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Effects of dietary polyamines and clofibrate on metabolism of polyamines in the rat

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The activities of catalase, polyamine oxidase, diamine oxidase, ornithine decarboxylase, and peroxisomal β-oxidation were assayed in homogenates from liver and small intestinal mucosa of rats which had been fed either a diet very low in polyamines or a diet containing five times the levels of dietary polyamines (putrescine, spermine, and spermidine) found in a standard rat diet. In rats fed the high polyamine diet, hepatic activities of catalase and polyamine oxidase were significantly decreased. Levels of the other activities were unchanged, except that intestinal ornithine decarboxylase was decreased. In rats treated simultaneously with clofibrate, the high polyamine diet restored activities of catalase, ornithine decarboxylase, and polyamine oxidase back to levels found in rats fed the low polyamine diet. The expected increase in activity of peroxisomal β-oxidation was observed, although this was somewhat diminished in rats fed the high polyamine diet. Intestinal diamine oxidase activity was stimulated by clofibrate, particularly in rats fed the high polyamine diet. For the duration of the experiment (20 days), levels of putrescine, spermine, and spermidine in blood remained remarkably constant irrespective of treatment, suggesting that polyamine homeostasis is essentially independent of dietary supply of polyamines. It is suggested that intestinal absorption/metabolism of polyamines is of significance in this respect. Treatment with clofibrate appeared to alter polyamine homeostasis. (J. Nutr. Biochem. 10:700–708, 1999)
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Introduction

Polyamines (i.e., putrescine, spermidine, and spermine) are found in every living cell and are believed to participate in cellular proliferation and differentiation.[1,2] Several food ingredients contain large quantities of polyamines.[3] It appears that cellular polyamines are derived from endogenous biosynthesis, as well as well as from the diet and from intestinal micro-organisms.[4]

In the mammalian organism, ornithine is the biosynthetic precursor of polyamines. Ornithine is initially decarboxylated by ornithine decarboxylase (ODC) (EC 4.1.1.17), forming putrescine. Spermidine is subsequently produced directly from putrescine by the action of spermidine synthase (EC 2.5.1.16), transferring a propylamine moiety from adenosyl-5′-methylthiopropylamine to putrescine. Spermine is similarly formed from spermidine by spermine synthase (EC 2.5.1.22), which catalyzes a reaction analogous to that of spermidine synthase. The mobilization and catabolism of polyamines is primarily carried out by polyamine oxidase (EC 1.5.3.3), although the N-acetylated polyamines now are the preferred substrates. An outline of polyamine metabolism is shown in *Figure 1*. A detailed account of polyamine metabolism has been given by Seiler.[5]

Polyamine metabolism has a peroxisomal connection because hepatic polyamine oxidase is localized to the peroxisomes in both liver and duodenum.[6] Fibrate-type drugs (e.g., clofibrate) bring about a dramatic proliferation of peroxisomes, most markedly in the liver, but also in the intestine.[7] Therefore, it is of interest to investigate whether peroxisomal proliferation and induction of peroxisomal metabolism in any way influences polyamine metabolism. Possible links between peroxisomal and polyamine metabolism have been presented previously (for a review see

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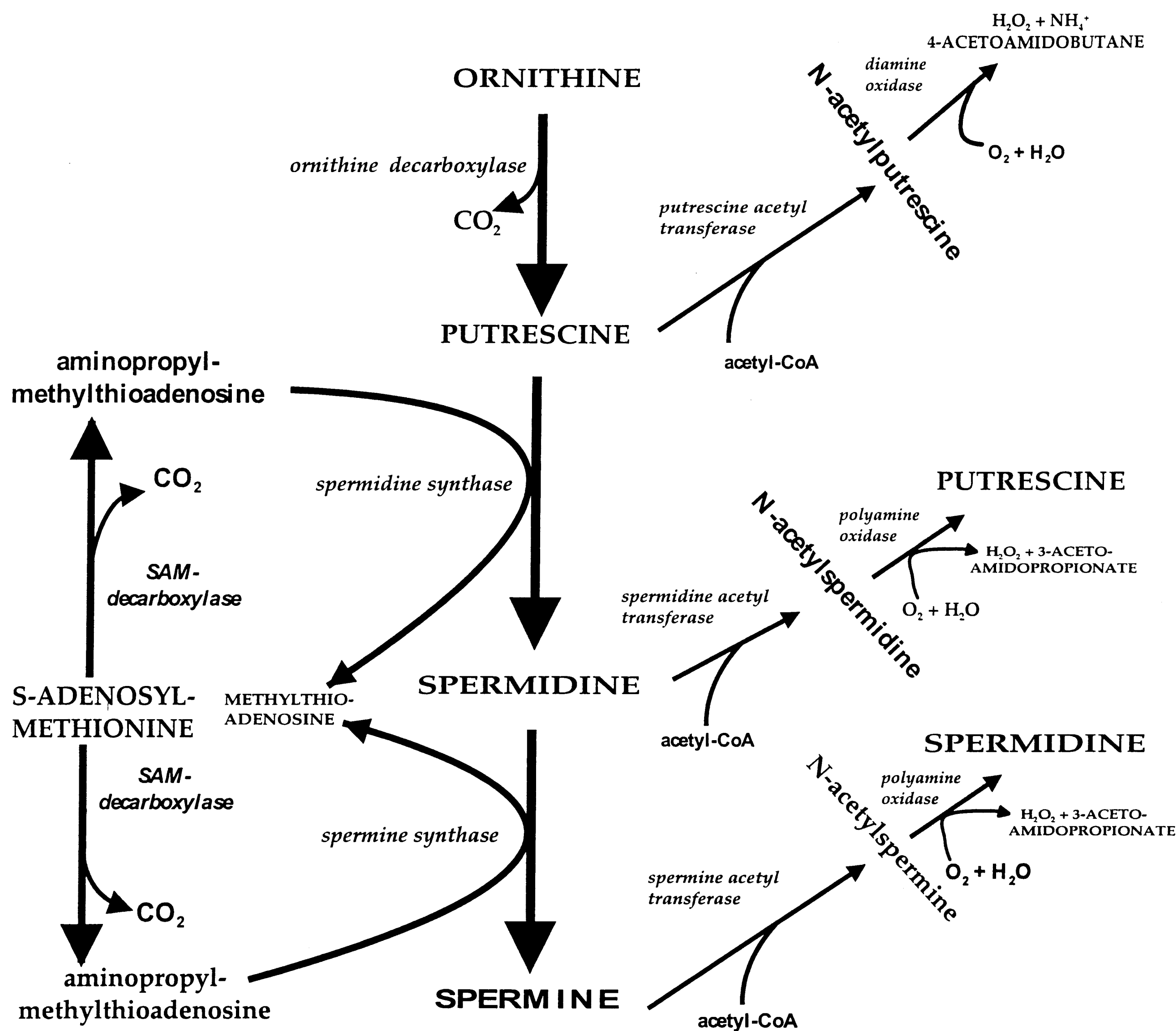

