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Thyromimetic effect of peroxisomal proliferators in rat liver

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Amphipathic carboxylates, of varying hydrophobic backbones, which act as peroxisomal proliferators (aryloxyalkanoic acids, methyl-substituted dicarboxylic acid) induce in euthyroid or thyroidectomized rats, as well as in rat hepatocytes cultured in 3,5,3′-tri-iodo-L-thyronine (T_3)-free media, liver enzyme activities that are classically considered to be thyroid-hormone-dependent (malic enzyme, mitochondrial α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase and S14). The dose required *in vivo* for the thyromimetic effect of peroxisomal proliferators was 10^3-fold higher than the dose of T_3 required. Similarly, peroxisomal proliferators were active in culture in the range 1–100 μM compared with 1 nM for T_3. Their maximal inductive capacities were, however, similar to or greater than that of T_3. The thyromimetic effect of peroxisomal proliferators was only partially correlated with their capacities as inducers of liver peroxisomal enzymes. The thyromimetic effect with respect to liver malate dehydrogenase and S14 resulted from an increase in their mRNA contents. The increase in liver S14 mRNA was accounted for by transcriptional activation of the S14 gene. T_3 binding to isolated liver nuclei or nuclear extract was competitively displaced by some but not all of the non-thyroidal inducers of the above liver activities. In contrast with the thyromimetic effect induced in liver cells, no increase in growth hormone mRNA was observed in cultured GH_1 pituitary cells incubated in the presence of non-thyroidal amphipathic carboxylates. The characteristics of the thyromimetic effect of amphipathic carboxylic peroxisomal proliferators indicate that these agents may act as transcriptional activators of thyroid-hormone-dependent genes in the rat liver.

INTRODUCTION

Amphipathic carboxylates of varying structural characteristics, e.g. aryloxyalkanoic acids or esters (clofibrate [1], bezafibrate [2], nafenopin [3]), phthalate plasticizers (di-2-ethylhexylphthalate [4]), native long-chain fatty acids [5] or substituted long-chain dicarboxylic acids [6,7] have been repeatedly reported to induce liver peroxisomal proliferation and specific peroxisomal enzyme activities in rodents and other species (reviewed in [8,9]). The peroxisomal activities induced by peroxisomal proliferators were reported to result from a selective increase in the transcription rate of peroxisomal β-oxidative genes, with concomitant increases in their respective mRNAs [10,11]. Thyroid hormones (reviewed in [12,13]) appear to share some of the basic characteristics of peroxisomal proliferators. Indeed, thyroid hormones may be generally classified as amphipathic carboxylates, having a carboxyl function carried on a hydrophobic iodinated diphenoxy backbone. Also, similarly to peroxisomal proliferators [14,15], thyroid hormones may induce conversion of preadipocytes into matured fat cells [16], as well as serving as effective hypolipidaemic agents (reviewed in [17]). Moreover, thyroxine was recently reported to induce peroxisomal proliferation in the rat liver [18], even though this was significantly less extensive than that induced by typical peroxisomal proliferators. These characteristics that are shared by thyroid hormones and peroxisomal proliferators have tempted us to question whether typical members of the group of peroxisomal proliferators might act as thyromimetic agents, capable of directly inducing liver activities classically considered to be thyroid-hormone-dependent. An increase in liver malic enzyme (EC 1.1.1.40) activity in clofibrate-treated rats has been previously reported [19,20] but proposed to be secondarily mediated by competitive displacement of thyroid hormones from their plasma binding sites or by modulation of the insulin/glucagon hormonal balance. The present report indeed shows that various peroxisomal proliferators, which are structurally not related to thyroid hormones, directly induce thyroid-hormone-dependent liver functions.

MATERIALS AND METHODS

Animals

Euthyroid male albino rats weighing 150–200 g were fed for 6 consecutive days with laboratory chow diet containing nafenopin, bezafibrate, dehydroepiandrosterone (DHEA) or 3,3,14,14-tetramethylhexadecanedioic acid (MEDICA 16) as stated [% (w/w) of the administered diet]. For hormonal induction, the animals were injected intraperitoneally for 6 consecutive days with 15 μg of tri-iodothyronine (T_3) or tri-iodothyroacetic acid (Triac)/100 g body per day. Hypothyroid rats were obtained either by surgical parathyrothyroidectomy or by methimazole treatment (0.025% methimazole added to the drinking water) of male rats weighing 120 g. A hypothyroid state was reflected by the cessation of weight gain and confirmed by measurement of serum T_3 levels, which were 38 ± 12 μg/dl on the day of death. At 5 weeks after thyroidectomy or methimazole treatment the animals were treated either with the above drugs administered by stomach tube for 14 days as stated or with T_3 or Triac as described above for euthyroid animals.

