

PAPERS

Dietary polyamines are essential luminal growth factors for small intestinal and colonic mucosal growth and development

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Abstract

Background—Polyamines are essential for cell growth. Dietary and probably gut bacterial derived polyamines contribute significantly to the polyamine body pool.

Aims—To evaluate the influence of dietary, luminal polyamines on growth and development of different gastrointestinal organs in normally growing rats.

Methods—Male suckling Wistar rats were randomly allocated to four treatment groups: polyamine deficient diet (PDD); PDD plus antibiotics (neomycin 2 g/kg and metronidazole 34 mg/kg); PDD plus polyamine supplementation at normal concentrations; or normal standard laboratory chow. After a six month feeding period 7–10 animals/group were sacrificed.

Results—No differences in body weight gain, food consumption, or general behaviour could be observed between the four groups of animals. Feeding of PDD alone or PDD plus antibiotics resulted in a highly significant decrease in organ weight, protein content, and DNA content in small intestinal and colonic mucosa whereas no alterations were found in the liver.

Conclusions—Long term feeding of polyamine deficient diets resulted in a significant hypoplasia of small intestinal and colonic mucosa. Dietary, luminal polyamines are important local factors for growth and the development of small intestinal and colonic mucosa.

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Keywords: colon; gut; nutrition; ornithine decarboxylase; polyamines; polyamine deficient diet

Polyamines are important constituents of all mammalian cells and are essentially involved in a variety of regulatory steps during normal, adaptive, and malignant cell proliferation.^{1–3} Intracellular polyamine homeostasis is growth dependently regulated by intracellular polyamine de novo synthesis and interconversion as well as supply from extracellular sources.^{3–6}

The gastrointestinal tract is the major source of extracellular polyamines and luminal, gastrointestinal polyamines are derived either from the diet or from the bacterial flora.^{6–10}

Because of its high proliferation rate, intestinal and colonic mucosa has a special demand for polyamines.^{11–12} Uptake of extracellular polyamines via luminal or basolateral membrane either by distinct polyamine carriers^{13–14} or passive diffusion^{15–16} was found to be an important regulatory mechanism of polyamine metabolism during small intestinal and colonic adaptation.^{15–18} Oral administration of spermine or spermidine was able to induce precocious morphological and functional maturation of the small bowel and accelerated early intestinal development.^{19–21} On the other hand, deprivation of gastrointestinal polyamines by feeding polyamine deficient diets and reducing luminal polyamine producing bacteria by oral antibiotics caused a significant reduction in solid tumour proliferation and confirmed the relevance of luminal polyamines for malignant growth.^{8–10, 22}

While the importance of luminal gastrointestinal polyamines for adaptive and malignant growth is well documented, little is known about their function in normal, physiological organ growth. The present study was designed to evaluate the long term effects of dietary and gut bacterial derived polyamines on growth and development of various gastrointestinal organ systems in normally growing rats.

Materials and methods

CHEMICALS

The following substances were purchased from Sigma Chemical Co. (St Louis, Missouri, USA): *o*-phthalaldehyde, 1,7-diaminoheptane and polyamine standards, Brij 35, bovine serum albumin, calf thymus DNA, sucrose, Tris buffer, dithiothreitol, pyridoxal phosphate, hyamine hydroxide, neomycin, metronidazole, and ammonium formate. Acetonitrile, glycerol (87%), disodium phosphate, and phenylmethylsulphonyl fluoride were from Merck (Darmstadt, Germany). DL-[1-¹⁴C] ornithine, S-adenosyl-L-carboxyl-¹⁴C methionine, 1-¹⁴C-acetyl CoA, and methyl-³H-thymidine triphosphate were

Abbreviations used in this paper: AB, antibiotics; ODC, ornithine decarboxylase; PA, polyamines; PDD, polyamine deficient diet; SAM-DC, S-adenosylmethionine decarboxylase; SAT, spermidine/spermine N¹-acetyltransferase.

