

Neurotensin-like immunoreactivity after intestinal resection in the rat

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SUMMARY Neurotensin is a tridecapeptide located mainly in the distal small intestine. The present study was carried out in order to investigate the neurotensin response after proximal small intestinal resection in the rat. After resection, the median plasma concentration of neurotensin like immunoreactivity (NTLI) was unchanged compared with sham operated rats. Intra-gastric instillation of fat increased the plasma concentration of NTLI from 45 pmol/l (34-63) in sham operated rats to 92 pmol/l (46-121) in resected rats. No significant increase in the plasma concentration of NTLI was found after intra-gastric instillation of amino acids or glucose. The tissue concentration of NTLI increased significantly in the jejunum and ileum after proximal small intestinal resection, while the number of immunoreactive neurotensin cells was unchanged. This study shows that the adaptive responses in the distal small intestine after proximal small intestinal resection also involve the neurotensin producing cells.

Neurotensin is a tridecapeptide localised in the brain, peripheral nerves, and endocrine cells of the intestinal mucosa.¹⁻³ Neurotensin immunoreactive cells occur throughout the gastrointestinal tract with increasing density towards the ileum.⁴ Increased concentration of circulating neurotensin occurs after ingestion of fat, whereas glucose or amino acids produce a minor or no secretory response in man and rat.^{5,6}

Resection of the proximal small intestine in rats induces adaptive changes in the remaining small intestine. These include an increased crypt cell production rate with increased rate of cell migration to the villi and consequently enlargement of the villi.⁷ Macroscopically, the residual small intestine is dilated, thickened, and elongated.⁸ Functionally, there is increased absorption per unit of length and a reduction in intestinal transit time.^{9,10} Food intake, pancreatic juice and bile seem to be important factors in initiating the adaptive response, these effects may

be mediated by regulatory peptides.¹¹⁻¹³ In the distal small intestine enteroglucagon cells and neurotensin containing cells are present.¹⁴ The plasma concentration of enteroglucagon is persistently raised after proximal small intestinal resection, and it has been suggested that enteroglucagon may be involved in initiation of the postresectional ileal hyperplasia.¹⁵ The present study was undertaken to investigate the NTLI response after proximal small intestinal resection in the rat. In addition the concentration of NTLI in the distal jejunum and ileum were compared before and after resection.

Methods

EXPERIMENTAL PROCEDURE

Twenty male Wistar rats weighing approximately 300 g were studied. Before each experiment the rats were fasted overnight but allowed free access to water. The rats were divided into two groups of 10 rats. In the first group approximately 60% of the proximal small intestine was resected as measured from the ligament of Treitz, leaving the distal jejunum and ileum intact. The other group under-

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went transection and reanastomosis at the ligament of Treitz and served as controls. Intestinal continuity was restored with an end to end anastomosis. Four weeks later all rats had a polyethylene catheter placed in a jugular vein. At intervals of one week, 2 ml blood was drawn from the jugular vein 20 minutes after intragastric instillation of 2 ml of one of the following agents: Intralipid (Kabi Vitrum, Sweden), Aminess (Kabi Vitrum), glucose 20% and saline 0.154 mol/l. Plasma was stored at -20°C for later determination of NTLI. Ten days after the last investigation all rats were killed and specimens from the distal jejunum and distal ileum were removed for quantification and immunohistochemical localisation of neurotensin.

LABORATORY ANALYSES

The concentration of NTLI was determined by radioimmunoassay in unextracted plasma as previously described.¹⁶ The antiserum used (code no 759A-4) requires the entire sequence of neurotensin for the antigen-antibody reaction and does not react with smaller fragments of neurotensin.¹⁶ The antiserum does not crossreact with other known gastrointestinal peptides. Antiserum was raised in rabbits by immunisation with neurotensin 1-13. Monoiodinated neurotensin (¹²⁵I (Tyr-3)-NT) was used as tracer and neurotensin 1-13 (Beckman, Ca-USA) as standard.¹⁷ Detection limit of the assay is 3 pmol/l and the working range 3–100 pmol/l. Intra-assay and interassay variation is below 15%.

