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 whereas no differences were found in the liver.

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): α-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-carboxy-14C methionine, L-14C-acetyl CoA, and methyl-14H-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 N'-acyetyltransferase.
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obtained from Amersham (Little Chalfont, UK). Millex-GS filters, pore size 0.22 mm, 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 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).

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 10,000 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-lysine (1.78 TBq/nmol) according to the method of Pegg and Pösö. Enzyme activity was expressed 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/nmol) 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 3H labelled N’-acyetylsperrmidine from 3H-acetyl CoA (2.07 GBq/nmol) plus spermidine. SAT activity was calculated as picomoles N’-acyetylsperrmidine/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. 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 (FLUO1000 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.

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 (PDD + PA) and (4) normal standard laboratory chow. 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.

ANIMALS
Male suckling Wistar rats (30–40 g, Harlan Winkelmann, Borchen, 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.
DNA assay
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
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.
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Dietary polyamines are essential for intestinal growth and development even in normally growing animals. The diets used in the present study were identical as described by Sarhan 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. 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. The amount of polyamines produced by gut bacteria is however not yet defined and their importance is controversial. Oral administration of metronidazole and neomycin almost completely eliminates Gram negative bacteria, while Gram positive bacteria are only reduced in number. 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. 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. 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 Bardocz and colleagues 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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