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obtained from Amersham (Little Chalfont, UK). Millex-GS-filters, pore size 0.22 µm, were from Millipore (Molsheim, France). Whatman glass fibre filters (GF/C) and Whatman DE 81 ion exchange filters were from Whatman International (Maidstone, UK). Bisbenzimidazole (Hoechst H-33258) was from Hoechst (Frankfurt, Germany) and Bio-Rad protein reagent was purchased from Bio-Rad Laboratories (Munich, Germany).

DIETS

Standard laboratory chow as well as the different special diets were obtained from Altromin (Lage/Lippe, Germany). Polyamine deficient diet (PDD), polyamine deficient diet plus antibiotics (neomycin 2 g/kg and metronidazole 34 mg/kg) (PDD + AB), as well as polyamine deficient diet plus normal, physiological supplementation with polyamines (putrescine 80 mg/kg, spermidine 300 mg/kg, spermine 100 mg/kg) (PDD + PA) were identically prepared as previously published by Seiler and colleagues⁹ and Sarhan *et al.*²² The polyamine deficient diets used in this study were fully balanced, well tolerated animal diets.^{9, 22} HPLC analysis revealed a putrescine, cadaverine, and spermine content below the detection limit of the method; spermidine content was less than 15 nmol/g diet which is a reduction of more than 98% compared with standard laboratory chow (560 nmol/g).

ANIMALS

Male suckling Wistar rats (30–40 g, Harlan Winkelmann, Borcken, Germany) were housed at 24°C and exposed to a 12 hour light/12 hour dark cycle. Animals had free access to water and food. Water and food consumption as well as body weight were registered once a week for 24 hours. The study was approved by the Board of Ethics of the Christian-Albrechts University of Kiel, Germany.

EXPERIMENTAL DESIGN

Male suckling Wistar rats were randomly allocated directly after weaning into one of four different treatment groups: (1) polyamine deficient diet (PDD); (2) polyamine deficient diet plus antibiotics (PDD + AB); (3) polyamine deficient diet plus supplementation with putrescine, spermidine, and spermine at physiological concentrations (PDD + PA); and (4) normal standard laboratory chow. Once a week the following parameters were registered: body weight, water and food consumption, animal behaviour, and stool characteristics. After a six month feeding period 7–10 animals/group were killed and the following parameters were analysed in small intestine, colon, and liver: organ wet weight; single blinded, detailed histological analysis of all organs by a skilled pathologist (DH); protein and DNA content; DNA polymerase activity; polyamine concentrations; and activities of ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (SAM-DC), and spermidine/spermine *N*'-acetyltransferase (SAT). For statistical analysis the PDD + PA fed animals are regarded as the control group.

ANALYTICAL PROCEDURE

Homogenisation

The whole small intestine, colon, and liver were removed and homogenised 1:5 on ice, firstly in a buffer solution consisting of 10 mM Tris buffer (pH 7.9), 25 mM KCl, 5 mM MgCl₂, 0.25 M sucrose, 5 mM dithiothreitol, and 1 mM phenylmethylsulphonyl fluoride with a Potter S homogeniser at 1000 rpm (Braun, Melsungen, Germany; 10 up and down strokes) and then with a Dounce glass/glass homogeniser (Kontes Glass, Vineland, New Jersey, USA; 15 up and down strokes). Aliquots were taken and stored at –20°C until required for DNA protein and polyamine analyses. The remainder of the raw homogenate was centrifuged at 1000 *g* for 10 minutes; the supernatant was removed and ultracentrifuged at 110 000 *g* (2°C) for 50 minutes. Aliquots of the resulting cytosol fraction were taken for the determination of ODC, SAM-DC, and DNA polymerase and frozen at –20°C until analysis. ODC and SAM-DC activities were analysed the same day.

Ornithine decarboxylase

ODC activity was calculated by measuring the picomoles of ¹⁴CO₂ liberated from the 1-¹⁴C-labelled substrate ornithine (2.11 GBq/mmol) as recently described in detail.²³ Assays were run in triplicate and the results were calculated as picomoles ¹⁴CO₂/h/mg DNA.