For quantification of neurotensin all tissue samples were weighed at -20°C , homogenised in 10-fold weight of cold 2.0 mol/l acetic acid for five minutes. Homogenates were bathed in boiling water for 10 minutes. Insoluble material was removed by centrifugation at 3000 rpm for 20 minutes at 4°C , and the supernatant lyophilised. Radioimmunoassay of lyophilised extracts, reconstituted in 0.02 mol/l phosphate buffer pH 7.4 with 0.2% albumin and 0.01% thiomersal (assaybuffer), were carried out at five dilutions to span the sensitive part of the dose response curves. The reading from the middle of the curve was used and results expressed as pmol/g tissue. Recovery of synthetic neurotensin added to tissue before extraction was $97.6 \pm 3.9\%$ (mean \pm SD, $n=10$). Tissue extracts from all rat studies were assayed in random order within one assay.

Gel filtration was done at room temperature using Sephadex G-25 superfine column (1.6 \times 100 cm) (Pharmacia Fine Chemicals, Sweden). Crude extracts of pooled ileal and jejunal specimens (30 g) were reconstituted with 1 ml of 0.1 mol/l acetic acid. The column was eluted with 0.1 M acetic acid with a flow of 10 ml/h. The eluate of fractions of 1.1 ml were collected, lyophilised and reconstituted in assay

buffer. Column was calibrated with human serum albumin, ²²Na⁺, and NT 1-13. Trace amounts of albumin and ²²Na⁺ were added to all samples for internal calibration. Recovery was above 84%.

Specimens for immunohistochemical localisation of neurotensin were fixed by perfusion in Bouin's fixative without acetic acid. The immunohistochemical technique used was the unlabelled peroxidase-antiperoxidase method (PAP). The antiserum 3844 was diluted 1:1600. In control sections the antibody was preincubated with synthetic neurotensin 1-13 10 $\mu\text{mol/l}$. Quantification of the number of immunoreactive cells was carried out on perpendicularly oriented sections consisting of the entire thickness of the mucosa. The number of immunoreactive cells per 10 crypt-villi and per unit length of muscularis mucosae were counted. In the latter a graticule oriented parallel to the muscularis mucosae was used and all immunoreactive cells were counted.

STATISTICAL ANALYSIS

Statistical evaluation of the results was performed by Wilcoxon's test for paired differences and the Mann-Whitney test for unpaired samples. Results are given as medians and total ranges.

Results

In sham operated rats the median plasma concentration of NTLI was 18 pmol/l (range 13–34) after intragastric instillation of saline. Instillation of Intralipid significantly increased the median plasma concentration of NTLI to 45 pmol/l (range 34–63), whereas instillation of glucose 20% or Aminess had no significant effect (Table 1). After proximal small intestinal resection the concentration of NTLI was 26 pmol/l (range 20–38). Intralipid increased the concentration of NTLI to 92 pmol/l (range 46–121) while instillation of glucose 20% or Aminess had no significant effect. In resected rats the plasma concentration of NTLI after instillation of Intralipid was significantly higher than in sham operated rats. The

Table 1 Effect of proximal small intestinal resection on concentration of NTLI in plasma after intragastric administration of saline, glucose, amino acids or fat

Treatment	Sham operation (n=10) pmoll	Resection (n=10) pnoll
Saline	18 (13–34)	26 (20–38)
Glucose 20%	25 (22–37)	35 (17–58)
Aminess	19 (13–32)	23 (15–38)
Intralipid	45* (34–63)	92† (46–121)

Values are given as medians and total ranges. * $p<0.01$ compared with sham operated rats given saline. † $p<0.01$ compared with resected rats given saline and sham operated rats given Intralipid.

