues were prepared and assays were conducted as described under Methods.

[b] Values are the mean ± SD of four rats.

[c] 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 carboxylesterase(s). I_{50} values presented in Table 3 clearly indicate the inhibitory potency of the tested substituted trifluoroketones on the hydrolysis 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 carboxylesterase(s) in normal mouse and rat. In mouse, compound 7 was the most potent one followed by 5 and then 6, while in rat 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 mouse, while the opposite was true for the rest of the inhibitors.

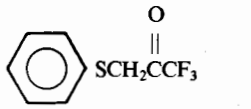
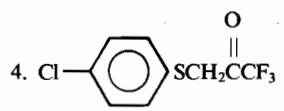
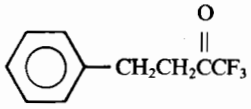
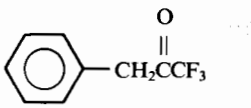
DISCUSSION

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 (Hammock and Ota, 1983; Reddy and Lalwani, 1983; Berge and Aarsland, 1985). Induction of smooth endoplasmic reticulum suggested that microsomal carboxylesterase activities may also be affected by peroxisome proliferators.

Five substrates were selected to study the response of microsomal carboxylesterases in liver, kidney, and testes to the peroxisome proliferator, clofibrate, in mouse and rat.

TABLE 3

THE INHIBITORY POTENCY OF SUBSTITUTED TRIFLUOROKETONES ON THE HYDROLYSIS OF CLOFIBRATE BY MOUSE AND RAT LIVER MICROSOMES

Structure no.	I_{50}[a] (M)		
	Mouse		
	Normal	Clofibrate-fed[b]	Normal rat
1. $CH_3(CH_2)_9SCH_2C(=O)CF_3$	2.5×10^{-7} (1.7)[d]	3.7×10^{-7} [c,*] (2.0)	1.9×10^{-7} (0.8)
2. $CH_3(CH_2)_8SCH_2C(=O)CF_3$	3.8×10^{-8} (0.6)	5.6×10^{-8} (0.4)	1.6×10^{-7} [c,*] (0.5)
3. 	3.5×10^{-7} (0.7)	2.0×10^{-7} (0.3)	8.8×10^{-7} (0.4)
4. 	5.7×10^{-7} (1.2)	2.4×10^{-7}* (0.6)	2.3×10^{-7}* (0.6)
5. $CH_3(CH_2)_9C(=O)CF_3$	4.7×10^{-10} (0.6)	1.0×10^{-9}* (0.5)	1.1×0^{-9}* (0.6)
6. $CH_3(CH_2)_5C(=O)CF_3$	1.4×10^{-9} (1.3)	3.1×10^{-9}* (0.9)	9.6×10^{-9}* (0.4)
7. 	3.0×10^{-10} (0.4)	3.9×10^{-10} (0.5)	8.7×10^{-8}* (1.1)
8. 	9.6×10^{-9} (0.4)	1.1×10^{-8}* (0.5)	6.2×10^{-8}* (0.8)

[a] I_{50} values were obtained from at least three separate determinations of two assays per treatment. The substrate concentration was 1×10^{-4} M, and protein concentrations were 50 and 12.5 mg/ml for Swiss–Webster mice and Sprague–Dawley rats, respectively.

[b] Male mice were fed 0.5% (w/w) clofibrate in ground chow. Control mice and rats were fed ground chow only. Tissues were prepared and assays were conducted as described under Methods.

[c] I_{50} values which are significantly different from those obtained for untreated mice are indicated by *. Significance is assumed if there is no overlap of fiducial limits at the I_{50} as calculated by the method of Lieberman (1983) with 95% confidence. As an example, in compound 4 95% CI were 7.4×10^{-7}–4.4×10^{-7}, 4.1–1.5×10^{-7}, and 4×10^{-7}–1.7×10^{-7} M for normal mouse, treated mouse, and normal rat, respectively.

[d] 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 hepatocellular damage and malathion carboxylesterase solubilization are related, and they explored the use of

diethylsuccinate 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-packing 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; Gollamudi *et al.*, 1985). *p*-Nitrophenylacetate is a model ester rapidly metabolized by esterases from a variety of sources (Ljungquist and Augustinsson, 1971; Kao *et al.*, 1985).

The response of xenobiotic-metabolizing carboxylesterases to peroxisome proliferators has been limited to a very recent study by Mentlein *et al.* (1986) who reported that the specific activities of acetanilide carboxylesterase and decanoyl-DL-carnitine hydrolase increased more than three-fold in clofibrate-treated rat liver microsomes, while the specific clofibrate hydrolase 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 dependent. The induction is higher in mouse 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 inductions are 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 hydrolases 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 benzo[*a*]anthracene, benzo[*a*]pyrene, and 3-methylcholanthracene 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 (Lalwani *et al.*, 1983).

The value of inhibitors in classifying and distinguishing among families of carboxylesterases is well documented; however, such inhibitors have been limited to irreversible inhibitors such as organophosphates and carbamates (Heymann, 1982). Trifluoromethylketones are thought to be potent, reversible inhibitors of esterases due to their resemblance to a teterahedral 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 that 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 clofibrate. On the other hand the significantly different I_{50}s may support 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 enzyme(s) 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 toward 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).

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