Pergamon

Biochemical Pharmacology, Vol. 47, No. 3, pp. 515–519, 1994.

0006-2952/94 $6.00 + 0.00

CETABEN AND FIBRATES BOTH INFLUENCE THE ACTIVITIES OF PEROXISOMAL ENZYMES IN DIFFERENT WAYS

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(*Received* 9 *April* 1993; *accepted* 28 *September* 1993)

Abstract—The effects of cetaben and clofibric acid were compared on the activities of peroxisomal enzymes in the liver and kidney of male Wistar rats. Cetaben at 200 mg/kg body wt increased the activities of all of the enzymes in the liver that were studied two to eight times, whereas the changes induced by the same dose of clofibric acid increased some of the enzymes and decreased others. In the kidney, cetaben increased the activities of all investigated peroxisomal enzymes, while clofibric acid only increased the activity of palmitoyl-CoA oxidase. The data obtained in the dose–response study of cetaben revealed a significant rise in the activities of peroxisomal enzymes in both the liver and kidney at doses of 50–100 mg/kg body wt administered over 10 days, but the maximal effect was observed at 250 mg/kg. Palmitoyl-CoA oxidase and D-amino acid oxidase respond most markedly to cetaben. Cetaben could represent an atypical peroxisomal proliferator, since it increased the activities of all peroxisomal enzymes investigated. The fact that the individual components localized in the peroxisomes do not change markedly could be of importance with respect to the function and physical properties of peroxisomes.

In a number of papers published during the last few years it has been established that a broad range of compounds differing in chemical structure, including certain hypolipidemic drugs, induce peroxisomal proliferation in the liver of rodents [1–3]. Although various biochemical changes were induced by peroxisomal proliferators, those for the alternative route of β-oxidation of fatty acids seem to be the most important [4–6]. While searching for potential antiatherosclerotic and hypolipidemic agents it was found that this effect was also typical for certain alkylaminobenzoic acids. From the point of view of potential ability to reduce the concentration of cholesterol and triglycerides in the serum of experimental animals effectively, cetaben (sodium *p*-hexadecyaminobenzoate) was selected as the most suitable substance of this class [7]. The lipid lowering effect of cetaben, accompanied by peroxisomal proliferation and increased catalase activity, has been described already [8], but there is still a lack of detailed data which would clarify the biochemical correlates of the proliferative effect of cetaben. The main aim of our study was therefore to supplement the missing data as well as to characterize cetaben as a peroxisomal proliferator.

MATERIALS AND METHODS

Sodium cetaben was synthesized by the Slovak Drug Research Institute (Modra, Slovakia). Clofibric acid was obtained from the Sigma Chemical Co. (Poole, U.K.). All the other substances used in biological and analytical procedures were reagent grade purity obtained from Sigma and Serva (Heidelberg, Germany). Male Wistar rats from the Velaz farm (Prague) were used. The animals were fed *ad lib.* with commercial feed and had free access to drinking water. Each experimental group, either in the study aimed at the comparison of effects of cetaben and clofibric acid, or in the dose–response study of cetaben, consisted of six to seven randomly distributed animals, weighing initially 230–270 g. Cetaben was prepared in suspension and clofibric acid as a neutralized solution. Identical doses of cetaben and clofibric acid (200 mg/kg body wt) were used in the comparative study based both on the data of Fort *et al.* [8] and the already established fact that such doses of fibrates were sufficient, for the induction of peroxisomal proliferation. These drug doses were administered to the animals by gavage for either 3 or 10 successive days. In the next part of the experimental study the animals were given cetaben by gavage for 10 successive days in amounts of 10, 25, 50, 100, 250 and 500 mg/kg body wt. After the experimental period the animals were killed by cervical dislocation and liver and kidney homogenates were prepared in 0.25 mol/L of sucrose, containing Na-EDTA in a final concentration of 1 mmol/L.

Biochemical analyses. Catalase activity was determined according to Sinha [9]. D-Amino acid oxidase was assayed spectrophotometrically [10] using D-alanine as a substrate. Urate oxidase was assayed by a kinetic method [11]. Palmitoyl-CoA oxidase was estimated according to Walusimbi-Kisitu and Harrison [12] using a fluorimetric assay.