Table 2 Concentration of NTLI in the distal jejunum and ileum in sham operated rats and in rats subjected to proximal small intestinal resection

Organ	Sham operation (n=8) pmol/g	Resection (n=8) pmol/g
Jejunum	1.76 (0.88-38.9)	26.7*† (9.1-67.3)
Ileum	1.47 (0.18-3.27)	4.16* (0.55-46.6)

Values are given as medians and total ranges. *p<0.01 compared with the corresponding group of sham operated rats. †p<0.01 compared with the concentration of NTLI in the ileum of resected rats.

differences found after instillation of saline, Aminess or glucose 20% were not significantly different from the controls (Table 1). The concentration of NTLI in the distal jejunum was not significantly different from the concentration of NTLI in the distal ileum (Table 2). After proximal small intestinal resection the amount of NTLI in the jejunum increased 15 times, but only by a factor 3 in the distal ileum (Table 2). Using gel chromatography, NTLI in the pooled hypertrophied jejunum and ileum specimens eluted at the same position as intact neurotensin 1-13 (Fig. 1). The number of NTLI cells per unit length of the muscularis mucosae and per 10 crypt-villi in the hypertrophied jejunum and ileum was not significantly different from controls (Table 3). Histologically the height of the villi and the depth of the crypts increased after proximal small intestinal resection (Fig. 2).

Discussion

In mammals NTLI is predominantly found in the jejunum and ileum, but the distribution of NTLI along the gastrointestinal tract differs among species. In man and dog the highest concentration of NTLI is found in the distal ileum.^{18,19} In the rat, Carraway and Leeman²⁰ reported nearly equal concentrations in the ileum and jejunum, while others found the highest concentration in the ileum.²¹ In the present study no difference was found between the concentration of NTLI in the distal jejunum and the ileum in sham operated rats. After proximal small intestinal resection the concentration of NTLI increased by a factor of 15 in the distal jejunum, but only by a factor 3 in the ileum. The increase in plasma concentration of NTLI after instillation of fat was more pronounced in resected rats than in sham operated rats. These results suggest that the adaptive changes in the distal intestine after proximal small intestinal resection may also involve the NTLI cells.

The basal plasma concentrations of NTLI were unchanged after intestinal resection. This is different from the increased concentration of enteroglucagon found in plasma after proximal intestinal resection in

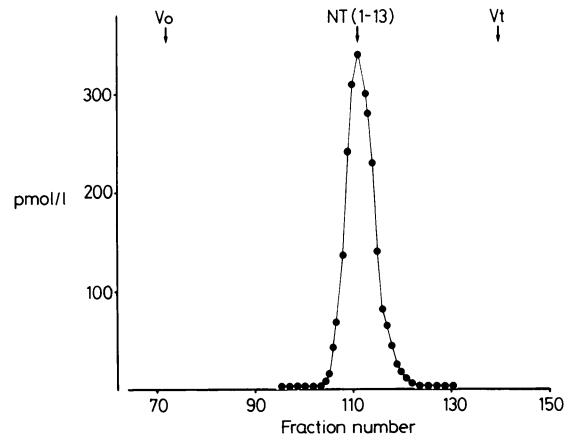


Fig. 1 Gel filtration of pooled jejunal and ileal extract from resected rats. Abscissa: Fraction number. Ordinate: Neurotensin in pmol/l determined by radioimmunoassay. V_o : Void volume, V_t : Total volume, NT (1-13): Elution volume of synthetic NT (1-13).

the rat.²² This rise in the level of circulating enteroglucagon was not combined with increased tissue concentration of enteroglucagon in the distal small intestine.²³ Immunohistochemically, a relative decrease in the enteroglucagon cell number was found.²³ Ultrastructural studies showed a decrease in the number of secretory granules and more prominent Golgi complexes and endoplasmic reticulum, which suggest a hyperfunction of the enteroglucagon cells in the ileum of resected rats.²⁴ In the resected group of rats in the present study we found an increased level of NTLI in the jejunum and ileum, no increase in the number of NTLI cells and only increased levels of circulating NTLI in response to fat. This suggests that the increased tissue concentration of NTLI accumulates in the neurotensin cell and is released only in response to a physiological stimulus – for example, fat.

