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FACTORS WHICH MAY BE SIGNIFICANT REGARDING REGULATION OF THE CLOFIBRATE-DEPENDENT INDUCTION OF HEPATIC PEROXISOMAL β-OXIDATION AND HEPATOMEGALY

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Abstract—The stimulation of hepatic polyamine metabolism observed 5 hr following intraperitoneal injection of clofibrate to rats was completely abolished following prior treatment with α-difluoromethylornithine. No induction of peroxisomal β-oxidation could be observed 5 hr after injection of clofibrate, although appreciable induction occurred 10 hr after injection. Prior treatment with difluoromethylornithine partially inhibited this induction. On chronic treatment with clofibrate together with difluoro-methylornithine, clofibrate-dependent induction of peroxisomal β-oxidation, as well as of the hepatomegaly, was partially inhibited. In hypophysectomized rats, no stimulation of polyamine metabolism was found following acute administration of a single dose of clofibrate. In thyroidectomized and in adrenalectomized animals, this stimulation was apparent, although the levels of activity were only some 10% of control levels. In hypophysectomized, in thyroidectomized and in adrenalectomized rats, appreciable induction of peroxisomal β-oxidation occurred on chronic treatment with clofibrate. However, no hepatomegaly was observed in these animals.

Administration of the hypolipidaemic drug clofibrate [2-(4-chlorophenoxy)-methylpropionate] causes profound alteration of lipid metabolism in the liver (for review see ref. [1]) as well as in other organs [2]. Clofibrate, like several other hypolipidaemic agents, has also been found to cause hepatomegaly and marked proliferation of rat liver peroxisomes [3, 4]. The activity of the peroxisomal β-oxidative system is markedly increased as well [5, 6].

Ornithine decarboxylase (EC 4.1.1.17), the initial and rate-limiting enzyme in polyamine biosynthesis, is rapidly induced in response to a variety of growth-promoting stimuli such as trophic hormones [7, 8], mitogens [9] and partial hepatoectomy [8, 10]. Ornithine decarboxylase activity appears to reach a maximum 4–5 hr after stimulation. A similar observation has been made following injection of clofibrate [11]. Several different conditions which cause changes in ornithine decarboxylase activity can be correlated with changes in protein biosynthesis [12]. Thus it is possible that induction of peroxisomal β-oxidation may be dependent on an initial stimulation of polyamine metabolism.

Some experiments designed to elucidate these possibilities are reported here. These studies include experiments with D,L-α-difluoro-methylornithine, which has been recently shown to be an enzyme-activated, irreversible inhibitor of ornithine decarboxylase [13–15]. Difluoro-methylornithine has been reported to be a selective inhibitor of ornithine decarboxylase, and to be non-toxic when given to experimental animals [15, 16].

Thyroidectomy has been shown to diminish some of the pharmacological effects of clofibrate [17]. Both thyroidectomy and adrenalectomy, however, appear to have only marginal effects on clofibrate-dependent peroxisomal proliferation [18]. We have therefore examined the effects of hypophysectomy, adrenalectomy and thyroidectomy on some of the clofibrate-dependent changes observed in rat liver.

MATERIALS AND METHODS

Reagents. D,L-[1-^{14}C]-Ornithine hydrochloride was purchased from The Radiochemical Centre (Amersham, U.K.) and *S*-[carboxyl-^{14}C]adenosyl-L-methionine from New England Nuclear (Boston, MA). Peroxidase (type II) was purchased from Sigma Chemical Co (St. Louis, MO). Palmitoyl-CoA was synthesized and assayed as described previously [19]. Dimethyl sulphoxide (Puriss) and clofibrate were obtained from Fluka A.G. (Buchs, Switzerland). D,L-α-Difluoro-methylornithine was kindly donated by Centre de Recherche, Merell International (Strasbourg, France).