S-adenosylmethionine decarboxylase

SAM-DC activity was calculated by measuring the picomoles of ¹⁴CO₂ liberated from the substrate *S*-adenosyl-L-carboxyl-¹⁴C-methionine (2.07 GBq/mmol) according to the method of Pegg and Pösö.²⁴ Enzyme activity was expressed as picomoles ¹⁴CO₂/30 min/mg DNA.

Spermidine/spermine *N*'-acetyltransferase

SAT activity was measured according to the method described by Matsui and colleagues²⁵ by determining the formation rate of ¹⁴C-labelled *N*'-acetylspermidine from ¹⁴C-acetyl CoA (2.07 GBq/mmol) plus spermidine. SAT activity was calculated as picomoles *N*'-acetylspermidine/min/mg DNA.

DNA polymerase

DNA polymerase activity was calculated as picomoles methyl-³H-thymidine triphosphate (1.78 TBq/nmol) incorporated/30 min/mg DNA as described in detail by Haarstad *et al.*²⁶ Methyl-³H-thymidine 5-triphosphate (20 pmol) and 25 µg activated calf thymus DNA were used as substrate. All assays were run in triplicate.

Polyamines

For polyamine separation an ion pairing reversed phase HPLC (Merck-Hitachi, Tokyo, Japan) method followed by postcolumn derivatisation with *o*-phthalaldehyde and consecutive fluorescence detection (F1000 fluorescence photometer) was used as previously published in detail.²⁷ In contrast to the previously published method, the final dilution of pancreatic tissue for polyamine analysis was 1/10. Putrescine, spermidine, and spermine concentrations were calculated as nmol/mg DNA.

DNA assay

DNA content was measured using the fluorescent dye H-33258 according to the method of Labarca and Paigen.²⁸ Calf thymus DNA was used as standard. DNA content was expressed as milligrams per total organ.

Protein

Protein content was determined according to the method of Bradford²⁹ using bovine serum albumin as standard. Protein content was expressed as milligrams per total organ.

STATISTICS

Results were calculated as mean (SD) values. The between group statistical significances were evaluated by Student's *t* test for unpaired values adapted for multivariate comparisons according to Holm.³⁰

Results

Feeding of the PDD reduced alimentary polyamine intake by 98% (0.20 $\mu\text{mol/day}$). Average daily polyamine intake in PDD plus PA fed animals (18.8 $\mu\text{mol/day}$) was similar to those fed with standard laboratory chow (15.3 $\mu\text{mol/day}$).

ANIMAL DEVELOPMENT

Food consumption and course of body weight during the 26 week feeding period revealed no significant differences between the four different treatment groups. Furthermore, no alterations in general animal behaviour or stool characteristics were observed during the half year feeding period. Long term feeding of all diets was well tolerated by the animals.

HISTOLOGICAL OBSERVATIONS

Tissue samples of small intestinal and colonic mucosa as well as the liver were taken from all animals after the 26 week feeding period for single blinded histological examination by a skilled pathologist. Apart from a slight hypotrophic appearance of small intestinal and colonic mucosa in PDD and PDD + AB fed rats no significant histopathological changes, and particularly no inflammation or necrosis, were observed in the organs of any diet fed group compared with standard laboratory chow fed controls.

TROPHIC PARAMETERS

Feeding of PDD + AB resulted in a significant ($p < 0.005$) decrease in wet organ weight in small intestine and colon, while feeding of PDD significantly ($p < 0.01$) decreased small intestinal mucosal weight (fig 1). Protein content of colonic mucosa and liver was not significantly different in the four treatment groups, but was significantly ($p < 0.01$) decreased in small intestinal mucosa of PDD fed animals compared with PDD + PA fed controls (fig 2). Feeding of PDD or PDD + AB resulted in a significant decrease in DNA content in small intestinal ($p < 0.01$) and colonic ($p < 0.005$) mucosa, while DNA content was not altered in the liver in any treatment group (fig 3). In the liver no significant alterations were found in any of the different treatment