For detection of glycolate oxidase activity the reaction product-glyoxylate was determined by coupling with 2,4-dinitrophenylhydrazine. Kidney L-

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Table 1. Effects of cetaben and clofibric acid administered for 3 days on the activities of peroxisomal enzymes in rat liver

Parameter	Control (N = 7)	Cetaben (N = 7)	Clofibric acid (N = 7)
Catalase	3.13 ± 0.09	4.45 ± 0.05*	4.07 ± 0.02*
Palmitoyl-CoA oxidase	1.46 ± 0.16	3.31 ± 0.28	20.36 ± 1.41*
D-Amino acid oxidase	9.72 ± 0.44	13.47 ± 0.97*	4.31 ± 0.47*
Glycolate oxidase	30.95 ± 0.59	45.03 ± 1.47*	16.67 ± 1.39*
Urate oxidase	22.50 ± 1.22	31.61 ± 1.35*	20.11 ± 1.21

Cetaben and clofibric acid were given at 200 mg/kg body wt. The activities of all enzymes are given in nkat/g tissue with the exception of catalase which is expressed in mkat/g tissue.

Values are means ± SEM.

* Significantly different from control ($P < 0.01$).

Table 2. Effects of cetaben and clofibric acid administered for 10 days on the activities of peroxisomal enzymes in rat livers

Parameter	Control (N = 7)	Cetaben (N = 7)	Clofibric acid (N = 7)
Catalase	3.00 ± 0.08	4.29 ± 0.15*	4.78 ± 0.22*
Palmitoyl-CoA oxidase	1.45 ± 0.15	11.26 ± 0.60*	24.35 ± 0.43*
D-Amino acid oxidase	13.29 ± 1.31	29.21 ± 3.94*	4.32 ± 0.51*
Glycolate oxidase	32.90 ± 0.64	59.95 ± 1.86*	16.98 ± 1.61*
Urate oxidase	24.24 ± 0.67	48.43 ± 1.43*	19.48 ± 0.44*

Cetaben and clofibric acid were given at 200 mg/kg body wt. The activities of all enzymes are given in nkat/g tissue with the exception of catalase which is expressed in mkat/g tissue.

Values are means ± SEM.

* Significantly different from control ($P < 0.01$).

hydroxyacid oxidase was determined using the same method but L-α-hydroxyisocapronic acid was used as the substrate. Proteins were determined according to Lowry *et al.* [13]. Statistical analysis was performed using the unpaired test included in the Statgrafic program.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the data on the influence of cetaben and clofibric acid upon the activities of enzymes located in peroxisomes. The results show that cetaben increases the activities of all peroxisomal enzymes 2–8-fold, with the exception of catalase. The above results also indicate that a prolonged time was necessary to obtain clear-cut biochemical changes. In accordance with known data, clofibric acid similar to other fibrates induced contradictory changes in the activities of peroxisomal enzymes. While palmitoyl-CoA oxidase increased several-fold and an increase in catalase activity was also observed. D-amino acid oxidase, glycolate oxidase and urate oxidase were markedly decreased. These changes were already well developed after a short period of drug application. Substantial differences in the effects of both studied compounds were also observed with respect to kidney enzyme parameters (Table 3). Cetaben increased markedly the activities of all peroxisomal enzymes. However, changes induced by clofibric acid were characterized by increased activities of palmitoyl-CoA oxidase only, while activities of other peroxisomal enzymes only changed insignificantly. The effectiveness of different doses of cetaben in increasing the activities of peroxisomal enzymes in the liver and kidney was determined over a 10-day period to allow for significant expression of changes. The results shown in Figs 1 and 2 are in accordance with the data presented previously in this study. Significant increases in the activities of peroxisomal enzymes in both the liver and kidney were observed in animals receiving 25–100 mg of cetaben/kg body wt. The dose needed to achieve the maximal inductive effect of cetaben upon peroxisomal enzymes was 250 mg/kg body wt. The changes are expressed better in liver tissue, and palmitoyl-CoA oxidase and D-amino acid oxidase are the most responsive enzymes for cetaben.