Figure 1 Polyamine metabolism.

Osmundsen et al.[8]), but much remains to be established. To this end we investigated the effects of clofibrate-induced peroxisomal proliferation on activities of enzymes involved in polyamine metabolism and on levels of polyamines in tissues.

Clofibrate-dependent induction of peroxisomal enzymes is associated with hepatomegaly in rats, and both of these events are markedly diminished when endogenous polyamine biosynthesis is inhibited by α-difluoromethylornithine.[9] Therefore, we also attempted to establish whether the level of dietary polyamines influenced clofibrate-dependent induction of peroxisomal enzymes. In addition, it is not clear if treatment with a peroxisomal proliferator (e.g., clofibrate) in conjunction with diets very low or high in polyamines can cause specific changes in activities of these enzymes and/or modify levels of polyamines in tissues. Therefore, we carried out experiments using rats fed a low or a high polyamine diet, with or without simultaneous treatment with clofibrate. Although liver was the primary tissue studied, some studies were also carried out on intestinal mucosa.

The results show that a high polyamine diet caused significantly decreased activities in hepatic catalase (EC 1.11.1.6) and polyamine oxidase, as well as intestinal ornithine decarboxylase activity. When a high polyamine diet was given together with clofibrate the observed decrease in activities were restored. The activity of intestinal diamine oxidase was markedly stimulated. The relative liver weight was unaltered by the polyamine diets.

Even in mice overexpressing ornithine decarboxylase, which is rate limiting for polyamine biosynthesis, homeostasis is maintained.[10] In general, it is likely that enhanced input of polyamines leads to a compensatory increased rate of catabolism. Likewise, when one route of supply is diminished, an alternative route of supply will be correspondingly enhanced.[5] Although α-difluoromethylor-

nithine, which is an irreversible inhibitor of ornithine decarboxylase, efficiently inhibits the enzyme in cell cultures, causing depletion of cellular polyamines and cessation of cell growth.[11] However, in vivo inhibition of ODC is less complete, as is the arrest of tissue growth.[9,12,13] Therefore, previous data suggests polyamine homeostasis to be remarkably resilient to external influence.

In view of the essential role for polyamines in cell growth it would appear likely that nature had ensured that polyamine homeostasis will be maintained unless an unlikely set of unfavorable phenomena occur simultaneously. In these experiments we have investigated if dietary level of polyamines alone, or combined with treatment with a peroxisomal proliferator (clofibrate), affects homeostasis as expressed by levels of putrescine, spermine, and spermidine in various tissues. Clofibrate is known to cause powerful induction of hepatomegaly and of peroxisomal proliferation and also may influence polyamine metabolism. Therefore, the combined treatment with a high polyamine diet and clofibrate may yield interesting data about polyamine homeostasis.

Materials and methods

Reagents

Horseradish peroxidase (type VI-A, 250 to 330 U/mg), N^1-acetylspermine, 4-aminoantipyrine, palmitoyl-CoA, NAD^+, FAD, HEPES, hexandiamine, putrescine 2HCl, spermidine 3HCl, spermine 4HCl, and mannitol were purchased from Sigma Chemical Co. (St. Louis, MO USA). Perhydrol (H_2O_2) was obtained from E. Merk (Darmstadt, Germany). Clofibrate was purchased from Fluka AG (Buchs, Switzerland). All other reagents were of analytical grade. [1-^{14}C]-Putrescine dihydrochloride (specific radioactivity 2.11 GBq/mmol) and DL-[1-^{14}C] ornithine HCl (specific radioactivity 4.00 GBq/mmol) were obtained from Amersham International plc. (Rainham, Essex, UK).

Rat diets

AIN-76A, a semi-synthetic, low polyamine diet, was purchased from Special Diets Services Ltd. (Essex, UK). This diet contained 50, 10, and 1 nmol/g of putrescine, spermidine, and spermine, respectively. A fraction of this diet was supplemented with additional putrescine, spermidine, and spermine. The polyamines were dissolved in enough water to cover a pre-weighed amount of AIN-76A fodder. After thorough mixing, the wet powdered fodder was shaped into small cakes and dried in a ventilated oven set at 50°C.