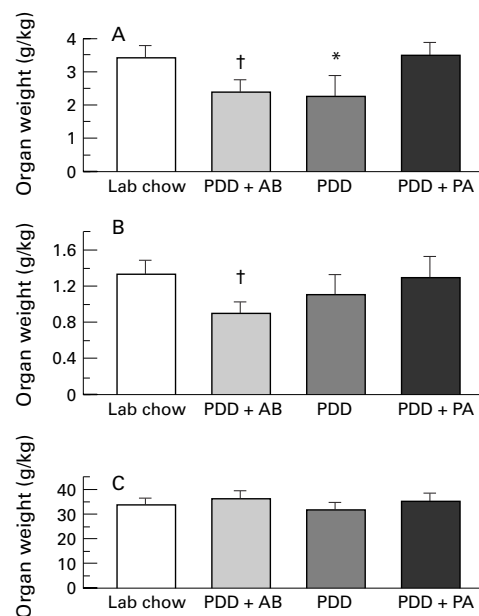


Figure 1 Organ weight of (A) small intestinal mucosa, (B) colonic mucosa, and (C) liver in rats ($n=7-10$) after a 26 week feeding period with either PDD, PDD + AB, PDD + PA, or standard laboratory chow. Significant differences versus PDD + PA: * $p < 0.01$; † $p < 0.005$.

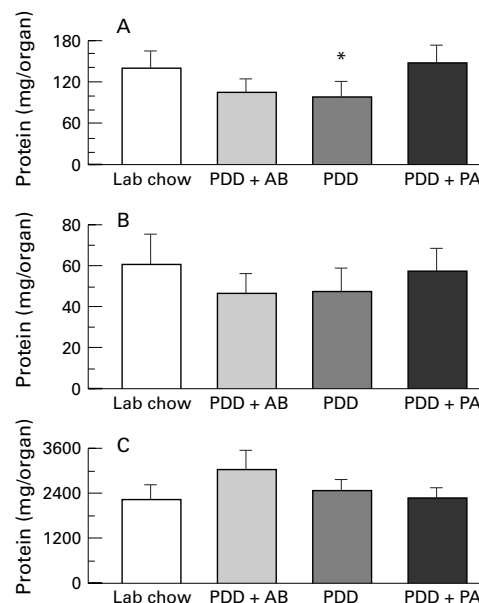


Figure 2 Protein content of (A) small intestinal mucosa, (B) colonic mucosa, and (C) liver in rats ($n=7-10$) after a 26 week feeding period with either PDD, PDD + AB, PDD + PA, or standard laboratory chow. Significant differences versus PDD + PA: * $p < 0.01$.

groups (figs 1, 2, and 3). DNA polymerase activity was not significantly altered in any of the three organs of the animals fed with the different diets (data not shown).

POLYAMINE METABOLISM

No significant changes in the activities of ODC, SAM-DC, or SAT, or concentrations of putrescine, spermidine, and spermine (data not shown) were observed in small intestinal mucosa, colonic mucosa, and liver between any of the four different treatment groups.

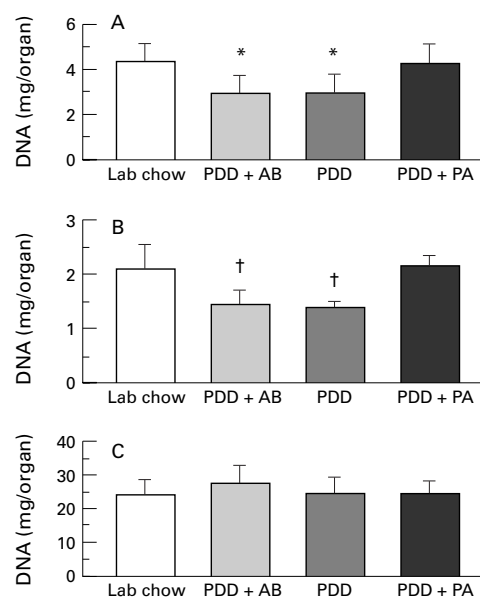


Figure 3 DNA content of (A) small intestinal mucosa, (B) colonic mucosa, and (C) liver in rats ($n=7-10$) after a 26 week feeding period with either PDD, PDD + AB, PDD + PA, or standard laboratory chow. Significant differences versus PDD + PA: * $p<0.01$; † $p<0.005$.