Cultured cells

Cultured rat hepatocytes were prepared as previously described [5]. Cells were plated on rat-tail-collagen gel prepared according to [21] and grown in RPMI 1640 medium containing 10% (v/v) fetal calf serum (rendered T_3-free by charcoal treatment), 100 munits of insulin/ml, 10 μg of cortisol/ml, 50 μg of streptomycin sulphate/ml and 50 μg of penicillin G/ml. Peroxisomal

Abbreviations used: DHEA, dehydroepiandrosterone; MEDICA 16, 3,3,14,14-tetramethylhexadecanedioic acid; T_3, 3,5,3′-tri-iodo-L-thyronine; Triac, 3,5,3′-tri-iodothyroacetic acid.

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proliferators or T_3 were added to the culture medium in dimethyl sulphoxide kept at a final concentration of 0.5%. The culture medium was changed every 48 h. After 6 days in culture the cells were harvested by collagenase treatment, washed with modified Hanks buffered saline and suspended in homogenization buffer containing 0.25 M-sucrose, 10 mM-Tris/HCl, pH 7.4, and 5 mM-$MgCl_2$.

GH_1 pituitary cells (ATCC CCL82) were cultured in RPMI 1640 medium containing 15% (v/v) horse serum and 2.5% (v/v) fetal calf serum. For the induction of growth hormone, the culture medium was replaced 48 h before the experiment by RPMI 1640 medium containing 10% (v/v) fetal calf serum rendered T_3-free by charcoal treatment and then further incubated for 48 h in the presence of either T_3 or peroxisomal proliferators as stated.

Enzyme activities

Liver homogenates were prepared in 4 vol. of 0.25 M-sucrose and centrifuged at 700 *g* for 10 min. The mitochondria-enriched fraction was prepared by centrifuging the 700 *g* supernatant at 4500 *g* for 10 min followed by washing the pellet once. The 4500 *g* supernatant was centrifuged at 100000 *g* for 1 h to yield the cytosolic fraction.

Cultured liver cells suspended in homogenization buffer were homogenized using a Teflon pestle. The membrane and cytosolic fractions were obtained by centrifuging the homogenate at 100000 *g* for 1 h.

Malic enzyme (EC 1.1.1.40) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49) activities were assayed as described in [22] and [23] respectively, using the cytosolic fraction from either whole liver or cultured hepatocytes. Mitochondrial α-glycerophosphate dehydrogenase (EC 1.1.99.5) activity was measured according to [24] using either the mitochondria-enriched fraction of rat liver or the 100000 *g* pellet of cultured hepatocytes. Peroxisomal enoyl-CoA hydratase (EC 4.2.1.17) in liver or cultured cell homogenates was determined as previously described [6]. Protein was determined according to [25,26].

RNA preparation and analysis

Liver or GH_1 cell total RNA was prepared using guanidium thiocyanate and was centrifuged through a CsCl cushion according to [27]. The RNA was analysed by Northern blot analysis [28] and quantified by dot-blot hybridization [29]. For Northern blot hybridization 25–40 μg of total RNA was loaded on the agarose gel. For dot-blot analysis 1–16 μg of total RNA was denatured with formaldehyde and blotted on Nytran. Hybridization values were linearly related to the blotted RNA content. After hybridization with the respective molecular probes the blots were exposed to X-ray film and the autoradiograms were evaluated densitometrically.

Run-on transcription assay

Rat liver nuclei were isolated as described in [30]. Nuclei were stored at −70 °C in storage buffer containing 50% (w/v) glycerol, 50 mM-Hepes (pH 7.8), 1 mM-$MgCl_2$, 4 mM-$MnCl_2$ and 0.1 mM-EDTA. Transcription assays were carried out as described in [10], using nuclei samples amounting to 15 absorbance units (260 nm) and 250 μCi of [^{32}P]UTP in a final volume of 0.1 ml. Incubation was carried out at 26 °C for 30 min followed by addition of 20 units of RNAase-free DNAase I and then 50 μg of proteinase K. [^{32}P]RNA was extracted according to [31] and hybridized for 48 h at 65 °C in 1 ml of 50 mM-Hepes (pH 7.5)/0.5 M-NaCl/10 mM-EDTA/0.2% SDS/0.5 mM-UTP/0.2% Ficoll/0.02% polyvinylpyrrolidone/0.2% BSA/300 μg of salmon-sperm DNA/ml to 2 μg of the respective plasmid DNAs (pAlb1, pS14-C2, pMJ26, pMJ125, pBR322) affixed to Nytran. The washed blots were exposed to X-ray film and the radioactivity was then quantified by liquid scintillation counting. Radioactivity hybridized to pBR322 served as background and was subtracted from each experimental value. Transcriptional activity was expressed relative to that of albumin.