Animals. All rats (male) were of the albino Wistar breed. Normal rats (220–350 g), if not otherwise stated, were obtained from the Unit for Laboratory Animals, The Norwegian College of Veterinary Medicine. Hypophysectomized (140–260 g), adrenalectomized (145–240 g) and thyroidectomized rats (140–210 g) and some untreated control rats (140–260 g) were purchased from Veterinær Möllegaards Avlsstation (Havdrup, Denmark) (all male Wistar albino rats). These were used *ca.* 10 days after surgery. All rats were given a standard pelletted laboratory diet and water *ad lib.* Adrenalectomized rats were given drinking water containing 1% (w/v) NaCl. The rats were kept at 22°, relative humidity being between 40 and 60%, and with lighting between

8 a.m. and 8 p.m. All animals were sacrificed between 1 and 2 p.m.

Single doses of clofibrate (285 mg/kg body wt, dissolved in dimethyl sulphoxide) and difluoromethylornithine (100 mg/kg body wt, dissolved in physiological saline) were injected intraperitoneally.

For chronic treatment with clofibrate, rats were fed a standard pelleted diet supplemented with 0.3% (w/v) clofibrate, prepared as described [1]. For chronic treatment with difluoro-methylornithine, the substance was added to drinking water (2%, w/v). Animals treated chronically with difluoro-methylornithine showed an increase in body wt which was about half that of animals treated with clofibrate alone, or of control animals. These animals appeared healthy otherwise.

Control animals were always injected with identical volumes of saline or dimethyl sulphoxide.

Enzyme assays. Ornithine decarboxylase activity was assayed by measuring the amount of $^{14}CO_2$ produced from [1-^{14}C]ornithine. The assay mixture contained [1-^{14}C]ornithine (sp. act. 2.5 μCi/μmole) at a concentration of 0.1 mM. The assays were otherwise carried out as described [20, 21].

S-Adenosyl-methionine decarboxylase activity (EC 4.1.1.50) was measured as described for ornithine decarboxylase activity, except that the assay mixture contained *S*-[carboxyl-^{14}C]adenosyl-L-methionine (sp. act. 1.0 μCi/μmole) at a concentration of 0.2 mM, together with 2.5 mM putrescine and 40 μM pyridoxal phosphate. For ornithine decarboxylase assays, 33% (w/v) liver homogenates were prepared using a medium composed of 250 mM sucrose, 2 mM EDTA, 5 mM dithiothreitol and 0.2 mM pyridoxal phosphate. The homogenization medium used for assays of *S*-adenosyl-methionine decarboxylase had only 0.40 μM pyridoxal phosphate, but was otherwise identical to that used for the homogenization of liver samples for ornithine decarboxylase assays. Homogenization was carried out for 15 sec using an Ultraturrax homogenizer. The homogenates were centrifuged at 20,000 *g* for 30 min, and the resulting supernatants used for enzyme assays. All operations were carried out at 0–4°.

Catalase (EC 1.11.1.6) was assayed as previously described [22]. Peroxisomal β-oxidation was measured as palmitoyl-CoA-dependent NAD^+ reduction [19], with the exception that 25 μM flavine-adenine-dinucleotide was added to ensure optical rates of peroxisomal β-oxidation. Hepatic capacities for peroxisomal β-oxidation were estimated from measurements of peroxisomal β-oxidative and catalase activities. Peroxisomal β-oxidative activity was estimated in gradient fractions with high β-oxidative activity derived from density gradient centrifugation of a subcellular fraction in self-generated Percoll gradients. Measurements of catalase activity were performed in the crude liver homogenate. The details of this procedure have been given elsewhere [23, 24]. In addition, peroxisomal β-oxidation was measured as acyl-CoA oxidase activity as described [25], using 100 μM palmitoyl-CoA as substrate.

Protein assays. Proteins were assayed using the Bio-Rad protein assay kit (Bio-Rad Laboratories), with freeze-dried bovine γ-globulins as standards.

Measurements of liver content of polyamines. The hepatic contents of putrescine, spermidine and spermine were measured by means of high performance liquid chromatography of the dansylated derivatives. A direct dansylation method was used [26] as modified in ref. [27]. The chromatographic procedure was essentially similar to that described in ref. [28], but was carried out on a Radial-PAK-A column using a Waters Associates chromatograph equipped with a fluorescence detector. The chromatograph was also fitted with a data system which integrated the eluted mass peaks.

Statistical analysis. The statistical significance of differences between population means was evaluated by one-way analysis of variance, using the Bonferroni modification of the *t*-test [29].