Discussion

Dietary and luminal gastrointestinal polyamines contribute significantly to the polyamine body pool^{16 31 32} and are essentially involved in early small intestinal maturation and functional development,¹⁹⁻²¹ and stimulate intestinal growth and adaptation,^{11 12 15-18 33 34} as well as the proliferation of various malignant solid tumours.^{8-10 22} Extending these observations, the results of the present long term study reveal that feeding of polyamine deficient diets induces significant hypoplasia of small intestinal and colonic mucosa. Our data prove the importance of dietary and luminal polyamines as local growth factors for mucosal nutrition and development even in normally growing animals.

The diets used in the present study were identically prepared as described by Sarhan and colleagues⁸ and Seiler *et al.*⁹ This balanced diet has a highly significantly reduced polyamine content and the long term results of the present six month study confirm previous short term observations^{8 9 22} by showing that this diet was well tolerated by the animals, caused no obvious side effects, and no differences in food intake compared with animals fed with normal laboratory chow. Therefore, feeding of this diet proved to be an excellent experimental tool to evaluate the effects of dietary polyamine deprivation on various physiological and pathophysiological conditions *in vivo*.

Gut bacteria are known to produce considerable amounts of polyamines. There is also experimental evidence that polyamines derived from bacteria resident in the gut contribute to the body polyamine pool.^{7 8 34} The amount of polyamines produced by gut bacteria is however not yet defined and their importance is controversial. Oral administration of metroni-

dazole and neomycin almost completely eliminates Gram negative bacteria, while Gram positive bacteria are only reduced in number.⁸ Our data confirm and extend earlier observations by showing that even long term administration of both antibiotics over six months causes no significant side effects and is well tolerated by the animals.^{8 9 22} Nevertheless, to what extent bacterial derived intraluminal polyamines are eliminated and whether there is any adaptation of the gastrointestinal flora during long term feeding of both antibiotics is not known and is difficult to verify experimentally.^{8 9 22} Simultaneous administration of these antibiotics resulted in no significant additional alterations to any of the trophic parameters measured compared with polyamine deficient diet fed animals. Therefore, the data of the present study do not suggest that bacterially derived polyamines are more important than those derived from the diet.

Deprivation of luminal polyamines resulted in a significant hypoplasia of small intestinal and colonic mucosa; however no significant alterations to the intracellular polyamine metabolism were observed in either organ compared with controls. The lack of significantly increased activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase indicates that at least after a six month feeding period intracellular *de novo* synthesis is not activated as a compensatory mechanism to maintain intracellular polyamine homeostasis in small intestinal and colonic mucosa. This is in accordance with observations in short term intestinal growth models where Bardóc and colleagues^{15 18 35} showed that uptake of polyamines through the basolateral membrane is the important regulatory mechanism for maintenance of small intestinal polyamine concentrations during adaptive growth. Based on these observations, uptake of polyamines via the basolateral membrane is the best candidate for compensation and maintenance of polyamine concentrations in the gastrointestinal mucosa in polyamine deficient chow fed animals. Nevertheless, the present data reveal that dietary polyamines are important local factors for small intestinal and colonic mucosal growth, irrespective of whether there was intracellular compensation of polyamine deficiency. The local mechanisms, however, are not yet known.

In conclusion, long term feeding of polyamine deficient diets resulted in a significant hypoplasia of small intestinal and colonic mucosa, while no effects were found in the liver. Dietary, luminal polyamines are important local growth factors for the nutrition and development of small intestinal and colonic mucosa in normally growing rats.

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