Molecular probes

S14 mRNA levels were determined using the 610 bp *Pst*I restriction fragment excised from the recombinant DNA pS14-C2 [32] obtained from H. Towle (University of Minnesota, Minneapolis, MN, U.S.A.). Malic enzyme mRNA levels were determined using the 1000 bp pair *Eco*RI restriction fragment excised from the recombinant DNA pME6 [33] obtained from V. Nikodem (NIH, Bethesda, MD, U.S.A.). Albumin mRNA levels were determined using the 1000 bp *Hin*dIII restriction fragment excised from the recombinant DNA pAlb1 [34] obtained from S. M. Tilghman (Princeton University, Princeton, NJ, U.S.A.). Peroxisomal enoyl-CoA hydratase mRNA levels were determined using the 2324 bp *Eco*RI restriction fragment excised from the recombinant DNA pMJ26 [35]. Peroxisomal palmitoyl-CoA oxidase mRNA was probed by using the pMJ125 plasmid [36]. Growth hormone mRNA was determined using the 800 bp *Pst*I restriction fragment excised from the recombinant DNA pGH1 [37]. cDNA probes were ^{32}P-labelled by nick-translation [38].

Nuclear binding of L-[3′-^{125}I]T_3

Receptor binding *in vitro* was determined using either isolated liver nuclei or a nuclear extract. Nuclei prepared by sedimentation of rat liver homogenate through high-density sucrose solution [30] were further washed twice with 0.25 M-sucrose containing 2 mM-$MgCl_2$, 20 mM-Tris/HCl, pH 7.5, and 0.5% Triton X-100. For binding studies with isolated nuclei the pellet was suspended in 0.25 M-sucrose containing 50 mM-NaCl, 20 mM-Tris/HCl, pH 7.5, 1 mM-EDTA and 5% (v/v) glycerol. L-[3′-^{125}I] T_3 binding in the presence of increasing concentrations of either non-radioactive T_3 or peroxisomal proliferators was determined according to [39]. DHEA was either suspended in the reaction mixture by sonication or dissolved first in ethanol and then added to the reaction mixture. For binding studies with solubilized receptors the nuclei pellet was solubilized and the binding of L-[3′-^{125}I]T_3 to the nuclear extract was determined according to [40].

Materials

MEDICA 16 was synthesized according to [7]. Nafenopin was from Ciba–Geigy. Bezafibrate was from Boehringer Mannheim. T_3, Triac and DHEA were from Sigma. [α-^{32}P]UTP (3000 Ci/mmol) and L-[3′-^{125}I]T_3 (> 1200 μCi/μg) were from Amersham. [α-^{32}P]dCTP (3000 Ci/mmol) was from New England Nuclear.

Statistics

Significance of differences was analysed by the Mann–Whitney U test.

RESULTS

The thyromimetic effects of peroxisomal proliferators were studied in the rat *in vivo* by measuring the increase in the hepatic specific activities of several thyroid-hormone-dependent enzymes, e.g. malic enzyme, mitochondrial α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase and S14, induced by peroxisomal proliferators of varying structural characteristics. These included bezafibrate and nafenopin, which present two fibrate carboxylates of variable hydrophobic backbones, MEDICA 16, consisting of a substituted long-chain

Table 1. Enzyme activities and mRNA content in the livers of euthyroid rats

Euthyroid rats were treated for 6 days with either T_3 (15 μg/day per 100 g body wt.) or Triac (15 μg/day per 100 g body wt.) injected intraperitoneally or with nafenopin (0.1 %, w/w), bezafibrate (0.2 %, w/w), MEDICA 16 (0.25 %, w/w) or DHEA (0.6 %, w.w) mixed in the diet. Liver enzyme activities were determined as described in the Materials and methods section in between five and eight non-treated rats and in groups of treated rats each consisting of three animals. The mRNA contents were determined by dot-blot hybridization with their respective probes as described in the Materials and methods section in non-treated and treated groups consisting of three animals per group. Total RNA per dot amounted to 4–16 μg for malic enzyme and 1–4 μg for S14 and enoyl-CoA hydratase. mRNA results are presented in densitometric units relative to the respective non-treated values. Values are means ± S.D.; *significantly different from the respective non-treated value; $P < 0.05$.