A common characteristic in the effects of industrial plasticizers, chlorinated alkanes and acids as well as several hypolipidemics, mainly fibrates, is their influence upon the induction of peroxisomal enzymes. Numerous papers show that the activities of three enzymes of peroxisomal β-oxidation increased many-fold [14, 15] the activity of catalase rose only

Table 3. Effects of cetaben and clofibric acid administered for 10 days on the activities of peroxisomal enzymes in rat kidney

Parameter	Control (N = 7)	Cetaben (N = 7)	Clofibric acid (N = 7)
Catalase	1.04 ± 0.13	2.07 ± 0.09†	1.15 ± 0.06
Palmitoyl-CoA oxidase	0.29 ± 0.02	0.59 ± 0.06†	0.48 ± 0.07*
D-Amino acid oxidase	145 ± 12.3	288 ± 3.3	151 ± 12.8
L-Hydroxyacid oxidase	29.3 ± 2.2	52.5 ± 3.3+	27.0 ± 1.22

Cetaben and clofibric acid were given at 200 mg/kg body wt. The activities of all enzymes are given in nkat/g tissue with the exception of catalase which is expressed in mkat/g tissue.

Values are means ± SEM.

* Significantly different from control ($P < 0.05$).

† Significantly different from control ($P < 0.01$).

