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Lipid-metabolizing enzymes, CoASH and long-chain acyl-CoA in rat liver after treatment with tiadenol, nicotinic acid and niadenate

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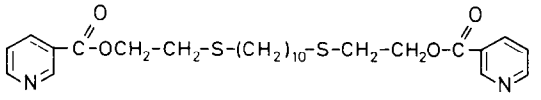
Tiadenol [bis(hydroxy-ethylthio)-1,10-decane] is highly effective in lowering the serum concentration of cholesterol, triglycerides and the VLDL + LDL/HDL ratio in different types of hyperlipoproteinemias [1–3]. However, the drug is also more potent that the hitherto most widely used hypolipidemic drug, clofibrate, in inducing hepatomegaly associated with peroxisomal proliferation and increased activity of some hepatic lipid-metabolizing enzymes in the rat [4–7]. A number of hypolipidemic drugs which induce hepatomegaly and peroxisomal proliferation have been shown to cause liver tumors in rats and mice [5, 8].

Nicotinic acid represents a different class of hypolipidemic drugs and is thought to act mainly by inhibiting peripheral lipolysis thereby making less free fatty acids available for the synthesis of triglycerides, VLDL and eventually LDL [9–11]. Niadenate is a prodrug of nicotinic acid and tiadenol:



The hydrolysis of the ester thus yields two active compounds with distinct mechanisms of action. Since marked increase and subcellular redistribution of peroxisomal enzyme activities occur after clofibrate or tiadenol treatment [6, 12] we have carried out a study with niadenate and nicotinic acid including tiadenol as a reference drug.

Materials and methods. Male Wistar rats weighing 250–270 g were randomly selected for drug treatment or for control experiments. Tiadenol, nicotinic acid or niadenate were added to the diet by soaking commercially available rat food with an acetone solution as described previously [6] leaving pellets containing 0.3, 0.25 and 0.5% (w/w), respectively, of the drugs. The concentration of tiadenol and niadenate in the diets was equimolar whereas that of nicotinic acid matched the amount produced by hydrolysis of niadenate (two molecules of nicotinic acid per molecule of the prodrug). All drugs were obtained from Laboratorios Almirall S.A. (Barcelona, Spain).

Pairs of animals were housed in separate cages and fed the experimental diets with water *ad lib.* for 10 days. The food intake was measured daily for each pair of rats to get an estimate of the average daily drug dose. The rats were killed by decapitation and the livers were homogenized.

The homogenates were fractionated into nuclear (N), mitochondrial (M), light-mitochondrial (L), microsomal (P) and particle-free supernatant (S) fractions according to DeDuve *et al.* [13] with a few modifications [12]. All procedures were performed at 0–4°, and the fractions were stored below −20° until analyzed. The enzyme assays were carried out as described elsewhere [12]. CoASH and long-chain CoA were determined using a published high-pressure liquid chromatographic method [14] with some modifications [12].

Results and discussion. The liver weight increased considerably in the tiadenol- and niadenate-treated animals, and the hepatomegaly was accompanied by a marked increase in protein content (Table 1). The drugs caused little change in the DNA content per gram liver, but there was a trend towards lower relative amounts (μg/mg protein) in the tiadenol-treated animals. Like the liver enlargement produced by clofibrate and some other lipid-lowering drugs [5, 15] the findings of the present study may be interpreted as indicating both an increase in cell size and an increased number of hepatocytes.

Both tiadenol and niadenate elicited a large increase in the amount of long-chain acyl-CoA and free CoASH whereas nicotinic acid influenced the levels to a much smaller extent (Table 1). The concentration of free CoASH per gram liver increased almost 10-fold after niadenate feeding. The total amounts as well as the activities per gram liver of palmitoyl-CoA hydrolase went up several-fold after treatment with tiadenol or niadenate (Table 2). The increase in the palmitoyl-L-carnitine hydrolase and sedimentable palmitoyl-CoA hydrolase activities was less pronounced. A 10–20-fold increase was observed in peroxisomal palmitoyl oxidation after tiadenol or niadenate administration. Total catalase was increased after treatment with tiadenol or niadenate, but the sedimentable enzyme activity was almost unchanged. The activity of urate oxidase per gram liver was not elevated after drug treatment, and there was rather a trend toward lower values in the tiadenol and niadenate experiments.