Treatment	Malic enzyme (munits/mg of protein)	Mitochondrial α-glycerophosphate dehydrogenase (munits/mg of protein)	Peroxisomal enoyl-CoA hydratase (units/mg of protein)	mRNA (units)		
				Malic enzyme	S14	Peroxisomal enoyl-CoA hydratase
Not treated	9.4±2.4	11.4±4.6	0.5±0.3	1.0±0.2	1.0±0.3	1.0±0.3
T_3	85.0±21.0*	41.0±4.0*	0.5±0.2	34.0±2.8*	4.0±1.0*	1.0±0.2
Triac	32.0±3.8*	22.0±5.0*	0.4±0.2	5.0±2.0*	–	1.0±0.2
Nafenopin	72.0±3.6*	62.0±7.5*	4.6±0.2*	40.0±15.0*	1.0±0.5	133.3±30.0*
Bezafibrate	53.3±5.8*	23.0±4.0*	5.0±0.4*	17.0±9.5*	0.7±0.3	153.3±3.7*
MEDICA 16	35.0±3.9*	27.0±4.6*	3.5±0.3*	6.5±4.0*	1.3±0.2	94.7±25.0*
DHEA	58.0±7.0*	52.0±11.0*	4.4±0.8*	18.0±9.0*	0.8±0.2	120.0±11.3*

Table 2. Enzyme activities and mRNA content in the livers of thyroidectomized rats

Thyroidectomized rats were treated for 6 days with T_3 (15 μg/day per 100 g body wt.) or Triac (15 μg/day per 100 g body wt.) injected intraperitoneally, or treated for 14 days with nafenopin (7.5 mg/day per 100 g body wt.) or MEDICA 16 (15 mg/day per 100 g body wt.) administered intragastrically. Enzyme activities and mRNAs were determined as described in the Materials and methods section. Total RNA per dot was as specified in Table 1. mRNA results are presented in densitometric units relative to the respective non-treated values. Values are means ± S.D. ($n = 3$); *significantly different from the non-treated value, $P > 0.05$.

Treatment	Enzyme activity (munits/mg of protein)		mRNA (units)		
	Malic enzyme	Mitochondrial α-glycerophosphate dehydrogenase	Malic enzyme	S14	Peroxisomal enoyl-CoA hydratase
Non-treated	< 1.5	1.6±0.3	1.0±0.6	1.0±0.1	1.0±0.4
T_3	30.0±10.0*	36.0±5.5*	62.2±11.1*	3.0±0.5*	1.0±0.2
Triac	17.0±3.0*	15.7±4.6*	23.3±3.3*	2.2±0.2*	1.0±0.2
Nafenopin	24.5±5.0*	14.4±7.0*	58.9±5.0*	3.6±1.5*	36.2±4.7*
MEDICA 16	4.2±0.8*	3.7±1.0*	22.2±8.9*	2.9±0.1*	40.0±15.0*

dioic acid, and DHEA, which lacks a carboxyl function altogether but was recently reported to induce peroxisomal proliferation in rats [41]. As shown in Table 1, MEDICA 16, DHEA and the two fibrate drugs induced increases in the specific activities of hepatic malic enzyme and mitochondrial α-glycerophosphate dehydrogenase similar to those induced by maximal doses [42] of Triac or T_3. Nafenopin also proved to be a potent inducer of hepatic glucose-6-phosphate dehydrogenase, being induced 16-fold, compared with a 20-fold induction by T_3 treatment (results not shown). The thyromimetic potencies of the four peroxisomal proliferators were only partially correlated with their capacities as inducers of peroxisomal enzymes (Table 1) or their capacities as hypolipidaemic agents (results not shown). The thyromimetic effect of peroxisomal proliferators was accompanied by an increase in malic enzyme mRNA which was correlated with their respective capacities for inducing malic enzyme activity (Table 1). However, peroxisomal proliferators did not induce any increase in S14 mRNA in the euthyroid rat under conditions where peroxisomal enoyl-CoA hydratase mRNA was increased by 100–150-fold. It is worth noting that, in contrast with the previously reported peroxisomal proliferative capacity of thyroxine [18], T_3 did not induce an increase in peroxisomal enoyl-CoA hydratase under conditions where its effects with respect to malic enzyme, mitochondrial α-glycerophosphate dehydrogenase and S14 were clearly observed (Table 1).

The thyromimetic effect of peroxisomal proliferators as reported here was not mediated by an increase in the overall production of thyroid hormones nor in the availability of the free hormone due to competition between peroxisomal proliferators and thyroid hormone binding proteins. Thus the thyromimetic effects of nafenopin and MEDICA 16 were evident in thyroidectomized (Table 2) and methimazole-treated (results not shown) rats, where the capacity of nafenopin to induce malic enzyme and mitochondrial α-glycerophosphate dehydrogenase approached that of maximal doses of T_3. However, in contrast with their effects in euthyroid rats (Table 1), nafenopin and MEDICA 16 induced 3–4-fold increases in S14 mRNA in thyroidectomized rats as compared with a 3-fold increase induced by T_3 treatment (Table 2).