RESULTS

Effects of acute administration of clofibrate on the hepatic activities of ornithine decarboxylase and S-adenosyl-methionine decarboxylase, and on the liver content of polyamines

The hepatic activities of ornithine decarboxylase and *S*-adenosyl-methionine decarboxylase were measured at various times after intraperitoneal injection of clofibrate (285 mg/kg body wt). The results are shown in Fig. 1. As expected, a peak of ornithine decarboxylase activity was observed 5 hr post-injection. The activity of *S*-adenosyl-methionine decarboxylase showed a small but statistically significant increase 8 hr post-injection.

Experiments with an inhibitor of protein biosynthesis (cycloheximide, 50 mg/kg body wt) showed that $t_{\frac{1}{2}}$ for ornithine decarboxylase activity after clofibrate stimulation was *ca* 15 min (data not shown). This finding suggests that the increased ornithine decarboxylase activity was due to *de novo* synthesis of the enzyme, and is in agreement with previous reports regarding stimulated ornithine decarboxylase activity [30].

In Fig. 2 the results showing the effects of intraperitoneal injection of clofibrate on hepatic levels of putrescine, spermidine and spermine at various times after injection are presented. These assays were carried out in parallel with the measurements of enzymic activities shown in Fig. 1. The concentration of putrescine increased following the injection, also reaching a peak at *ca* 5 hr. Hence changes in putrescine concentrations reflect alterations in ornithine decarboxylase activity (Fig. 1). With the exception of a small but significant decrease in the concentration of spermidine three hours after injection, there were no marked changes in the concentrations of spermidine or spermine during the first 8 hr after injection of clofibrate.

Effects of acute administration of difluoro-methylornithine on clofibrate-dependent stimulation of ornithine decarboxylase, and on the hepatic levels of polyamines

Table 1 shows that intraperitoneal injection of difluoro-methylornithine 2 hr prior to intraperitoneal injection of clofibrate completely abolished the clofibrate-dependent stimulation of both ornithine decarboxylase activity and the increase in putrescine

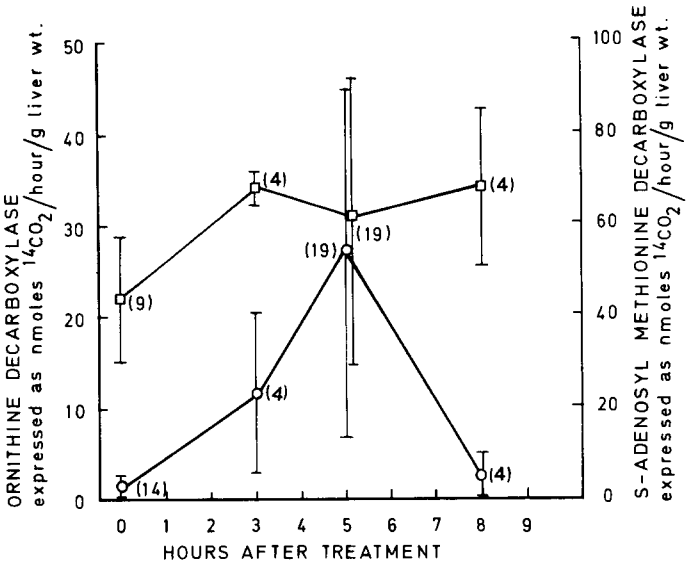

Fig. 1. Effects of intraperitoneal injection of clofibrate on liver activities of ornithine decarboxylase and *S*-adenosyl-methionine decarboxylase. The activities of ornithine decarboxylase (○–○) and *S*-adenosyl-methionine decarboxylase (□–□) were measured in rat liver preparations at various time intervals after intraperitoneal injection of clofibrate (285 mg/kg body wt). The plotted values represent the mean values derived from 4–19 rats, with S.D. indicated. The experimental details are described in Materials and Methods.