The increase in palmitoyl-CoA hydrolase activity after tiadenol or niadenate treatment occurred mainly in the M- and S-fractions with a concomitant reduction in the P-fraction (Fig. 1). The relative distribution of sedimentable palmitoyl-CoA hydrolase was less affected. Palmitoyl-L-carnitine hydrolase increased mainly in the supernatant S-fraction at the expense of the P-fraction which normally contains the highest activities.

A large increase in peroxisomal palmitoyl-CoA oxidation was observed in the supernatant after tiadenol or niadenate feeding. A marked reduction occurred in the L-fraction resulting in higher percentages of sedimentable activity in the heaviest fractions (N and M). A redistribution of catalase activity was observed with higher amounts in the supernatant and a lower relative sp. act. in the L-fraction after tiadenol or niadenate administration. These drugs also elicited a redistribution of urate oxidase from the L-

Table 1. Liver weight, protein, DNA, CoASH and long-chain acyl-CoA levels in total homogenate*

Parameter	Control		Tiadenol		Nicotinic acid		Niadenate	
Mean food intake (g/day)	24		26		22		21	
Dose (g/day/kg body weight)			0.27		0.18		0.39	
Δ rat weight (g)	30	43	16	23	14	30	−3	−4.5
Liver weight (g)	10.2	11.8	17.3	18.9	12.2	12.4	14.9	15.9
Liver weight (g/100 g body weight)	3.7	3.9	6.1	6.5	4.0	4.3	5.6	5.8
Protein [whole liver (mg)]	1400	1710	2800	3250	1950	2200	2600	2720
Protein (mg/g liver)	149	179	169	178	165	173	190	192
DNA (mg/g liver)	2.3	2.5	2.0	2.2	2.5	2.7	2.6	2.7
CoASH (acid-soluble) (nmoles/g liver)	107	135	700	800	135	160	780	1180
Long-chain acyl-CoA (acid-insoluble) (nmoles/g liver)	88	118	165	194	113	130	175	198

* The values of the two rats in each treatment group are given.

Table 2. Enzyme activities in total homogenate*

Enzymes	Control		Tiadenol		Nicotinic acid		Niadenate	
Palmitoyl-CoA hydrolase (nmoles/min/g liver)	3360	3880	13,360	13,380	4090	4480	13,310	14,730
Sedimentable palmitoyl-CoA† hydrolase (nmoles/min/g liver)	2940	2960	6380	6580	3300	3800	6160	6350
Palmitoyl-L-carnitine hydrolase (nmoles/min/g liver)	1230	1320	1570	1690	1450	1830	1820	1880
Peroxisomal palmitoyl-CoA oxidation (nmoles/g liver)	320	350	3950	4150	390	430	5700	6300
Catalase (μmoles/min/g liver)	58	60	103	118	44	52	104	119
Sedimentable catalase† (μmoles/min/g liver)	31	35	32	38	33	40	28	30
Urate oxidase (μmoles/min/g liver)	0.5	0.8	0.3	0.5	0.6	0.7	0.3	0.4
Succinate-PMS-oxidoreductase (μmoles/min/g liver)	3.9	4.5	4.1	4.5	4.3	4.5	4.1	4.7
Glutamate dehydrogenase (μmoles/min/g liver)	102	116	128	132	118	132	128	136
NADPH–cytochrome *c* reductase (nmoles/min/g liver)	750	900	1110	1470	1100	1300	1520	1560

* The values of the two rats in each treatment group are given.
† Measured in the combined N–M–L- and P-fractions.

to the P-fraction, but little or no activity appeared in the supernatant. An increase in NADPH–cytochrome *c* reductase occurred in the microsomal (P) fraction accounting for the major part of the enhancement in total activity after tiadenol or niadenate. With these drugs there was an increased floating layer on top of the S-fraction after the final centrifugation. This floating layer contained high activities of acyl-CoA hydrolase, catalase and peroxisomal palmitoyl-CoA oxidation.

The massive increase and the subcellular redistribution of peroxisomal enzyme activities after treatment with tiadenol or niadenate is in keeping with the well-known peroxisome-proliferating properties of the drugs [4, 5, 15, 16]. A discussion of the changes in relation to the biogenesis of the peroxisomes has been presented elsewhere [6, 12].

It should be noted that the increase in free CoASH after tiadenol or niadenate treatment was attributable particularly to the M-, L- and S-fractions (unpublished results). Interestingly, palmitoyl-CoA hydrolase and other acyl-CoA hydrolases increased concomitantly in the M- and S-fractions. Since this was a consistent finding also with clofibrate [12], the changes may reflect a common mechanism of action.