The induction of malic enzyme and mitochondrial α-glycerophosphate dehydrogenase activities was also observed in cultured rat hepatocytes incubated in T_3-free medium in the presence of nafenopin, bezafibrate or MEDICA 16 added to the culture medium (Figs. 1*a* and *b*). With respect to both activities,

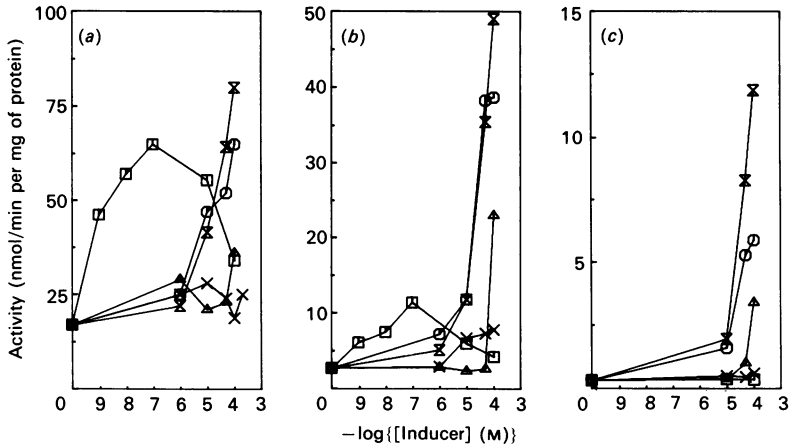

Fig. 1. Thyromimetic effects of peroxisomal proliferators in cultured rat hepatocytes

Cultured rat hepatocytes prepared as described in the Materials and methods section were incubated for 6 days with T_3 (□), nafenopin (⧖), bezafibrate (Ⓘ) MEDICA 16 (△) or DHEA (×) as indicated. Activities of (*a*) malic enzyme, (*b*) glycerophosphate dehydrogenase and (*c*) peroxisomal palmitoyl-CoA oxidase were measured. Values are means of two experiments which differed by less than 10%.

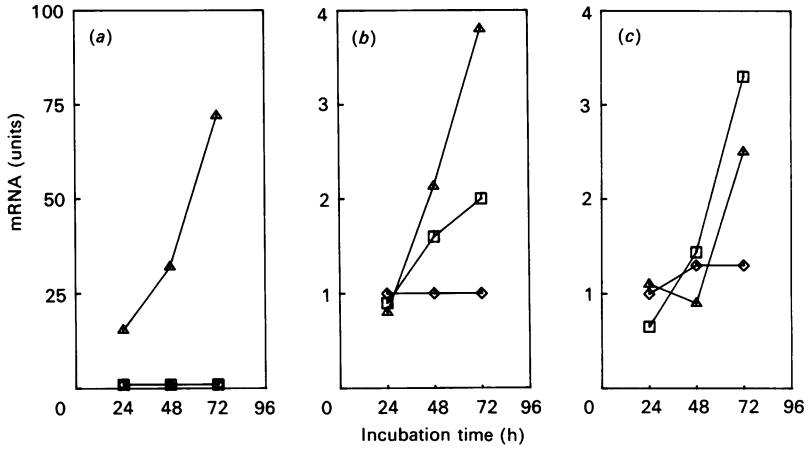

Fig. 2. Thyromimetic effects of peroxisomal proliferators in cultured rat hepatocytes: time courses

Cultured rat hepatocytes prepared as described in the Materials and methods section were incubated for the indicated time periods in the presence of 0.5 μM-T_3 (□) or 0.1 mM-nafenopin (△), or in their absence (◇). Peroxisomal enoyl-CoA hydratase mRNA (*a*), malic enzyme mRNA (*b*) and S14 mRNA (*c*) were determined by dot-blot hybridization with their respective probes as described in the Materials and methods section. mRNA results are presented in densitometric units relative to the respective non-treated values at 24 h (1.0). The Figure shows one experiment representative of two.