The amount of polyamines added to the fodder were defined according to a scale where one unit of polyamines (1PA) corresponded to the combination of 230 nmol/g of putrescine, 251 nmol/g of spermidine, and 30 nmol/g of spermine. One unit of polyamines corresponded to the levels of these polyamines as measured in a standard rat and mouse chow diet purchased from B & K Universal Ltd. (North Humberside, UK).[9] A fraction of the AIN-76A fodder was added to the polyamines to a level of 5PA units [i.e., corresponding to five times the levels found in regular fodder (high polyamine fodder)]. Analysis of polyamines in fodder supplemented with polyamines showed a variation of 4% between 10 g samples.

A pre-weighed amount of AIN-76A fodder was soaked in acetone containing clofibrate to a final level of 0.5% (w/w of fodder). The acetone was evaporated off, leaving the clofibrate-containing fodder. Fractions of clofibrate-supplemented fodder were subsequently added to the polyamines, as described above.

Experimental animals

Male Fisher (F344) rats were obtained from Halan Ltd. (UK). They were randomized and housed in plastic cages, with three animals per cage. The animal stable had light-dark cycles of 12 hours (8:00 AM to 8:00 PM). The relative humidity was 60%; the temperature was 21 to 22°C.

Groups of these animals were fed a semi-synthetic low polyamine or high polyamine fodder. Other groups were fed low or high polyamine fodder supplemented with clofibrate. The rats were maintained on these diets for 20 days. Water and fodder were given ad libitum. Their body weights ranged from 90 to 120 g at the start of the experiment, increasing to approximately 170 to 190 g at the end of the experimental period. The specific rates of growth were similar for all treatments groups (approximately 2.9 g/day), although the groups fed clofibrate showed a somewhat lower rate of growth.

Preparation of experimental samples

The rats were sacrificed by decapitation using a guillotine. Tissues for analysis were removed as soon as possible after decapitation. A 20-cm long proximal segment of the small intestine, starting approximately 0.5 cm beyond the pyloric sphincter, was removed and rinsed with ice-cold 0.9% (w/v) NaCl, blotted against a filter paper, and placed on an ice-cold glass surface. The intestinal segment was cut open longitudinally using a pair of fine scissors, and the mucosal layer was scraped off with a small glass slide. The resulting mucosa was weighed and suspended in 4 volumes of ice-cold mannitol medium [mannitol (300 mmol/L), HEPES (25 mmol/L), EGTA (1 mmol/L), pH 7.2]. This was immediately homogenized for 15 seconds with an Ultra Turrax (Janke and Kunkel, Staufen, Germany) at 25,000 rpm, and stored at −20°C. Prior to assay one part of ice-cold mannitol medium was added to one part of the homogenate, and the resulting suspension was made 0.05% (v/v) with regard to Triton X-100. It was subsequently centrifuged at 1,075 × g for 15 minutes. The resulting supernatant was used for all assays, except the ornithine decarboxylase assay.

Homogenates for ornithine decarboxylase assays were prepared as follows[14]: To 1 part of mucosa, 9 volumes of 0.1 mol/L phosphate buffer, pH 7.2, containing ethylenediaminetetraacetic acid (EDTA; 2 mmol/L), dithiothreitol (DTT) (5 mmol/L), and pyridoxalphosphate (0.2 mmol/L) was added, and homogenized with an Ultra Turrax at 25,000 rpm for 15 seconds. For liver 1 part tissue and 3 volumes of buffer were used. The homogenate was centrifuged for 30 minutes at 20,000 × g, and the supernatant was frozen at −80°C until used.

For other assays livers were rapidly removed, weighed, and transferred nto ice-cold mannitol medium (as described for preparation of intestinal homogenates). A 10% (w/v) homogenate was prepared by using two strokes in a Potter-Elvehjem homogenizer equipped with a Teflon piston. The homogenates were centrifuged for 1 minute at 1,075 × g. The resulting supernatants were divided into small vials and stored at −20°C. These were used in most enzyme assays.

The colon content for polyamine determination was prepared as follows: The colon was emptied by flushing with 10 mL ice-cold phosphate buffer saline, and the content was collected on a nylon net and was stored at −80°C until analyzed.

Enzyme assays

Catalase. H_2O_2:H_2O_2 oxidoreductase (catalase) activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm and at

25°C, essentially as described by Bergmeyer et al.[15] The assay mixture contained in a final volume of 2.55 mL: 2.0 mL potassium phosphate buffer (50 mmol/L), pH 7.4, 0.05 mL homogenate, and 0.5 mL H_2O_2 in potassium phosphate buffer (50 mmol/L). Because catalase is labile in diluted solutions, the homogenates were diluted 10 times with 20 mmol/L K-phosphate buffer, pH 7.4, immediately prior to assay.

Peroxisomal β-oxidation. Peroxisomal β-oxidation was assayed as palmitoyl-CoA-dependent NAD^+ reduction as described previously.[16]. An assay mixture of 900 μL, pH 7.5, consisted of phosphate buffer (30 mmol/L), FAD (20 μmol/L), NAD^+ (250 μmol/L), dithiotreitol (1 mmol/L), CoASH (200 μmol/L), Triton X-100 (0.005% v/v), and KCN (1 mmol/L). To the mixtures was added 50 μL homogenate, and the reaction started with addition of 10 μL palmitoyl-CoA (5 mmol/L). The reaction was performed at 30°C and the reaction rate was followed at 340 nm against a blank.