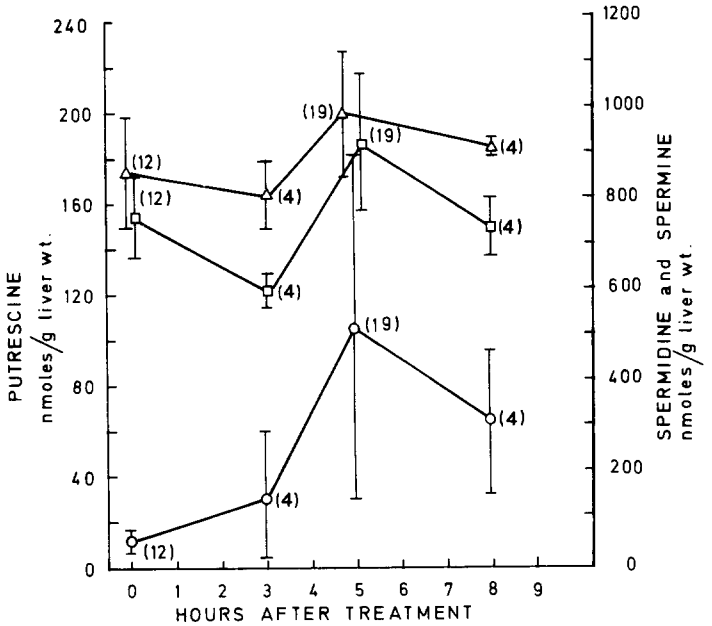

Fig. 2. Effects of intraperitoneal injection of clofibrate on hepatic levels of polyamines. The hepatic levels of putrescine (○–○), spermidine (□–□) and spermine (△–△) were measured at various time intervals following injection of clofibrate (285 mg/kg body wt). The plotted values represent means derived from measurements with 4–19 rats, with S.D. indicated. Experimental details are as described in Materials and Methods.

Table 1. Effects of intraperitoneal injection of α-difluoro-methylornithine on clofibrate-dependent stimulation of polyamine formation

Treatment	Ornithine decarboxylase activity (nmole $^{14}CO_2$/hr per g liver wt)	Putrescine	Spermidine (nmole/g liver wt)	Spermine
Clofibrate	26 ± 19* (3)	106 ± 76*	933 ± 150*	1002 ± 135
Clofibrate + difluoro-methylornithine	0.8 ± 0.3 (3)	14 ± 1	896 ± 25	1013 ± 20
Untreated	1.1 ± 0.7 (11)	13 ± 3	774 ± 90	873 ± 117

Liver ornithine decarboxylase activity, putrescine, spermidine and spermine levels were measured in the liver of rats which had been given intraperitoneal injections of α-difluoro-methylornithine (200 mg/kg body wt) 2 hr prior to intraperitoneal injection of clofibrate (285 mg/kg body wt). The rats were killed 5 hr after injection of clofibrate. The values presented represent mean values with S.E.M. indicated. The numbers in parentheses show the number of animals included in each experimental group. Experimental details are given in Materials and Methods.

* Indicates means significantly different from corresponding untreated mean.

levels, as measured 5 hr after clofibrate injection. No significant changes of the levels of spermine or spermidine were observed (Table 1). These findings are in agreement with the known inhibitory effect of difluoro-methylornithine [13].

Effects of clofibrate and difluoro-methylornithine on hepatic peroxisomal β-oxidation, and on polyamine metabolism

The results presented in Table 2 show that no increase in acyl-CoA oxidase activity was detectable 5 hr after a single intraperitoneal injection of clofibrate, although the expected stimulation of ornithine decarboxylase activity was apparent. Ten hours after the injection, a marked increase in acyl-CoA oxidase activity occurred, while the ornithine decarboxylase activity and polyamine levels (excepting spermidine) did not differ significantly from the control levels. In livers of rats treated with difluoro-methylornithine prior to injection of clofibrate, a significantly smaller increase in acyl-CoA oxidase activity was measured 10 hr after injection of clofibrate. Treatment with difluoro-methylornithine had no effect on the level of acyl-CoA oxidase activity measured 5 hr after injection of clofibrate (Table 2).

The effect of difluoro-methylornithine on clofibrate-dependent induction of peroxisomal β-oxidation was also investigated by treating rats with difluoro-methylornithine [2% (w/v) in drinking water] in parallel with chronic clofibrate treatment. After 9–11 days of treatment, hepatic peroxisomal β-oxidative activity was measured. From the results shown in Table 3 it can be seen that difluoro-methylornithine treatment led to a marked decrease in clofibrate-induced peroxisomal β-oxidation activity. This treatment did not, however, lower this activity to the level found in control rats (Table 3). Treatment with difluoro-methylornithine alone did not cause significant changes in peroxisomal β-oxidation as compared to the activity in control rats (data not shown). It can also be seen from the table that the mean liver wt, expressed as a percentage of the body wt, was significantly smaller in the group which had received difluoro-methylornithine in addition to clofibrate, compared to the rats which were given clofibrate alone. The relative liver wt was, however, still significantly higher than that for the untreated control group.