T_3 was found to have an apparent EC_{50} value (concn. causing half-maximal stimulation) of approx. 1 nM, whereas the threshold concentration for nafenopin and bezafibrate was approx. 1 μM. Within the concentration range 10–100 μM the induction of the two enzymes by the two fibrate drugs exceeded that observed in the presence of T_3 due to inhibition exerted by 10–100 μM-T_3 (added in the presence of dimethyl sulphoxide). The V_m values extrapolated from Lineweaver–Burk plots for malic enzyme in the presence of nafenopin and bezafibrate were similar to that in the presence of T_3 (calculated upon eliminating the inhibitory concentrations), whereas the extrapolated α-glycerophosphate dehydrogenase V_m values in the presence of the two fibrate drugs were 10-fold higher than that induced by T_3. The thyromimetic potencies of peroxisomal proliferators in culture were correlated well with their capacities as inducers of peroxisomal palmitoyl-CoA oxidase (Fig. 1*c*). T_3, however, was ineffective in inducing peroxisomal palmitoyl-CoA oxidase in culture, similarly to its ineffectiveness *in vivo* (Table 1).

In contrast with its peroxisome proliferative capacity and its thyromimetic effect *in vivo* (Table 1), DHEA added to the culture medium up to a concentration of 0.2 mM did not induce peroxisomal palmitoyl-CoA oxidase, malic enzyme or mitochondrial α-glycerophosphate dehydrogenase activities (Fig. 1).

The thyromimetic effects induced in cultured rat hepatocytes by nafenopin and MEDICA 16 were time-dependent and were accounted for by increases in the mRNAs of malic enzyme and S14 (Fig. 2). The time course of the thyromimetic effect in culture lagged behind that observed for the induction of enoyl-CoA hydratase mRNA (Fig. 2).

Malic enzyme and S14 mRNAs induced by peroxisomal proliferators were similar in size to the respective mRNAs induced by T_3 treatment. Thus Northern blot hybridizations of mRNA induced by T_3, nafenopin, bezafibrate and MEDICA 16 indicated 21 S and 27 S malic enzyme mRNAs, as previously reported by Dozin *et al.* for liver malic enzyme mRNA induced by T_3 [42]. Similarly, Northern blot hybridizations of mRNAs induced by nafenopin, bezafibrate and MEDICA 16 indicated a 16 S S14 mRNA, as previously reported for liver S14 mRNA induced by T_3 [43].

The role played by transcriptional activation and/or

Table 3. Transcription rates in thyroidectomized and euthyroid rats

In expt. A, thyroidectomized rats (three animals per treatment group) were treated with either nafenopin or MEDICA 16 as described in the legend to Table 2. In expt. B, euthyroid rats (5–6 animals per treatment group) were treated with T_3, nafenopin or MEDICA 16 as described in the legend to Table 1. Each liver sample was individually assayed as described in the Materials and methods section for the transcription rates of the indicated genes as compared with that of albumin. Values are means ± S.D. *Significantly different from the respective non-treated value, $P < 0.05$; †Significantly different from the thyroidectomized non-treated value, $P < 0.05$.

Expt.	Treatment	mRNA (units) S14	Peroxisomal enoyl-CoA hydratase	Peroxisomal palmitoyl-CoA oxidase
A	Non-treated	12.7±0.6	5.6±3.5	15.4±10.1
	Nafenopin	35.7±9.4*	89.0±19.0*	96.3±35.6*
	MEDICA 16	45.0±4.2*	96.3±22.7*	112.5±14.8*
B	Non-treated	38.0±3.4†	1.5±0.6	8.4±2.8
	T_3	28.2±9.4	2.2±1.6	6.2±1.8
	Nafenopin	27.2±12.2	170.3±53.3*	89.5±31.7*
	MEDICA 16	29.0±11.3	82.0±10.6*	92.7±48.6*

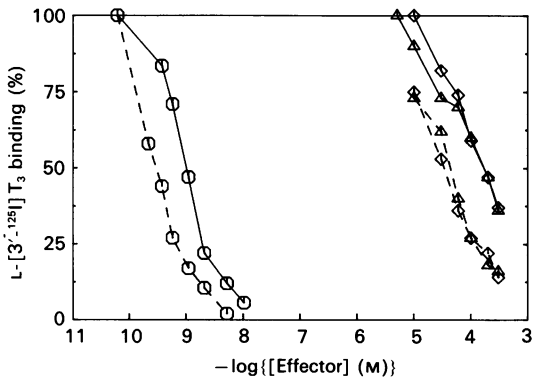

Fig. 3. Effects of peroxisomal proliferators on nuclear L-[3′-^{125}I]T_3 binding

Isolated rat liver nuclei (continuous lines) or a nuclear extract (broken lines), prepared as described in the Materials and methods section, were incubated for 2 h at 22 °C or for 22 h at 0 °C respectively in the presence of 0.06 nM-L-[3′-^{125}I]T_3 with increasing concentrations of unlabelled T_3 (○), nafenopin (△) or MEDICA 16 (◇). The specific binding of the L-[3′-^{125}I]T_3 tracer was determined as described in the Materials and methods section. Each individual point represents the mean of duplicate determinations that differed by no more than 5% of the mean value. The results are from an experiment representative of two.

