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

Chr Löser, A Eisel, D Harms, U R Fölsch

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.1–4 Intracellular polyamine homocostasis is growth dependently regulated by intracellular polyamine de novo synthesis and interconversion as well as supply from extracellular sources.5–6 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.5–10

Because of its high proliferation rate, intestinal and colonic mucosa has a special demand for polyamines.10–12 Uptake of extracellular polyamines via luminal or basolateral membrane either by distinct polyamine carriers13–14 or passive diffusion15–16 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.10–12 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): 3-phenylaldehyde, 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 phenylmethysulphonyl fluoride were from Merck (Darmstadt, Germany). DL-[1-14C] ornithine, S-adenosyl-L-carboxyl-14C methionine, 1-14C-acetyl CoA, and methyl-1H-thymidine triphosphate were purchased from Amersham, 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'-acyetyltransferase.
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spermine content below the detection limit of

analysis revealed a putrescine, cadaverine, and

control group.

the PDD + PA fed animals are regarded as the

acetyltransferase (SAT). For statistical analysis

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behaviour, and stool characteristics. After a six

weight, water and food consumption, animal

the following parameters were ana-

months feeding period 7–10 animals/group were

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logical concentrations (PDD + PA); and (4)

trescine, spermidine, and spermine at physio-

deficient diet plus supplementation with pu-

ment special diets were obtained from Altromin

(Lage/Lippe, Germany). Polyamine deficient

diet (PDD), polyamine deficient diet plus anti-

bios (neomycin 2 g/kg and metronidazole 34

mg/kg) (PDD + AB) were identically prepared

as polyamine deficient diet plus normal, physiological sup-

plementation 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.24 The polyamine

deficient diets used in this study were fully bal-

anced, well tolerated animal diets.8 23 HPLC

analysis revealed a putrescine, cadaverine, and

serine 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).

DIETS

Standard laboratory chow as well as the differ-

cent special diets were obtained from Altromin

(Lage/Lippe, Germany). Polyamine deficient

diet (PDD), polyamine deficient diet plus anti-

AD

EXPERIMENTAL DESIGN

Male suckling Wistar rats were randomly

allocated directly after weaning into one of four
different treatment groups: (1) polyamine defi-
cient diet (PDD); (2) polyamine deficient diet

plus antibiotics (PDD + AB); (3) polyamine

deficient diet plus supplementation with pu-

trescine, spermidine, and spermine at physio-

logical 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 ana-

lysed 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 centri-

fuged 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 determina-

tion of ODC, SAM-DC, and DNA poly-

merase 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 sub-

strate S-adenosyl-l-carboxyl-14C-methionine

(2.07 GBq/mmol) according to the method of

Pegg and Pösö.24 Enzyme activity was ex-

pressed as picomoles 14CO2/30 min/mg DNA.

Spermidine/spermine N'-acyt etransferase

SAT activity was measured according to the

method described by Matsui and colleagues9 by
determining the formation rate of 14C-

labelled N'-acetyl spermidine from 14C-acetyl

CoA (2.07 GBq/mmol) plus spermidine. SAT

activity was calculated as picomoles N'-

acetyl spermidine/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.26

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 deriva-
tisation 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 pancre-

atic tissue for polyamine analysis was 1/10.

Putrescine, spermidine, and spermine con-

centrations were calculated as nmol/mg DNA.

obtained from Amersham (Little Cha lfont, 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 Inter-
national (Maidstone, UK). Bisbenzimidazole

(Hoechst H-33258) was from Hoechst (Frank-

furt, Germany) and Bio-Rad protein reagent

was purchased from Bio-Rad Laboratories

(Munich, Germany).

ANIMALS

Male suckling Wistar rats (30–40 g, Harlan

Winkelmann, Borckcn, 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 defi-
cient diet (PDD); (2) polyamine deficient diet

plus antibiotics (PDD + AB); (3) polyamine

deficient diet plus supplementation with pu-

trescine, spermidine, and spermine at physio-

logical 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 ana-

lysed in small intestine, colon, and liver: organ

wet weight; single blinded, detailed histological

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(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.
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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**Discussion**

Dietary and luminal gastrointestinal 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 et al. 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 Bárdóczi 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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