In Table 4 are presented the data showing the effects of long-term treatment with clofibrate, and with clofibrate plus difluoro-methylornithine, on hepatic ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities.

Effects of hypophysectomy, thyroidectomy and adrenalectomy on clofibrate-dependent stimulation of hepatic ornithine decarboxylase activity, and on hepatic polyamine concentrations

The results shown in Table 5 demonstrate that ornithine decarboxylase activity is dependent on the presence of the hypophysis and the thyroid gland. Hypophysectomized animals showed ornithine decarboxylase activities of less than 10% of the level found in the normal controls, and the ornithine decarboxylase activity was not altered during the first 5 hr when hypophysectomized animals were injected with clofibrate (Table 5). Similar results have been reported [31] regarding the effect of hypophysectomy upon stimulation of ornithine decarboxylase activity following partial hepatectomy.

In thyroidectomized animals, the control ornithine decarboxylase activity was *ca* 10% of that found in normal animals, confirming the finding of Chideckel *et al.* [32].

Both in thyroidectomized and adrenalectomized animals, a small, absolute stimulation of ornithine decarboxylase activity was observed 5 hr after injection of clofibrate. This amounted to a 10- to 40-fold stimulation as compared to basal levels (Table 5). In contrast, the stimulation of ornithine decarboxylase activity caused by partial hepatectomy has been reported to be unaffected by these treatments [31].

In control adrenalectomized animals, ornithine decarboxylase activity was not significantly different from that found in normal animals. Three of the ten untreated adrenalectomized animals, however, showed markedly increased ornithine decarboxylase activity (data not shown). The reason why is not clear.

As was found for ornithine decarboxylase, no changes were observed in the hepatic levels of polyamines in hypophysectomized rats following in-

Table 2. Short-term effects of injections of clofibrate and difluoro-methylornithine on hepatic polyamine metabolism and acyl-CoA oxidase activity

Time after injection (hr)		Ornithine decarboxylase activity (nmole $^{14}CO_2$/hr per g liver wt)	Putrescine	Spermidine (nmole/g liver wt)	Spermine	Acyl-CoA oxidase activity (nmole H_2O_2 formed/min per mg protein)
0	(4)	5 ± 1	36 ± 6	1147 ± 45	1077 ± 49	0.62 ± 0.10
5	(3)	40 ± 10*	100 ± 2*	974 ± 66*	1310 ± 163	0.61 ± 0.05
5	(2)	0.6–1.0	10–12	875–898	945–1072	0.5– – 0.8
(+DFMO)						
10	(4)	4 ± 1	24 ± 8	1252 ± 36*	1337 ± 130	2.3 ± 0.10*
10	(3)	3 ± 0.2	20 ± 5	989 ± 8*	1300 ± 150	1.6 ± 0.20*
(+DFMO)						

Rats were given single intraperitoneal injections of clofibrate (285 mg/kg body wt). Five or 10 hr after injection, the animals were killed, and hepatic acyl-CoA oxidase and ornithine decarboxylase activities and the hepatic levels of polyamines were measured. Some rats were given intraperitoneal injections of difluoro-methylornithine 2 hr prior to injection of clofibrate. If measurements were to be carried out 10 hr after the clofibrate injection, the rats were also given a further injection of difluoro-methylornithine (DFMO) 5 hr post-injection. The clofibrate-dependent stimulation of polyamine metabolism was abolished by difluoro-methylornithine (see Table 1). The tabulated values represent mean values, with S.D. indicated. The number of animals is shown in parentheses. Experimental details are given in Materials and Methods.

* Values significantly different from zero-time values.

jection of clofibrate (Table 5). Compared with normal rats, the hypophysectomized animals showed significantly higher levels of spermine and lower levels of spermidine (Table 5). This is in agreement with previous findings [8, 33].