Diamine oxidase. Diamine:oxygen oxidoreductase (diamine oxidase; EC 1.4.3.6) was radiometrically determined by measuring [^{14}C]-pyrroline formed from [1-^{14}C]-putrescine.[17] The [^{14}C]-pyrroline was extracted by means of the scintillation liquid [toluene/PPO (0.35%)] and counted by a scintillation counter. The assay mixture (750 μL) contained phosphate buffer (200 mmol/L), pH 7.4, and [1-^{14}C]-putrescine (4.5 mmol/L, 8.22 kBq/μmol).

Polyamine oxidase. Polyamine:oxygen oxidoreductase (polyamine oxidase) was assayed spectrophotometrically.[18] The method was based on measurements of H_2O_2 generated during the oxidase reaction and converted to a chromophore by a peroxidase-coupled condensation of 4-aminoantipyrine (AAP) and phenol. The enzyme assay mixture was composed of glycine buffer (50 mmol/L), pH 9.5, AAP (82 mmol/L), phenol (10.6 mmol/L), peroxidase (4 iu), N^1-acetylspermine (5 mmol/L), 50 μL homogenate, and water to 1.0 mL. The reaction was performed at 30°C and started by addition of the homogenate, and the increase in absorbency at 500 nm was recorded.

Ornithine decarboxylase. Ornithine decarboxylase (EC 4.1.1.17) was assayed by measuring the amount of [$^{14}CO_2$] produced from [1-^{14}C]-ornithine. The enzyme assay of 2 mL was composed of phosphate buffer (0.1 mol/L), pH 7.2, EDTA (2 mmol/L), dithiotreitol (5 mmol/L), and pyridoxalphosphate (0.02 mmol/L). To the reaction mixture 0.1 mL [1-^{14}C]-ornithine of (18.5 MBq/mmol) was added. The ODC assay was otherwise carried out as described by Kobayashi et al.[19] and Maudsley et al.[20]

Assay of polyamines

The different tissue specimens and colon contents were weighed and homogenized in 4 volumes of 5% trichloroacetic acid, using an Ultra Turrax (25,000 rpm, for 30 sec). Hexandiamine was added as an internal standard, and the homogenate was kept on ice for 1 hour, followed by centrifugation at 2°C for 10 minutes at 5,000 × g. The supernatant was stored at −20°C until analyzed. The polyamines were dansylated[21,22] and separated by high performance liquid chromatography[17] on a Radial-PAK-A column (Waters, Milford, MA USA) by using a linear methanol/water gradient. The gradient composition was 65% methanol/35% water at start, increasing to 100% methanol in the course of 30 minutes. Dansylated polyamines were detected using a fluorescence detector, the excitation wavelength being 340 nm and emission wavelength being 510 nm.

Table 1. Effects of treatments on liver weight and relative liver weight

Treatment	Mean liver weight (g)	Relative liver weight (in % of bw)
Low PA (12)	8.35 ± 0.34	4.553 ± 0.075
5PA (6)	7.16 ± 0.19	4.273 ± 0.086
Clofibrate + low PA (9)	12.36 ± 0.51*	7.872 ± 0.157*
Clofibrate + 5PA (6)	13.43 ± 0.80*	8.072 ± 0.259*

Tabulated data represent means derived from the number of animals indicated by numbers in the parentheses. The low polyamine (PA) and clofibrate groups were given a diet very low in polyamines (AIN-76A diet), whereas 5PA and clofibrate + 5PA groups were given the same synthetic diet fortified with 5 times the concentration of polyamines found in a regular rat and mouse chow diet. Values for SEM are indicated.
*Significantly different from corresponding low polyamine means at levels of significance of $P < 0.01$. For details see Materials and methods.

Assay of proteins

Protein assays were carried out using the biuret assay,[23] with Boehringer Precimat (Boehringer, Mannheim, Germany) as protein standards.

Statistical analysis

The significance of differences between populations means was measured using Dunnett's multiple comparison test as a post-analysis of variance test. The GraphPad Prism v.2.0 program (GraphPad Software Inc., San Diego, CA USA) was used for the statistical analysis.

Results

Effects of dietary polyamines and clofibrate on relative liver weights

The results shown in *Table 1* demonstrate that a fodder supplemented with polyamines to a level that is five times higher than that of regular fodder had no significant effect on absolute, or relative, liver weights. On simultaneous treatment with clofibrate, the liver weights increased to the same extents as those in animals treated with clofibrate alone (*Table 1*). The relative liver weights of rats fed low polyamine fodder were similar to those found with rats fed on a regular fodder, suggesting that the dietary level of polyamines had no significant influence on this parameter (results not shown).

Effects of dietary polyamines and clofibrate on selected enzyme activities in liver and small intestine mucosa

Liver. Results presented in *Figure 2* show that hepatic catalase activity was affected by dietary polyamines. Rats treated with high polyamine diet alone exhibited an approximately 25% decrease in hepatic catalase activity. When treated with clofibrate alone, an expected increase in catalase activity was found, but there were no further significant changes in rats treated simultaneously with polyamines.

