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INDUCTION OF PEROXISOMAL ENZYMES AND PALMITOYL-CoA HYDROLASE IN RATS TREATED WITH CHOLESTYRAMINE AND NICOTINIC ACID

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Abstract—Male Wistar rats were given 200 mg/kg/day nicotinic acid or 1000 mg/kg/day cholestyramine by stomach tube for ten days. Peroxisomal palmitoyl-CoA oxidation (cyanide-insensitive) and the activities of palmitoyl-CoA hydrolase and urate oxidase were significantly increased in the total liver homogenate. Subcellular fractionation showed enhanced enzyme activities after drug treatment mainly in the peroxisome-containing fractions. The increase in urate oxidase activity and its subcellular distribution suggest that the tested drugs induce core-containing peroxisomes. The findings are similar to those previously reported with low doses of peroxisome-proliferating hypolipidemic drugs and with acetylsalicylic acid, a drug which is structurally similar to nicotinic acid. Since cholestyramine is not absorbed, its influence on hepatic enzymes probably occurs indirectly as a consequence of enhanced catabolism of cholesterol.

Proliferation of peroxisomes in the hepatocytes occurs in a number of animal species after treatment with clofibrate or other hypolipidemic drugs with similar pharmacodynamic properties [1, 2]. However, enzymic changes suggesting a peroxisomal reaction have also been demonstrated in rats given thyroid hormone (T_3) or high-fat diets suggesting that these organelles may play a physiological role in the regulation of lipid metabolism [3, 4].

Nicotinic acid is a lipid-lowering agent which has not been reported to induce peroxisomal proliferation and is thought to act mainly by inhibiting peripheral lipolysis [5]. The activity of the marker enzyme catalase is not significantly changed in rats treated with this drug [6], but a trend towards higher activities of some lipid-metabolizing enzymes was observed in a previously published study from our laboratory [7].

Cholestyramine, an anionic resin which is not absorbed, but traps bile acids and other acidic sterols in the intestinal lumen, reduces blood levels of cholesterol and low density lipoproteins (LDL) by reducing the negative feedback on cholesterol 7 alfa-hydroxylase [8, 9]. Since this is the rate-limiting enzyme in the catabolism of cholesterol, the clearance of LDL increases after cholestyramine administration [10].

The present study was carried out to test the hypothesis that lipid-lowering drugs not thought to act directly on the liver (cholestyramine), or considered to have at least partly a peripheral mechanism of action (nicotinic acid), are capable of producing secondary changes in peroxisomal enzyme systems. We chose to measure the enzyme activities (urate oxidase, catalase and cyanide-insensitive palmitoyl-CoA oxidation) which were reliable indicators of peroxisomal proliferation in our previous studies with clofibrate and tiadenol, and palmitoyl-CoA-hydrolase which is grossly induced by these drugs [7, 11–13].

MATERIALS AND METHODS

Drug administration. Male Wistar rats weighing 215–240 g were randomly selected for nicotinic acid or cholestyramine treatment and for control experiments. The rats were fed commercial pelleted ratfood [11] and kept under observation, handled and trained for 3–4 days prior to the study. The drugs were suspended (cholestyramine) or dissolved (nicotinic acid) in 0.5% w/v carboxymethylcellulose (CMS) and administered by stomach tube twice daily for 10 days. Groups of seven rats were given either nicotinic acid (200 mg/kg/day) or cholestyramine (1000 mg/kg/day) and control animals were given the vehicle (0.5% CMS) only. The animals were weighed and killed by decapitation 18–20 hr after the last dose.

Subcellular fractionation. The livers were removed, weighed and homogenized in ice-cold sucrose solution (0.25 M sucrose and 5 mM Hepes buffer, pH 7.4) in a Potter–Elvehjem-type homogenizer at 720 rpm and with two strokes of a loose-fitting Teflon pestle. The liver homogenate was fractionated into nuclear (N), heavy mitochondrial (M), light mitochondrial (L), microsomal (P) and supernatant (S) fractions according to the method of De Duve *et al.* [14] with a few modifications [12].