Five hours after injection of clofibrate into thyroidectomized rats, the hepatic levels for putrescine and spermidine exhibited a significant increase, compared to thyroidectomized control rats. The spermine levels, however, remained unaffected (Table 5). The increased putrescine level was still much lower than that found in normal rats treated with clofibrate.

In adrenalectomized rats, the hepatic spermidine and spermine levels were significantly higher than in normal rats, but the levels did not change 5 hr after clofibrate injection (Table 5). The hepatic putrescine level was similar to that of normal controls, and injection of clofibrate gave a minor increase in the putrescine level compared to that found in untreated animals. Treatment with clofibrate for 8 days resulted in no further changes in the hepatic content of polyamines (Table 5) except in the levels of putrescine in adrenalectomized rats.

Effect of hypophysectomy, thyroidectomy and adrenalectomy on clofibrate-dependent induction of peroxisomal β-oxidation

Rats which had been hypophysectomized, adrenalectomized, or thyroidectomized were fed a clofibrate-supplemented diet for 8 days. In Table 6 are presented the values for the specific activity of acyl-CoA oxidase measured in liver homogenates, as well as the values for body and liver wts. These results show that marked induction of acyl-CoA oxidase activity took place in the livers of all three groups. The highest activity was found in the thyroidectomized and the hypophysectomized animals, although the reason why is not clear. In spite of this increased activity, no hepatomegaly was found in any of the three groups.

For thyroidectomized animals, a relatively high rate of acyl-CoA oxidase activity was found in those rats which had not been given clofibrate (Table 6).

DISCUSSION

Clofibrate [11], as well as other hypolipidaemic agents [34], has been shown to cause stimulation of ornithine decarboxylase activity in rat liver following injection of a single dose. In addition, our data show that changes in both ornithine decarboxylase and *S*-adenosyl-methionine decarboxylase activities are mirrored in the levels of hepatic polyamines (Figs. 1 and 2).

Chronic treatment with clofibrate did not alter these two enzymic activities. When clofibrate was given together with difluoro-methylornithine (Table 4), the *S*-adenosyl-methionine decarboxylase activity was increased. This is in agreement with earlier findings after treatment with difluoro-methylornithine alone [35]. During 11 days of clofibrate treatment, however, increased hepatic levels of putrescine and spermidine were found, while the levels of spermine were lower than in the controls (unpublished data). This may be due to altered catabolism of spermine.

Table 3. Effects of chronic treatment with difluoro-methylornithine on clofibrate-dependent induction of hepatic capacity for peroxisomal β-oxidation

Treatment		Liver wt (g)	Liver wt (as % of body wt)	Peroxisomal β-oxidative capacity (μmole NAD^+ reduced/min) per liver	per g liver wt
Control	(4)	9 ± 1	3.1 ± 0.3	2.9 ± 0.5	0.3 ± 0.1
Clofibrate	(4)	15 ± 1*	5.6 ± 0.3*	54 ± 38*	3.6 ± 2.5*
Clofibrate + difluoro-methylornithine	(4)	11 ± 2	4.5 ± 0.5*	18 ± 3*	1.6 ± 0.4

Rats were fed a clofibrate-supplemented diet for 9–11 days. One group of animals was given drinking water supplemented with difluoro-methylornithine. A control group was given normal diet and drinking water. Hepatic capacities for peroxisomal β-oxidation were estimated, and the resulting means have been tabulated together with S.D. The numbers in parentheses represent the number of experimental animals in each group. Treatment with difluoro-methylornithine alone had no significant effect on β-oxidative capacity (data not shown). Means labelled with * are significantly different from those of the control group. On termination of treatment, the body wt of the animals treated with difluoro-methylornithine was *ca.* 84% of that of animals not given this drug. The daily food intake by difluoro-methylornithine-treated animals was, however, 83% of that of the other animals. Hence the dosage of clofibrate per kg of body wt should be very similar for all groups. Experimental details are described in Materials and Methods.