Perfusion of isolated rat livers with nicotinic acid and feeding of animals with the metabolite nicotinamide for 24 hr have been shown to induce small and inconsistent changes in some microsomal enzymes involved in drug metabolism [17, 18]. Our study comprising only two animals in each drug group was not designed to detect minor biochemical alterations. Although the hydrolase activities measured in the present study were a little higher after nicotinic acid administration than in the control experiments, the relative distribution was not appreciably altered. The same applies to peroxisomal palmitoyl-CoA oxidation and to NADPH–cytochrome *c* reductase. Therefore, the findings support the contention that this effective hypolipidemic drug acts mainly by mechanisms not primarily

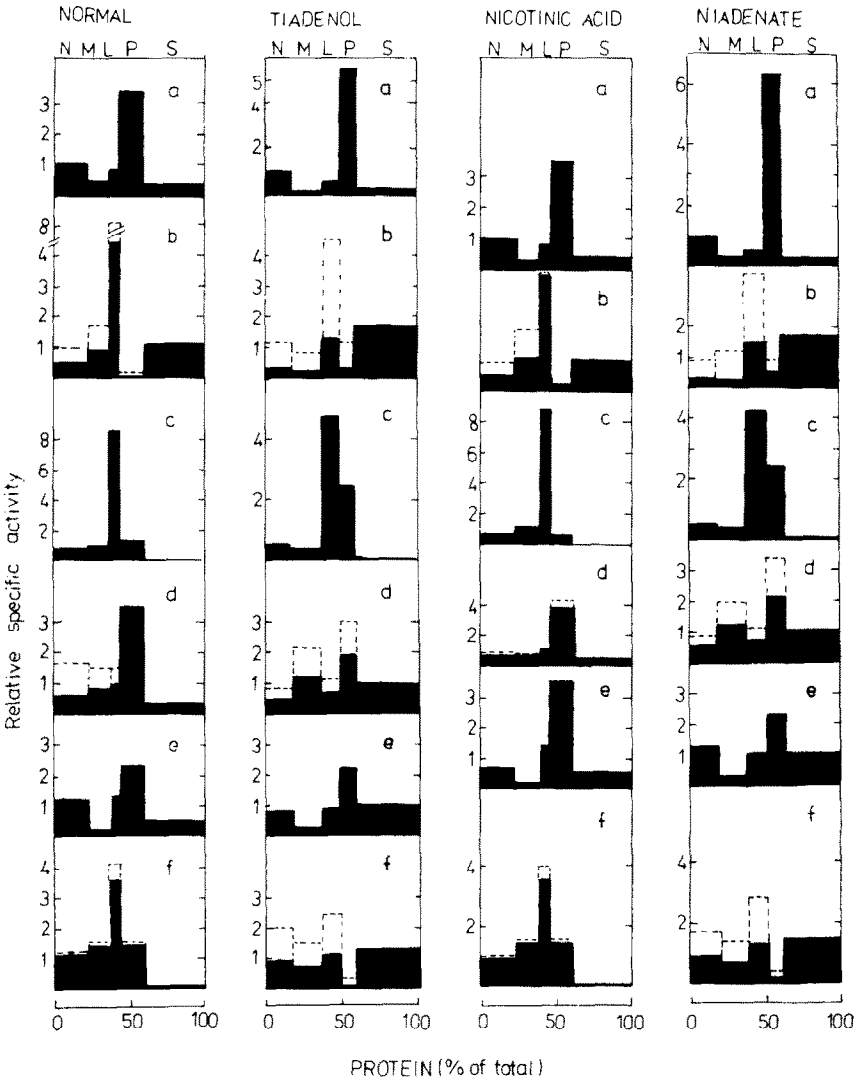


Fig. 1. Subcellular distribution of NADPH–cytochrome *c* reductase (a), catalase (b), urate oxidase (c), palmitoyl-CoA hydrolase (d), palmitoyl-L-carnitine hydrolase (e), and peroxisomal palmitoyl-CoA oxidation (f) in control and tiadenal, nicotinic acid or niadenate treated rats. The dotted lines (b, d and f) show the distribution of the sedimentable enzymes. The recoveries ranged from 85 to 128%.

related to peroxisomal proliferation [9–11].