Enzyme assays and other analytical methods. The palmitoyl-CoA hydrolysis was measured radiochemically as described previously [15]. The incubation medium contained 15 mM Hepes buffer, pH 7.5, 150 mM KCl, 2 mM EDTA, 0.01% Brij-58 and 100 μM palmitoyl-CoA, and the protein content was 30 μg. Cyanide-insensitive palmitoyl-CoA oxidizing activity [16], catalase [17] and urate oxidase [18],

were assayed according to published procedures. Protein was determined using the Bio–Rad protein assay kit (Bio–Rad Lab., U.S.A.) calibrated with freeze-dried bovine gamma globulin.

The Wilcoxon rank sum test was used to determine the significance of the differences between group means, treatment vs control.

Chemicals. [1-^{14}C] palmitoyl-CoA was purchased from New England Nuclear, Boston, MA, U.S.A. Unlabelled palmitoyl-CoA was obtained from Sigma Chemical Co., MO, U.S.A. All chemicals were of the highest purity commercially available. Nicotinic acid and cholestyramine were gifts from NAF-laboratoriene A/S (Oslo, Norway) and Novo Industri A/S (Copenhagen, Denmark), respectively.

RESULTS

Liver weight and protein content. Nicotinic acid and cholestyramine had no effect on liver weight or the relative liver size (Table 1). The total protein content was not appreciably affected by nicotinic acid treatment while cholestyramine administration caused a statistically significant increase in protein content per gram of liver.

Enzyme activities in liver homogenate. Table 2 shows the effects of nicotinic acid and cholestyramine on peroxisomal enzymes and palmitoyl-CoA hydrolase in rat liver homogenates. The specific activity and total activity of catalase was slightly elevated after nicotinic acid, but the difference compared to the controls did not reach statistical significance. Cholestyramine treatment increased the total catalase activity moderately (1.5-fold), but the specific activity was not appreciably changed. Interestingly, the activity of urate oxidase increased 2-fold after administration of nicotinic acid and also significantly after cholestyramine. The cyanide-insensitive palmitoyl-CoA oxidizing activity increased slightly after cholestyramine or nicotinic acid treatment. Nicotinic acid and cholestyramine also caused a small, but statistically significant increase of the activities of palmitoyl-CoA hydrolase.

Subcellular distribution. The subcellular distribution pattern of protein and some marker enzymes of rat liver homogenates was essentially as described previously [12, 15]. The recovery of protein and enzyme activities was in the range of 89–106%. The distribution of the marker enzymes for mitochondria, lysosomes and microsomes was similar in all groups of animals (data not shown).

As expected, the highest specific activity, as well as percentage recovery of catalase, urate oxidase and cyanide-insensitive palmitoyl-CoA oxidation, all

Table 1. The effect of nicotinic acid and cholestyramine treatment on liver weight and protein content in total homogenate (mean ± S.D.)

	Control	Nicotinic acid	Cholestyramine
Dose (mg/kg/day)		200	1000
Body weight increase (g)	36 ± 4	36 ± 3	41 ± 10
Liver weight (g)	12.4 ± 1.1	11.7 ± 1.9	12.0 ± 2.1
(g/100 g b.w.)	4.7 ± 0.3	4.5 ± 0.5	4.3 ± 0.5
Liver protein (g total)	1.77 ± 0.24	1.91 ± 0.25	2.09 ± 0.29
(mg/g liver)	143 ± 18	164 ± 7	176 ± 11*

* $P < 0.01$.

Table 2. The effect of nicotinic acid and cholestyramine on enzyme activities (mean ± S.D.) in rat liver homogenate

Enzyme activities	Controls (N = 7)	Nicotinic acid (N = 7)	Cholestyramine (N = 7)
Catalase			
nmol/min/mg protein	363 ± 33	421 ± 115	388 ± 91
μmol/min/g liver	37 ± 7	46 ± 14	54 ± 10†
Urate oxidase			
nmol/min/mg protein	4.1 ± 0.6	8.3 ± 1.3†	5.3 ± 0.8*
nmol/min/g liver	442 ± 97	917 ± 180†	756 ± 162†
Peroxisomal β-oxidation			
nmol/min/mg protein	1.5 ± 0.1	2.0 ± 0.3†	2.0 ± 0.4
nmol/min/g liver	152 ± 23	196 ± 49	264 ± 84†
Palmitoyl-CoA hydrolase			
nmol/min/mg protein	62 ± 9	80 ± 6†	74 ± 6*
μmol/min/g liver	6.1 ± 0.8	8.8 ± 1.0†	8.0 ± 1.5*

* $P < 0.05$.