Table 4. Effects of chronic treatment with clofibrate, or with clofibrate together with difluoro-methylornithine on hepatic activities of ornithine decarboxylase and *S*-adenosyl-methionine decarboxylase

Treatment	Ornithine decarboxylase activity	*S*-Adenosyl-methionine decarboxylase activity
	(nmole $^{14}CO_2$ produced/hr per mg liver)	
Control	1.1 ± 0.7 (14)	43 ± 14 (9)
Clofibrate	1.8 ± 1.1 (6)	39 ± 5 (6)
Control + difluoro-methylornithine	0.5–0.8 (2)	66–127 (2)
Clofibrate + difluoro-methylornithine	1.0 ± 0.7 (9)	84 ± 40* (9)

The activities of ornithine decarboxylase and *S*-adenosyl methionine decarboxylase were measured in liver homogenates derived from rats treated with clofibrate alone, or with clofibrate plus difluoro-methylornithine for 8–11 days. The tabulated values represent means, with S.D. indicated, for the number of animals indicated in parentheses. Experimental details are given in Materials and Methods.
* Means significantly different from control values.

Except for spermidine in hypophysectomized rats, the hepatic levels of polyamines in ectomized animals were similar to, or higher than, the levels found in untreated rats; this in spite of decreased hepatic ornithine decarboxylase activity. This suggests that ectomized animals can maintain their polyamine levels by other mechanisms.

The clofibrate-dependent stimulation of polyamine metabolism shows similarities to the stimulation caused by a more general growth promoting stimulus, like growth hormone or partial hepatectomy [7, 8]. Whether these stimuli also have similarities as regards the effects on hepatic peroxisomes is not clear, although partial hepatectomy may cause intermittent peroxisomal proliferation [36]. Data on peroxisomal β-oxidative activity, however, appear to be unavailable. It is known that partial hepatectomy causes no marked change in hepatic polyamine oxidative activity which is located at the peroxisome [37].

The results presented here show clearly also that hormones secreted by the adrenals, the hypophysis and the thyroid are not essential for clofibrate-dependent induction of peroxisomal β-oxidation (Table 6). Hormones from any one of these glands are, however, required for expression of clofibrate-mediated hepatomegaly.

The relatively high rate of acyl-CoA oxidase found in untreated thyroidectomized animals (Table 6) was surprising. The findings [17] that thyroidectomized rats have a markedly lower serum triglyceride level than normal rats may be of relevance in this context. This supports the suggestion that the hypertriglyceridaemic mechanism of action of clofibrate involves induction of peroxisomal β-oxidation, while hepatomegaly is not necessarily involved, as proposed [38]. To what extent the increased activity of peroxisomal β-oxidation observed in the ectomized animals also entails peroxisomal proliferation remains to be settled. Svoboda *et al.* [18] found that clofibrate-dependent peroxisomal proliferation did not appear to be markedly altered by thyroidectomy or adrenalectomy. However, Lazarow *et al.* [38] have reported that substantial induction of peroxisomal β-oxidation is possible without marked peroxisomal proliferation, although the drug they used was bezafibrate.

Table 5. Effect of hypophysectomy, thyroidectomy and adrenalectomy on clofibrate-dependent stimulation of ornithine decarboxylase activity and on liver levels of polyamines

Treatment	Ornithine decarboxylase activity (nmole $^{14}CO_2$/hr per g liver wt) Control		Clofibrate		Putrescine (nmole/g liver wt) Control	Clofibrate	Spermidine (nmole/g liver wt) Control	Clofibrate	Spermine (nmole/g liver wt) Control	Clofibrate
5 Hr										
Hypophysectomy	0.09 ± 0.07*	(7)	0.04 ± 0.1*	(4)	10 ± 6	11 ± 2*	572 ± 44*	578 ± 109*	1313 ± 90*	1290 ± 74*
Thyroidectomy	0.07 ± 0.02*	(3)	2.9 ± 1.7*	(8)	9.7 ± 1	16 ± 6*	701 ± 155	876 ± 108	1108 ± 34*	1058 ± 167
Adrenalectomy	0.53 ± 0.17	(6)	4.2 ± 3.2*	(10)	23 ± 9*	39 ± 19*	1104 ± 177*	1014 ± 124	1236 ± 193*	1273 ± 109*
Untreated	1.0 ± 0.5	(11)	34 ± 10	(13)	13 ± 3	139 ± 77	774 ± 90	971 ± 148	873 ± 117	959 ± 204
8 Days										
Hypophysectomy	—	(3)	—	(4)	19 ± 2	20 ± 3	650 ± 60	733 ± 88	1357 ± 209	1448 ± 89
Thyroidectomy	—	(3)	—	(4)	23 ± 12	23 ± 4	711 ± 72	1062 ± 83	1241 ± 292	1373 ± 129
Adrenalectomy	—	(3)	—	(4)	51 ± 4	26 ± 2	1158 ± 143	1218 ± 141	1338 ± 133	1353 ± 111
Untreated	—	(4)	—	(5)	17 ± 7	36 ± 7	1158 ± 85	1193 ± 114	1030 ± 118	940 ± 86