Polyamine oxidase activity also was affected by treat-

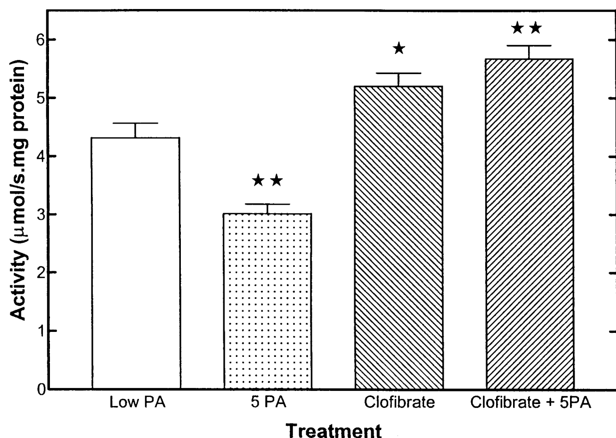

Figure 2 Effect of treatment with low or high (5PA) polyamine diets on hepatic catalase activity. Rats were treated with low or high polyamine diets as described in Materials and methods. Groups of animals were simultaneously also treated with clofibrate (striped columns). After 20 days of treatments the activity of hepatic catalase activity was assayed. Each experimental group consisted of at least 6 animals. The tabulated values are means with SEM indicated. Populations means that were significantly different from the low polyamine mean are denoted with * ($P < 0.05$) or ** ($P < 0.01$). Experimental details are otherwise given in Material and methods.

ment with polyamines. The activity was decreased by approximately 50% in livers of rats treated with a high polyamine diet (*Figure 3*). Clofibrate alone resulted in a significant decrease in polyamine oxidase activity. On the other hand, the polyamine oxidase activity was completely restored in rats treated with clofibrate plus polyamines.

The hepatic ornithine decarboxylase activity was not significantly altered by any of these treatments (results not shown).

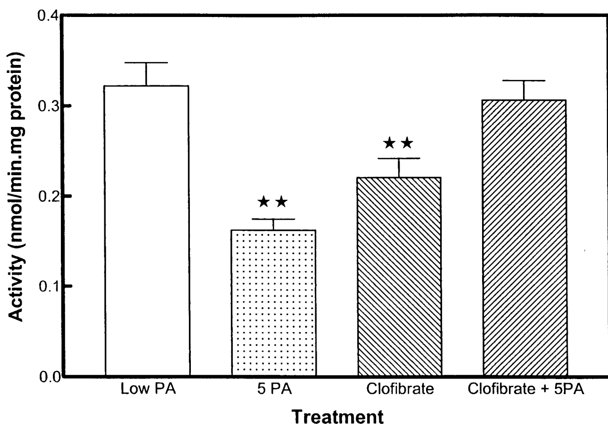

Figure 3 Effects of low (Low PA) or high (5PA) polyamine diets on hepatic polyamine oxidase activity. Rats were treated with low or high polyamine diets as described in Material and methods. Groups of animals were simultaneously also treated with clofibrate (striped columns). After 20 days of treatments the activity of hepatic polyamine oxidase activity was assayed. Populations means that were significantly different from the low polyamine mean are denoted with ** ($P < 0.01$). Experimental details are otherwise given in legend to *Figure 2*.

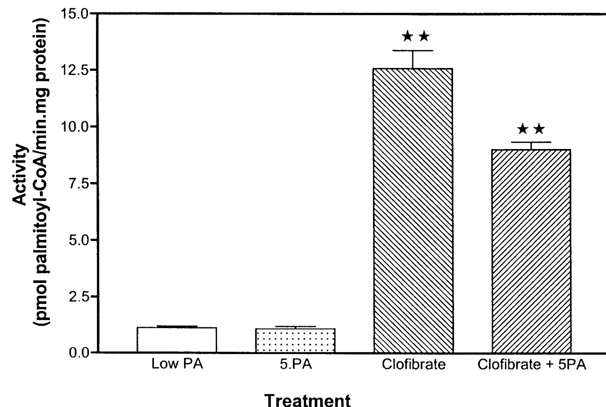

Figure 4 Effects of treatment with low (Low PA) or high (5PA) polyamine diets on activity of hepatic peroxisomal β-oxidation. Rats were treated with low or high polyamine diets as described in Materials and methods. Groups of animals were simultaneously also treated with clofibrate (striped columns). After 20 days of treatments the activity of hepatic catalase as assayed. Population means that were significantly different from the low polyamine mean are denoted with ** ($P < 0.01$). Experimental details are otherwise given in legend to *Figure 2*.

Neither a diet very low in polyamines nor high in polyamines caused significant changes in peroxisomal β-oxidation (*Figure 4*). Treatment with clofibrate gave an expected increase in activity of approximately 10-fold. However, in rats treated with both clofibrate and a high polyamine diet only an approximately 7-fold increase in this activity was observed (*Figure 4*).

Intestinal mucosa. We also examined effects of the various treatments on the corresponding activities in intestinal mucosa. An increase in catalase activity was always found when clofibrate was included in the treatment (*Figure 5*).

The intestinal ornithine decarboxylase activity, however, varied somewhat depending on treatment. In rats treated with a high polyamine diet alone the level of activity appeared markedly decreased, whereas treatments including clofibrate caused no significant changes in ornithine decarboxylase activity (*Figure 6*). This activity exhibited large inter-individual variation within each experimental group, giving population means with relatively large SEMs.

Diamine oxidase activity in the small intestine was not increased with the 5PA diet, but increased almost threefold following treatment with clofibrate alone and approximately eightfold after combined treatment of clofibrate with a high polyamine diet (*Figure 7*). Diamine oxidase activity in liver homogenates is known to be masked by competing reactions,[24] explaining why we observed very low activities (results not shown). No further diamine oxidase assays on liver homogenates were carried out.

These activities were also measured in rats fed on AIN-76A fodder contained 1PA unit (corresponding to the polyamine content of a standard chow). The results showed no significant differences between this group and that of rats fed the low polyamine diet (results not shown).