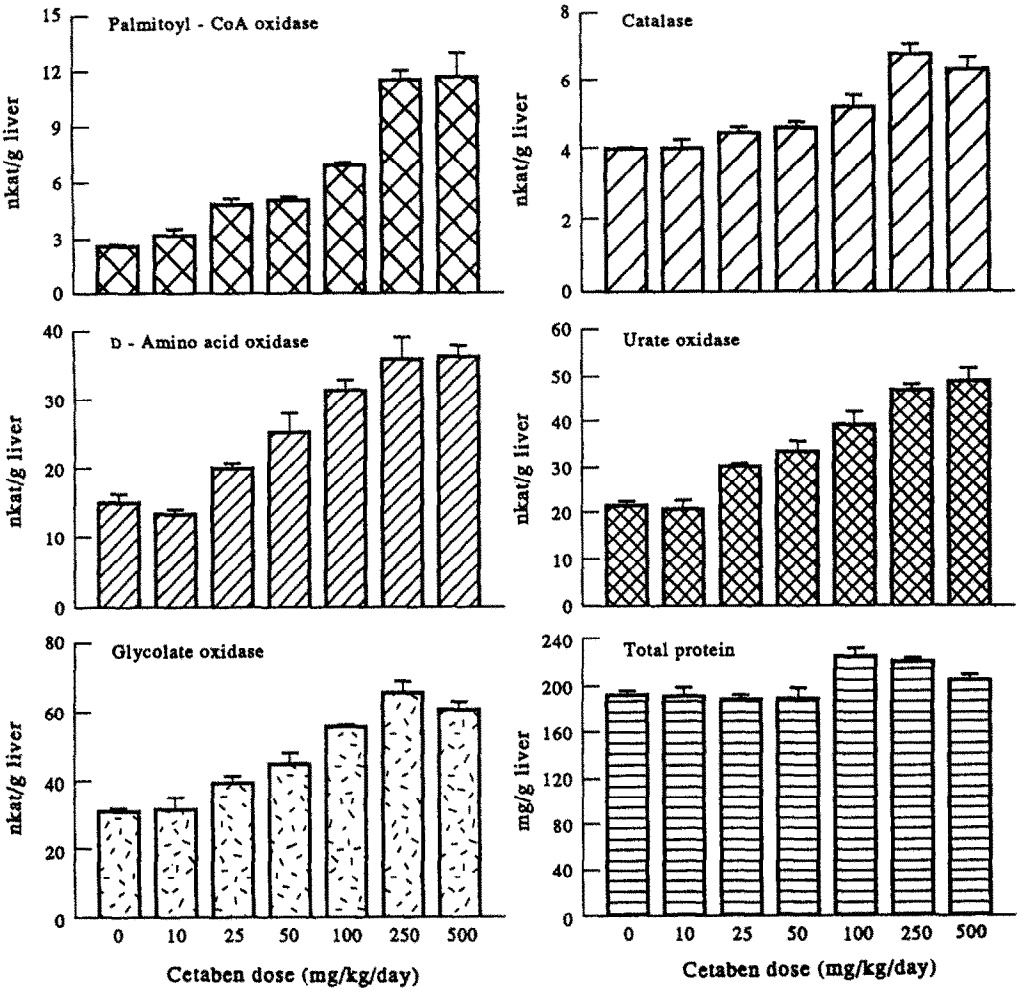

Fig. 1. Influence of cetaben on activities of peroxisomal enzymes in rat liver. At a cetaben dose of 50 mg/kg/day, all enzyme activities were statistically significantly different from control ($P < 0.05$). All data are presented as means ± SEM. In each experimental group there were six animals.

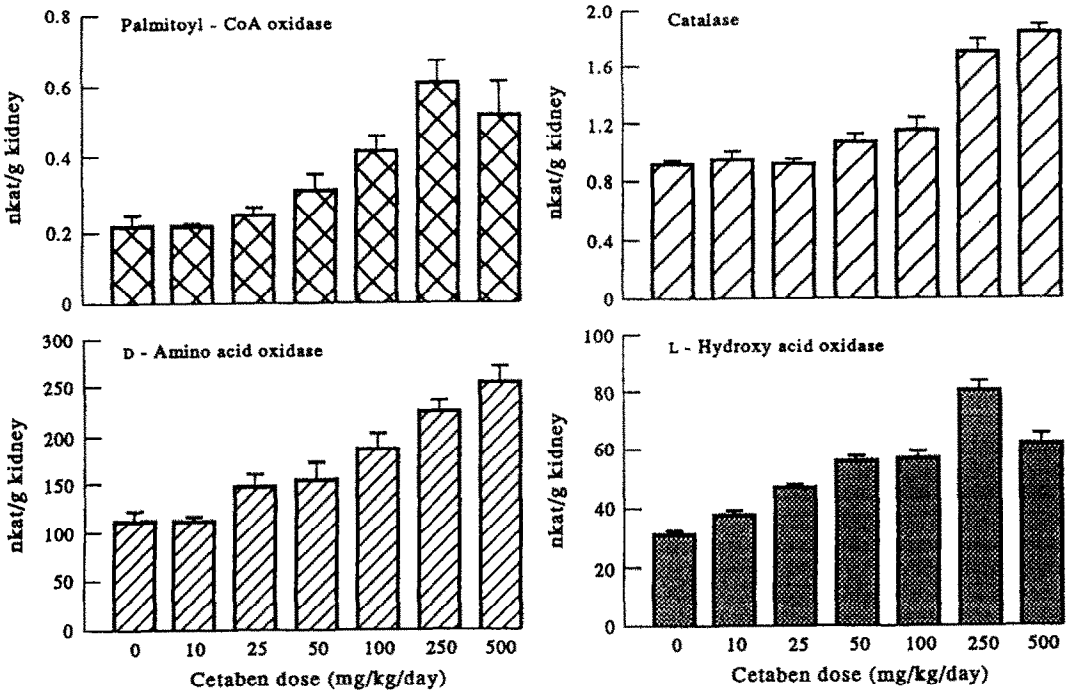

Fig. 2. Influence of cetaben on activities of peroxisomal enzymes in rat kidney. At the cetaben dose of 100 mg/kg/day all enzyme activities were statistically significantly different from control ($P < 0.05$). All data are presented as means ± SEM. In each experimental group there were six animals.

approximately 2-fold, while the activities of glycolate oxidase, D-amino acid oxidase and urate oxidase decreased substantially [16–18]. However, cetaben increases dose dependently the activities of all peroxisomal enzymes concerned in this study, not only in the liver but also in the kidneys. From our results we conclude that cetaben represents an atypical peroxisomal proliferator since it does not substantially change the proportionality of individual components located in the peroxisomes, a property which is characteristic of the majority of peroxisomal proliferators. This fact could be of primary importance with respect to the function and physical properties of peroxisomes.

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