† $P < 0.01$ compared to control group.

Table 3. The effect of nicotinic acid and cholestyramine on the subcellular distribution of enzyme activities*

Subcellular fractions	Palmitoyl-CoA hydrolase			Palmitoyl-CoA oxidation			Catalase			Urate oxidase		
	C	Nic	Chol	C	Nic	Chol	C	Nic	Chol	C	Nic	Chol
M-fraction	52	66	49									
L-fraction	56	60	58	3.2	4.6	4.6	724	775	932	38	70	74
P-fraction	123	137	127	0.1	0.5	0.4	49	81	90	3.9	6.6	4.0
S-fraction	33	31	32	0.1	0.2	0.1	267	303	384	No activity		

* Aliquots of the cytoplasmic extract from seven rats per group were pooled and fractionated as described in the text. The activities of the enzymes are expressed as nmol/min/mg protein.

C, control group; Nic, nicotinic acid-treated rats; Chol, cholestyramine-treated rats.

enzymes known to be localized in peroxisomes, were found in the L-fraction (Table 3). The specific activity of urate oxidase in the L-fraction was almost doubled and after cholestyramine or nicotinic acid. No urate oxidase activity was found in the S-fraction. The specific activity of catalase was markedly enhanced both in the L-, P- and S-fractions in the cholestyramine-treated rats. After nicotinic acid treatment the catalase activity in these fractions was also appreciably increased.

The specific activity of the peroxisomal palmitoyl-CoA oxidation after cholestyramine and nicotinic acid increased not only in the L-fraction, but also in the P-fraction. The specific activity of palmitoyl-CoA hydrolase was enhanced in the L- and P-fractions, and especially in the M-fraction, after treatment with nicotinic acid.

DISCUSSION

The enzymic changes observed with nicotinic acid and cholestyramine in the present study were much less pronounced than previously reported with moderate or high doses of clofibrate, tiadenol, and other members of this class of peroxisome proliferating hypolipidemic drugs [1, 2, 7, 11–13]. Increased activities of catalase, cyanide-insensitive palmitoyl-CoA oxidation and palmitoyl-CoA hydrolase and reduced urate oxidase activity are characteristic effects of these agents.

Earlier studies, which have failed to show consistent hepatic changes following treatment with nicotinic acid, either comprised small numbers of animals [7] or only determination of catalase as marker activity for the peroxisomes [6]. Moreover, administration of the drug admixed to the diet is less effective than by stomach tube [19], which is the method used in the present study.

The activities of cyanide-insensitive palmitoyl-CoA oxidation and catalase were increased in the peroxisome-containing L- and P-fractions. Interestingly, after the administration of nicotinic acid, urate oxidase also increased in the total homogenate, and in the peroxisome-containing L- and P-fractions. The increase in urate oxidase activity was less marked in the cholestyramine-treated animals. This activity is located in the dense core of the mature peroxisomes [20]. Although the morphology was not studied, the findings after treatment with nicotinic acid or cholestyramine are therefore suggestive of a modest peroxisomal proliferation, probably involving core-containing organelles.

In view of the small changes observed with the high doses of nicotinic acid and cholestyramine administered in the present study, it is unlikely that peroxisomal proliferation and increased fatty acyl-CoA oxidation represent the primary mechanism of action of these hypolipidemic drugs. The structural similarity of nicotinic acid, acetylsalicylic acid, and other aromatic carboxylic acids which cause peroxisomal proliferation [21] possibly by a mechanism involving formation of CoA esters [22], may be of some relevance for the interpretation of the present findings.

Peroxisomal proliferation, or in some cases a less marked reaction, occurs in response to increased dietary fat load [3] and in mildly hyperthyroid rats [4]. The present findings with cholestyramine, and possibly also with nicotinic acid, suggest that drugs which influence plasma lipids may indirectly induce minor changes in peroxisomal enzymes. There is, therefore, mounting evidence that peroxisome proliferation may be regarded as an adaptation mechanism in situations with altered lipid metabolism. Although quantitative differences undoubtedly exist with regard to their potency as inducers, a clear distinction between peroxisome-proliferating and non-proliferating hypolipidemic drugs can no longer be maintained.

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