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.

Because of its high proliferation rate, intestinal and colonic mucosa has a special demand for polyamines. Dietary polyamines via luminal or basolateral membrane either by distinct polyamine carriers or passive diffusion was found to be an important regulatory mechanism of polyamine metabolism during small intestinal and colonic adaptation. Oral administration of spermine or spermidine was able to induce precocious morphological and functional maturation of the small bowel and accelerated early intestinal development. 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.

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-l-14C-acetyl CoA, and methyl-1H-thymidine triphosphate were obtained from Amersham (Bucks, UK).

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

Keywords: colon; gut; nutrition; ornithine decarboxylase; polyamines; polyamine deficient diet.
**DIETARY POLYAMINES ARE ESSENTIAL FOR INTESTINAL GROWTH**

Dietary polyamines are essential for intestinal growth and development. Well tolerated animal diets were used in this study. Spermidine content was less than 98% compared with standard laboratory chow. The PDD + PA fed animals are regarded as the control group.

**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 colleagues and Sarhan et al. The polyamine deficient diets used in this study were fully balanced, well tolerated animal diets. 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, Borchern, 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 14 000 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. Assays were run in triplicate and the results were calculated as picomoles 14CO₂/30 min/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. 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 Matsu and colleagues by determining the formation rate of 14C-labelled N’-acetylpermidine from 14C-acetyl-CoA (2.07 GBq/mmol) plus spermidine. SAT activity was calculated as picomoles N’-acetylpermidine/min/mg DNA.

**DNA polymerase**

DNA polymerase activity was calculated as picomoles methyl-1H-thymidine triphosphate (1.78 TBq/nmol) incorporated/30 min/mg DNA as described in detail by Haarstad et al. Methyl-1H-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. 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 content was measured using the fluorescent dye H-33258 according to the method of Labarca and Paigen. Calf thymus DNA was used as standard. DNA content was expressed as milligrams per total organ.

Protein content was determined according to the method of Bradford 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.

**Results**
Feeding of the PDD reduced alimentary polyamine intake by 98% (0.20 µmol/day). Average daily polyamine intake in PDD plus PA fed animals (18.8 µmol/day) was similar to those fed with standard laboratory chow (15.3 µmol/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 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.
Dietary polyamines are essential for intestinal growth

Dietary polyamines contribute significantly to the polyamine body pool and are essentially involved in early small intestinal maturation and functional development, and stimulate intestinal growth and adaptation, as well as the proliferation of various malignant solid tumours. 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 colleagues and Seiler and colleagues. 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 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.

In conclusion, long term feeding of polyamine deficient diets 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 Bardocz and colleagues showed that uptake of polyamines through 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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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.
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