stabilization of the S14 transcript by peroxisomal proliferators was evaluated by studying the rate of transcription of the S14 gene as compared with the S14 mRNA content in animals treated with either nafenopin or MEDICA 16. The run-on transcription rate was normalized in each case to that of the albumin gene, since the rate of transcription of the latter was reported to remain unaffected by T_3 [44], and its mRNA content remained unaffected by nafenopin or MEDICA 16 treatment (results not shown). As shown in Table 3 (expt. A), the rate of transcription of S14 in nafenopin- or MEDICA 16-treated thyroidectomized rats was increased 3–4-fold compared with non-treated animals. This increase in the S14 transcription rate was similar in magnitude to the increases in S14 mRNA content induced by nafenopin or MEDICA 16 in thyroidectomized rats (Table 2). In contrast with thyroidectomized rats, the rate of transcription of S14 remained unaffected in euthryroid rats treated with nafenopin or MEDICA 16 (Table 3, expt. B), in line with their ineffectiveness in increasing the S14 mRNA content in these animals (Table 1). Hence the thyromimetic effects of nafenopin and MEDICA 16 with respect to S14 may essentially be accounted for by increased transcriptional activation of the S14 gene.

The possible binding of peroxisomal proliferators to the thyroid hormone nuclear receptor was evaluated by measuring the displacement of L-[3′-^{125}I]T_3 tracer using either rat liver nuclei or a nuclear extract. As shown in Fig. 3, L-[3′-^{125}I]T_3 binding to rat liver nuclei was 50% inhibited by 0.15 mM-nafenopin or -MEDICA 16, compared with 0.7 nM-T_3. Similarly, L-[3′-^{125}I]T_3 binding to the solubilized nuclear extract was 50% inhibited by 0.35 μM-nafenopin or -MEDICA 16, compared with 0.3 nM-T_3. Nafenopin inhibition of T_3 binding to isolated nuclei was further studied by varying the concentration of T_3 (0.06–0.5 nM) at variable fixed concentrations of nafenopin (0–300 μM), and was found to be competitive with respect to T_3 (results not shown). The K_i for nafenopin amounted to 219±49 μM, compared with a K_a of 0.75±0.09 nM for T_3. In contrast with nafenopin and MEDICA 16, L-[3′-^{125}I] T_3 binding to either rat liver nuclei or nuclear extract was not displaced by DHEA up to a concentration of 300 μM, or by bezafibrate up to a concentration of 1 mM. Furthermore, whereas T_3 added to fresh or cultured rat hepatocytes at a concentration of 1 nM could displace L-[3′-^{125}I]T_3 binding to the cell nuclei, no displacement was effected by bezafibrate added to the culture medium up to a concentration of 1 mM.

In contrast with the thyromimetic effect induced in liver cells by fibrate drugs or MEDICA 16, no increase in growth hormone mRNA was observed in cultured GH_1 pituitary cells incubated in the presence of 100 μM-nafenopin or 150 μM-MEDICA 16 under conditions in which a 8.5-fold increase in growth hormone mRNA was induced by 5 nM-T_3.

DISCUSSION

Peroxisomal proliferators of varying structural characteristics have been found here to induce, *in vivo* as well as in cultured rat hepatocytes, liver activities classically considered to be thyroid-hormone-dependent. The previously reported induction of malic enzyme by dichloroacetic acid [45], perfluorodecanoic acid [46] or halothane metabolites [47] may be explained within the framework of the thyromimetic effect of peroxisomal proliferators reported here. The variety of the functions induced by the variety of the compounds employed points to the general principle involved and may indicate that peroxisomal proliferators should be grossly considered as proper thyromimetic agents in the rat liver. However, in contrast with T_3, which is active *in vivo* and in culture in the range of micrograms/100 g body wt. and at 1 nM, respectively, the thyromimetic effects of peroxisomal proliferators were evident in the range of milligrams/100 g body wt. and at 1–100 μM, respectively. The maximal thyromimetic effect of peroxisomal proliferators, however, was similar to or exceeded that of T_3. The thyromimetic effects of peroxisomal proliferators were only partially correlated with their capacities as inducers of peroxisomal enzymes or liver growth, and in contrast with thyroxine [18], T_3 and Triac were completely ineffective as inducers of peroxisomal functions.