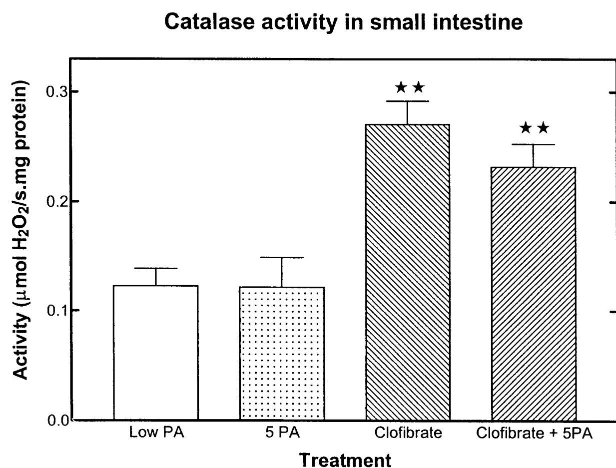

Figure 5 Effects of treatments with low (Low PA) or high (5PA) polyamine diets on intestinal catalase activity. Rats were treated with low or high polyamine diets as described in Material and methods. Groups of animals were simultaneously also treated with clofibrate (striped columns). After 20 days of treatments the activity of intestinal catalase as assayed. Populations means that were significantly different from the low polyamine mean are denoted with ** ($P < 0.01$). Experimental details are otherwise given in legend to *Figure 2*.

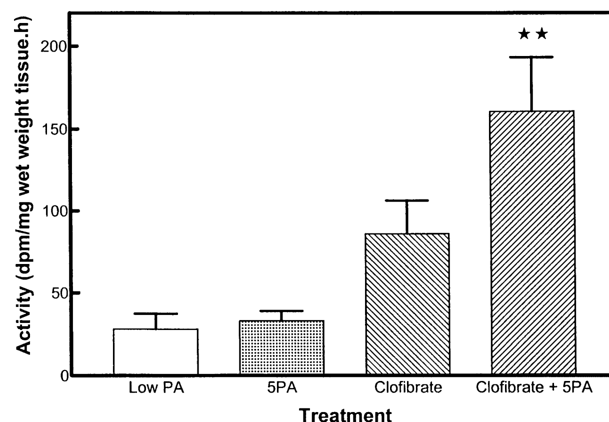

Figure 7 Effects of treatments with low (Low PA) or high (5PA) polyamine diets on intestinal diamine oxidase activity. Rats were treated with low or high polyamine diets as described in Material and methods. Groups of animals were simultaneously also treated with clofibrate (hatched columns). After 20 days of treatments the activity of intestinal diamine oxidase was assayed. Populations means that were significantly different from the low polyamine mean are denoted with ** ($P < 0.01$). Experimental details are otherwise given in legend to *Figure 2*.

Effects of the various treatments of levels of polyamines in colon content, liver, and blood

The high polyamine diet alone almost doubled the amounts of spermine and spermidine found in colon content (*Table 2*), whereas the amount of putrescine appears to have decreased. Treatment with clofibrate alone caused no significant changes in the amounts of polyamines in colon content, whereas the combined treatment of a high polyamine diet together with clofibrate also practically doubled the amounts of spermine and spermidine in colon content (*Table 2*) and the level of putrescine remained essentially unchanged.

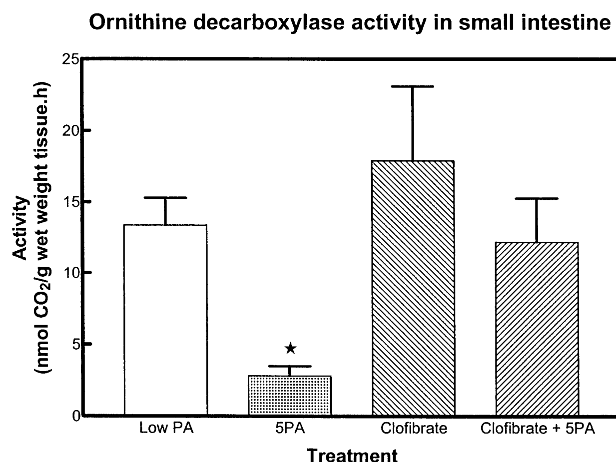

Figure 6 Effects of treatments with low (Low PA) or high (5PA) polyamine diets on intestinal ornithine decarboxylase activity. Rats were treated with low or high polyamine diets as described in Material and methods. Groups of animals were simultaneously also treated with clofibrate (striped columns). After 20 days of treatments the activity of intestinal ornithine decarboxylase was assayed. Populations means that were significantly different from the low polyamine mean are denoted with * ($P < 0.05$). Experimental details are otherwise given in legend to *Figure 2*.

Blood levels of polyamines, in contrast, remained much more invariant, irrespective of treatment. Treatment with clofibrate, or the combined treatment of clofibrate with a high polyamine diet, caused some a minor increase in levels of polyamines (*Table 2*). Only that of putrescine was statistically significant (*Table 2*).

In the liver the polyamine composition was rather different from that found in blood and in colon content where spermidine was by far the major constituent (*Table 2*). In the liver spermine and spermidine were present at similar levels (provided the rats had not been given a treatment including clofibrate), whereas that of putrescine was approximately 1/20 of their levels (*Table 2*). This is in agreement with previous findings.[25–27] Treatments involving clofibrate caused a significant increase in levels of putrescine and spermidine, whereas that of spermine was significantly decreased. This latter decrease was also observed in rats that had been treated with a high polyamine diet (*Table 2*).