Hypophysectomized, thyroidectomized or adrenalectomized rats were given intraperitoneal injections of clofibrate (285 mg/kg body wt). After 5 hr, liver ornithine decarboxylase activities were assayed, and the hepatic levels of polyamines measured. The tabulated values represent mean values, with S.E.M. indicated. The numbers in parentheses are the number of animals included in each experimental group. Values significantly different from those obtained with the untreated control group are labelled with an *. Also the ornithine decarboxylase activity found in thyroidectomized and in adrenalectomized rats which had been injected with clofibrate were significantly different from the values measured in animals not given clofibrate. Control animals were always injected with similar volumes of dimethyl sulphoxide. Experimental details are given in Materials and Methods.

Table 6. Effects of adrenalectomy, hypophysectomy and thyroidectomy on clofibrate-dependent induction of hepatomegaly and acyl-CoA oxidase activity

Treatment			Body wt (g)	Liver wt (g)	Relative liver wt (%)	Acyl-CoA oxidase activity (nmole H_2O_2 produced/min per mg protein)
Adrenalectomy	Control	(3)	245 ± 22	12.8 ± 2.1	5.2 ± 0.1	1.0 ± 0.6
	Clofibrate	(4)	202 ± 16	9.8 ± 1.4	4.8 ± 0.4	3.7 ± 0.4*
Hypophysectomy	Control	(3)	147 ± 4	5.6 ± 0.6	3.8 ± 0.3	0.8 ± 0.6
	Clofibrate	(4)	142 ± 4	5.6 ± 0.3	3.9 ± 0.4	6.6 ± 2.6*
Thyroidectomy	Control	(3)	194 ± 3	8.8 ± 2.0	4.5 ± 0.9	2.3 ± 0.5
	Clofibrate	(4)	209 ± 3	10.1 ± 1.2	4.8 ± 0.7	7.4 ± 2.7*
Untreated	Control	(11)	230 ± 26	10.9 ± 1.7	4.7 ± 0.2	0.8 ± 0.5
	Clofibrate	(14)	228 ± 28	13.0 ± 2.1	5.7 ± 0.4*	3.1 ± 0.7*

Rats which had been adrenalectomized, hypophysectomized or thyroidectomized were fed a diet supplemented with 0.3% clofibrate for 8 days. Acyl-CoA oxidase activity was measured in homogenates prepared from livers of these rats. The tabulated values represent means, with S.D. indicated. The numbers in parentheses are the number of rats in the various groups.

* Means which are significantly different from the corresponding control mean.

The inhibitory effect of treatment with difluoromethylornithine on induction of peroxisomal β-oxidation (Tables 3 and 4) suggests that the metabolic events associated with the transient, clofibrate-dependent stimulation or ornithine decarboxylase activity are related to the induction of peroxisomal β-oxidation. The effect of the difluoro-methylornithine treatment was to decrease both the induction of peroxisomal β-oxidative activity and the hepatomegaly, and was therefore different from that of the ectomies, which completely abolished the hepatomegaly only. However, the ectomies also abolished, or markedly diminished, the clofibrate-dependent stimulation of polyamine metabolism (Table 5).

The conclusions which emerge from the present data are that the extent of induction of peroxisomal β-oxidative activity by clofibrate can be decreased by inhibiting normal polyamine metabolism, and that this activity can also be induced via a mechanism which is not critically dependent on the normal polyamine metabolism. As far as expression of clofibrate-dependent hepatomegaly is concerned, this response clearly shows a more marked dependence on normal polyamine metabolism.

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