In contrast with the dual effect of T_3 on S14 expression, where transcriptional activation is accompanied by stabilization of the S14 nuclear transcript ([48,49]; note however the alternative view presented in [50]), the thyromimetic effect of peroxisomal proliferators with respect to S14 appears to be exclusively due to

transcriptional activation (Tables 2 and 3). In the absence of S14 transcript stabilization by peroxisomal proliferators, and since transcriptional activation of the S14 gene by T_3 approaches saturation at T_3 concentrations within the range prevailing in the euthyroid rat [48,49], transcriptional activation of the S14 gene by peroxisomal proliferators may be verified in hypothyroid rats only (Table 1 versus Table 2), where it is still not saturated by endogenous T_3, or alternatively in cultured rat hepatocytes grown in T_3-free medium. The induction of malic enzyme mRNA by peroxisomal proliferators even in euthyroid rats (Table 1) could perhaps reflect a higher K_m of the T_3-occupied nuclear receptor for transcriptional activation of the malic enzyme gene [51] compared with the K_m for transcriptional activation of the S14 gene. Thus T_3 concentrations prevailing in the euthyroid rat could still allow for further transcriptional activation of the malic enzyme gene by peroxisomal proliferators.

The thyromimetic effect of DHEA reported here may complement the findings recently reported by Song *et al.* [52] with respect to rat liver malic enzyme induction by DHEA. Thus rat liver malic enzyme induction by DHEA ([52], Table 1) was accounted for by transcriptional activation of its gene [52]. The overall effect of DHEA with respect to other thyroid-hormone-dependent functions (Table 1) further indicates that DHEA may be considered to be a proper thyromimetic agent. It is noteworthy, however, that in contrast with the amphipathic carboxylates employed here, DHEA lacks a carboxylic function; also, it is reported not to be catabolized into a carboxylic compound. Furthermore, the thyromimetic effect of DHEA was evident only in the euthyroid rat *in vivo* [52] but not in the hypothyroid rat [52] or in culture (Fig. 1). Moreover, in contrast with nafenopin and MEDICA 16, DHEA could not displace nuclear T_3, thus indicating perhaps that the thyromimetic effect of DHEA may be indirectly mediated in the euthyroid rat by a metabolite generated by DHEA treatment *in vivo*.

The T_3-competitive binding of nafenopin and MEDICA 16 to isolated liver nuclei or nuclear extracts (Fig. 3) could indicate that the thryomimetic effect induced by xenobiotic amphipathic carboxylates is mediated in a manner similar to that triggered by thyroid hormones proper. It should be noted, however, that in contrast with nafenopin or MEDICA 16, bezafibrate could not compete with T_3 for binding to isolated liver nuclei or nuclear extracts at concentrations at which it was a potent thyromimetic agent in euthyroid rats and in heptocytes cultured in T_3-free medium. Bezafibrate thus offers an example of a non-thyroidal amphipathic carboxylate which may induce liver thyroid-hormone-dependent genes while not directly involving the liver nuclear receptor for thyroid hormones. This dissimilarity between the modes of actions of T_3 and xenobiotic amphipathic carboxylates is further corroborated by the fact that neither nafenopin nor MEDICA 16 could induce pituitary growth hormone mRNA under conditions where T_3 proved to be a potent inducer. This apparent organ specificity of the thyromimetic effect induced by xenobiotic amphipathic carboxylates is indeed in line with the liver specificity reported for the transcriptional activation of malic enzyme by DHEA [52]. These dissimilarities between the characteristics of T_3 and peroxisomal proliferators may thus indicate that liver transcriptional activation induced by non-thyroidal amphipathic carboxylates does not directly involve the thyroid hormone nuclear receptor and is presumably mediated by a mechanism of action which differs from that initiated by thyroid hormones proper.

The thyromimetic effects of peroxisomal proliferators may indicate that, in spite of the structural diversity involved, thyroid hormones and xenobiotic amphipathic carboxylates may be grouped into a unified class of compounds structurally defined as having a carboxylic function carried on a hydrophobic backbone and functionally defined by the spectrum of liver activities induced by members of the series. The broad spectrum of compounds that may be considered to be members of the above class could serve as a basis for promoting and extending the synthesis of thyroid hormone functional analogues having a structure totally different from that of the iodinated diphenoxy backbone but that are still active as liver thyromimetic agents. The lower efficacy of these analogues as compared with thyroid hormones could be offset by the advantageous selectivity of such agents with respect to tissue specificity and differential action.

Note added in proof (received 29 January 1991)

A member of the steroid hormone receptor superfamily activated by a diverse class of peroximal proliferators has been recently cloned [53]. Being a member of the steroid hormone receptor superfamily makes this receptor a candidate of choice for transducing the thyromimetic effect of peroxisomal proliferators reported here.

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