These levels of polyamines were also measured in rats fed a fodder containing a "normal" level of polyamines (i.e., 1PA unit). The results showed no significant differences between this group and those of rats fed the low polyamine diet (results not shown). Therefore, the low polyamine diet does not appear to have caused significant permanent changes in levels of polyamines in liver and blood.

Discussion

Effects on enzyme activities

Dietary supplementation with polyamines, either alone or with clofibrate, had no influence on peroxisomal proliferation as judged by induction of hepatomegaly (*Table 1*). We

Table 2. Amounts of polyamines in blood, liver, and colon contents

Tissue/sample	Treatment	Putrescine (nmol/g weight)	Spermidine (nmol/g weight)	Spermine (nmol/g weight ± SEM)
Blood	Low PA	2.21 ± 0.13	44.670 ± 1.835	3.875 ± 0.332
	5PA	2.75 ± 0.25	39.170 ± 3.156	3.167 ± 0.477
	Clofibrate + low PA	2.57 ± 0.30	44.780 ± 3.635	4.000 ± 1.269
	Clofibrate + 5PA	4.50 ± 0.63*	52.000 ± 3.396	4.417 ± 0.779
Liver	Low PA	17.25 ± 2.05	806.50 ± 21.64	878.70 ± 23.66
	5PA	13.00 ± 0.73	797.80 ± 26.12	752.20 ± 45.19*
	Clofibrate + low PA	42.40 ± 5.88*	1009.00 ± 17.79*	647.10 ± 9.87*
	Clofibrate + 5PA	41.33 ± 3.32*	1046.00 ± 18.04*	703.30 ± 15.36*
Colon content	Low PA	58.95 ± 6.82	87.63 ± 5.84	39.72 ± 2.33
	5PA	37.83 ± 3.96	220.80 ± 20.31*	62.50 ± 4.37*
	Clofibrate + low PA	66.38 ± 6.79	90.98 ± 4.86	33.50 ± 3.85
	Clofibrate + 5PA	57.85 ± 5.67	230.60 ± 19.52*	63.95 ± 3.60*

The tabulated values are means derived from measurements on at least 6 experimental animals, with SEM indicated. For details see Materials and methods.
*Significantly different from corresponding low polyamines (PA) means at levels of significance of $P < 0.01$.
5PA–rats given a diet containing five fold the levels of polyamines found in a standard rat and mouse chow.

previously demonstrated that inhibition of polyamine biosynthesis in vivo, by administration of D,L-α-difluoromethylornithine, diminished the clofibrate-dependent induction of hepatomegaly.[9] The dietary level of polyamines appears to be of little significance in this respect. As far as the liver is concerned, this demonstrates that the endogenous supply of polyamines is more significant as regards hepatic polyamine homeostasis than exogenous polyamines. This contrasts the situation in intestinal tissues in which both endogenous and exogenous supplies of polyamines must be blocked to severely disrupt polyamine homeostasis.[28]

Peroxisomal oxidases

The activity of polyamine oxidase has generally been found to be invariant to conditions known to cause major changes in liver metabolism.[29] Our data show that enzymes are not induced by clofibrate (*Figure 3*), unlike many other peroxisomal oxidases. The straight chain palmitoyl-CoA oxidase is well known to be powerfully induced, and moderate induction of D-aspartate oxidase (EC 1.4.3.1) is observed (for a review see Kunau et al.[30]). On the other hand, other peroxisomal oxidases [e.g., glycollate oxidase (EC 1.1.3.15) or urate oxidase (EC 1.7.3.3)] are not induced.[31]

However, hepatic polyamine oxidase has been reported to be induced fourfold in iron-overloaded rats.[32] Most of the treatments used in the present experiments did not alter the activity of polyamine oxidase, except for a significantly decreased activity in rats on the high polyamine diet (*Figure 3*), which suggests that this activity is not entirely invariant. This finding concurs with the view that the primary function of polyamine oxidase is to oxidize N-acetylated spermine to spermidine, and N-acetylated spermidine to putrescine,[27,33,34] thereby mobilizing stores of polyamines (*Figure 1*). Hence, decreased activity of the enzyme is to be expected when the supply of dietary polyamines is abundant. Treatment with clofibrate alone, however, appeared to decrease polyamine oxidase activity somewhat (*Figure 3*). However, this decrease was completely reversed on treatment with both clofibrate and a high polyamine diet (*Figure 4*). The reason for this is not clear. With polyamine oxidase activity, no significant changes were found in the small intestine (results not shown).

Hepatic catalase exhibited an expected[35] small increase in activity on treatments involving clofibrate. By analogy with polyamine oxidase, a significant decrease in activity was also found in livers of rats given the high polyamine diet alone (*Figure 2*). The decrease in extent of induction of peroxisomal β-oxidation that was observed in rats given simultaneous treatment of clofibrate and a high polyamine diet (as compared with the level found in rats treated with clofibrate alone) was somewhat unexpected. The pattern of change was not similar to that of the other two oxidases that were assayed [e.g., polyamine oxidase (*Figure 3*) and catalase (*Figure 5*)]. This is not unusual, and serves to illustrate the degree of variation observed as regards the observed degrees of induction of hepatic peroxisomal oxidases on treatments causing peroxisomal proliferation.[30]

Although treatment with the high polyamine diet alone caused no significant change in intestinal diamine oxidase activity, an approximately threefold increase was observed in rats treated simultaneously with clofibrate and a high polyamine diet (*Figure 7*). The activity probably was also induced with treatment with clofibrate alone (*Figure 7*). Therefore, diamine oxidase and catalase activity responded similarly to these treatments (*Figure 5*), suggesting a similar control mechanism for these intestinal enzymes. Diamine oxidase is considered to function as a defense against high concentrations of diamines,[36] thus preventing uptake of polyamines (e.g., putrescine).[37] A high activity of diamine oxidase, however, results in high rates of production of H_2O_2. This would suggest that increased activity of catalase also is required in the intestine to avoid peroxidative damage to the mucosal cells. An increased dietary load of polyamines alone does not elicit this response. Our results indicate that a peroxisomal proliferator is required.