After proximal small intestinal resection, the part

Table 3 Number of neurotensin immunoreactive cells per unit length of muscularis mucosae and per 10 crypt villi in the distal jejunum and ileum of sham operated rats and rats subjected to proximal intestinal resection

Organs	Operation	Cells/ unit length	Cells/ 10 crypt villi
Jejunum	Sham operation	4 (2-6)	5 (4-8)
Jejunum	Resection	4 (3-7)	5 (2-6)
Ileum	Sham operation	5 (3-8)	6 (4-9)
Ileum	Resection	4 (2-5)	5 (3-8)

Results are given as medians and total ranges. In each group of rats 10 sections from each rat were counted. No statistical difference was found between the individual groups.

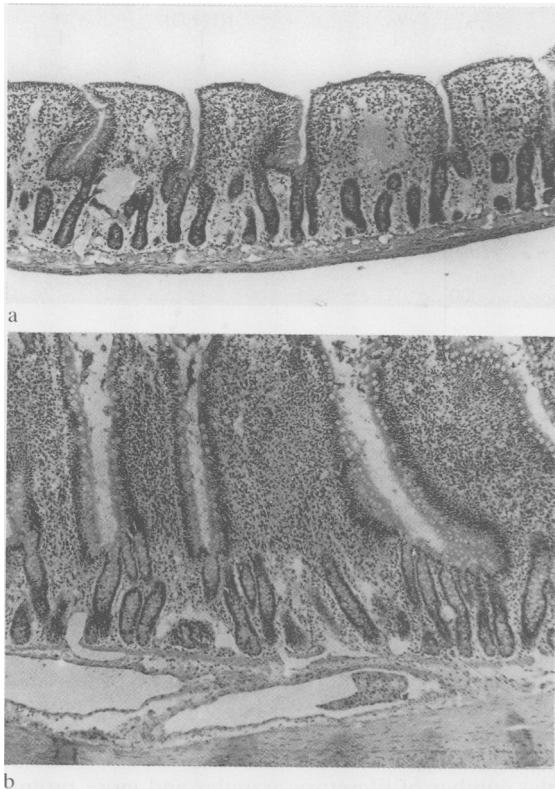


Fig. 2 (a) Transverse section of ileum from control rat. (b) Similar histologic section of ileum from resected rat. Crypt and villus height is considerably increased. Muscle layers are also thickened. (PAS-haematoxylin-Aurentia).

of the intestine richest in NTLI cells is transposed proximally and thereby exposed to a more nutrient rich chyme. This might be responsible for the increased NTLI response to fat. In addition, absorption of nutrients is further enhanced by a reduction in intestinal motility and transit time after resection, allowing prolonged contact between food and mucosa.¹⁰ On the other hand a large proportion of the neurotensin immunoreactive cells in the gastrointestinal tract is removed at the operation while the remaining small intestine develops increased concentrations of NTLI. The NTLI response to fat in rat and man occurs rapidly, within 10–20 minutes, which is less than a mixed meal requires to reach the distal ileum.^{25–27} Experimental studies have suggested that the early release of NTLI after oral administration of fat is partly mediated by a cholinergic non-vagal mechanism.^{28,29} These observations indicate that the increased plasma concentration of NTLI in resected rats receiving fat is a response to a larger input of nutrients to the distal small intestine.

In conclusion this study has shown, that after

proximal small intestinal resection an increased concentration of NTLI is found in the remaining small intestine, and that increased concentrations of NTLI can be elicited by intragastric instillation of fat. This study suggests that NTLI may play a role in the adaptive response occurring in the small intestine after resection.

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