The finding of principally the same biochemical changes in the liver after approximately equimolar doses of tiadenol and niadenate was to be expected since the latter is a prodrug of the former hypolipidemic substance. The present data are insufficient to determine whether there is a synergistic effect on hepatic enzymes and CoA levels of tiadenol and nicotinic acid produced from niadenate. However, combination of hypolipidemic drugs of the clofibrate type with nicotinic acid or congeners results in a synergistic effect on blood lipids in rats [19]. Clinical trials have also shown that the combination of clofibrate and nicotinic acid is highly effective in reducing serum cholesterol and triglycerides [20], and similar findings have been obtained with niadenate [data on file (Laboratorios Almirall S.A., Barcelona, Spain)].

In conclusion, niadenate has similar effects to those observed with tiadenol and clofibrate which cause hepatomegaly and gross induction of peroxisomal enzymes, some of which leak to the particle-free supernatant, probably due to altered membrane characteristics of newly induced peroxisomes [6, 21]. Free CoASH and long-chain acyl-CoA levels are also increased after tiadenol and niadenate treatment. The toxicological implications of the present findings depend on whether peroxisomal proliferation and carcinogenesis after high doses of hyperlipidemic compounds in rodents are relevant to the therapeutic use of these drugs in man [22].

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Analysis of the displaceable binding of the hypotensive drug R 28935 in rat brain

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A number of reports describe the pronounced hypotensive activity of R 28935, the racemic mixture of erythro 1-{1-[2-(1,4-benzodioxan-2-yl)-2-hydroxyethyl]-4-piperidyl}-2 benzimidazolinone (Fig. 1) in various animal models [1–7]. The hypotensive effect of R 28935 is probably of central nervous origin and stereospecific, since the threo form R 28914 is much less potent [2, 4, 5, 7, 8]. The central mechanism via which R 28935 induces hypotension is still unknown. The involvement of central α-adrenoceptors has been considered unlikely based on the observations that α-adrenoceptor-blocking agents, like yohimbine and piperoxan [2–4, 6] or phentolamine and tolazoline [1], were ineffective in inhibiting the hypotensive action of R 28935. However, it was found subsequently that the selective antagonist of α_1-adrenoceptors prazosin [9] strongly diminished the hypotension induced by R 28935 [7, 10, 11]. As a consequence thereof, central α_1-adrenoceptors have been suggested to play a role in the mechanism of action of R 28935. Although R 28935 possesses affinity for central [8] and peripheral α_1-adrenoceptors [7, 12], it is still very doubtful whether these sites represent the primary targets for R 28935 to cause hypotension, since, within a series of benzodioxane derivatives structurally related to R 28935, α_1-adrenoceptor affinity bears no relationship to hypotensive activity [7, 8].

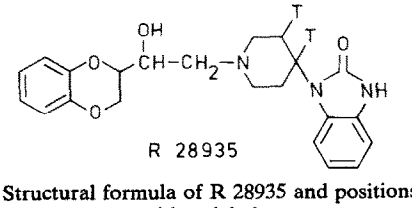

Fig. 1. Structural formula of R 28935 and positions of the tritium labels.

In the present study we have analyzed the specific binding of tritium-labeled R 28935 [13] to rat brain membranes in our efforts to learn more details of its mechanism of action. It was the main goal of the present investigation to establish whether the specific binding sites of [^{3}H]R 28935 identified in rat brain were associated with distinct receptor sites, possibly responsible for the initiation of the hypotensive action of this drug.

Materials and methods. Erythro [^{3}H]R 28935 (sp. act. 9 Ci/mmole) as well as unlabeled erythro R 28935 and threo R 29814 were gifts of Janssen Pharmaceutica (Beerse, Belgium). A number of benzodioxane derivatives structurally related to R 28935 indicated by the notation "R" (for structures see Ref. 8) were also provided by this company. Other drugs were obtained from normal commercial sources or the pharmaceutical companies of origin. All other chemicals were reagent grade.

Adult male Wistar rats (200–250 g) were decapitated, their brains (minus cerebella) removed and homogenized in 20 vol. (w/v) of 50 mM ice-cold Tris–HCl buffer (pH = 7.7 at 25°) using a Brinkman Polytron. The homogenate was centrifuged at 50,000 *g* for 10 min at 4°, resuspended in fresh buffer and centrifuged again. The final pellet containing the crude brain membranes was suspended in Tris–HCl buffer to give a concentration of 4 mg protein/ml and kept on ice until used. Protein concentration was assayed according to Lowry *et al.* [14].

Standard binding assays were run by incubating 500 μl of rat membrane suspension (see earlier) at 25° for about