Hepatic diamine oxidase activity is difficult to assay because of the presence of very active competitive reactions masking the diamine oxidase activity.[24] The low activity we

observed in this tissue (results not shown) was therefore regarded as an artefact.

Ornithine decarboxylase activity. Only intestinal ornithine decarboxylase activity was significantly changed by treatment with a high polyamine diet: It was decreased by approximately 50% (*Figure 6*). Treatment with clofibrate reversed this effect, also when combined with the high polyamine diet. Acute variations in ornithine decarboxylase activity are well-established.[2,3,38,39] The decrease observed here, however, must be of a more permanent nature. It could be argued that decreased activity is expected when the endogenous supply of putrescine is increased. Bardocz et al.[3] also found that high dietary concentration of putrescine inhibits ornithine decarboxylase. Treatment with clofibrate is likely to represent a growth stimulus and has been shown to cause the typical transient stimulation of hepatic ornithine decarboxylase.[9,40] The observed stimulation observed in the present study may be related to this phenomenon.

Effects of treatments on levels of polyamines

Blood levels of polyamines. The absence of an effect on blood levels of a high polyamine diet alone suggests that blood levels are well regulated, possibly by regulating intestinal absorption and catabolism. This does not, however, preclude the occurrence of a transient increase in blood levels of polyamines following the ingestion of a meal high in polyamines. Our blood sampling routine was not designed to detect this type of phenomenon. Treatments involving clofibrate significantly altered blood levels of polyamines, but the magnitude of change was not altered markedly by simultaneous treatment with a high polyamine diet. Therefore, it is probable that metabolic changes brought about by clofibrate are the likely causes of altered blood levels of polyamines.

Polyamine concentrations in colon contents

An increased dietary load of polyamines was clearly reflected in the increased amounts of spermine and spermidine in colon contents (*Table 2*). The colonic content of putrescine, in contrast, was not significantly altered. A more extensive intestinal metabolism of putrescine compared with that of spermine and spermidine is one possible reason for this phenomenon. The diminished level of intestinal ornithine decarboxylase (*Figure 6*), however, suggests that the high dietary load of putrescine caused a decrease in the biosynthetic capacity for putrescine. This line of reasoning is supported by the observed down-regulation of ornithine decarboxylase activity by increased cellular levels of polyamines.[41,42] The conversion of dietary putrescine into non-polyamine metabolites prior to being absorbed from the gut may be another contributing factor. Bardocz et al.[3] reported that approximately 80% of dietary putrescine can be catabolized in this way. Conversely, the markedly increased colonic content of spermine and spermidine observed in the present investigation suggest that a major fraction these polyamines is excreted. The polyamine levels in the colon content of rats fed low polyamine diet were approximately half of that found by Paulsen et al.,[14] suggesting variation between different batches of rats.

Intestinal metabolism of polyamines and intestinal diamine oxidase activity

Metabolism of spermine and spermidine, either in intestinal mucosa, or by the intestinal micro-flora, may also occur. Mucosal diamine oxidase, which may be involved in intestinal metabolism of polyamines,[36] was not increased in rats given the high polyamine diet alone, suggesting that the intrinsic activity adequately metabolized a high dietary load of putrescine. However, the activity was increased when treatments included clofibrate, particularly with combined treatment of clofibrate and a high polyamine diet. Clofibrate also has been shown to cause proliferation of intestinal microperoxisomes.[43] Therefore, these results suggest that intestinal diamine oxidase is a peroxisomal enzyme, or at least an enzyme that is induced on treatment with clofibrate. It is striking that hepatic polyamine oxidase activity, which is a peroxisomal enzyme, exhibited the same pattern of variation with the various treatments (*Figure 3*). Intestinal polyamine oxidase activity, however, showed no significant changes with these treatments (results not shown), suggesting that the intestinal enzyme is regulated by different mechanisms.

Clofibrate and polyamine homeostasis

Levels of putrescine and spermidine are always increased in livers of rats given a treatment involving clofibrate (*Table 2*). This increase amounts to an approximately threefold increase in putrescine levels and an approximately 30% increase in spermidine levels. Considering that liver weights in animals given these treatments are increased by almost 100% relative to those found in animals not given a treatment involving clofibrate (*Table 1*), the magnitude of increase in hepatic content of polyamine biosyntheses becomes greater by a factor of approximately 1.8. Taking differences in liver weights into consideration, it is also apparent that the total liver content of spermine is increased on treatments involving clofibrate (*Table 2*).

It is also apparent that when rats given clofibrate were also treated with high polyamine diet no further increases hepatic levels of polyamines were observed (*Table 2*). The modified polyamine homeostasis was therefore due solely to effects of clofibrate and was not influenced by the dietary load of polyamines. In colon content, in which metabolism presumably is not significantly influenced by clofibrate, a clear effect of the dietary polyamine load was observed, at least as regards spermine and spermidine (*Table 2*). Therefore, the results also demonstrate that hepatic polyamine homeostasis is remarkably resilient to changes in the dietary load of polyamines.

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