

Neurotensin induces hyperplasia of the pancreas and growth of the gastric antrum in rats

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SUMMARY We investigated whether chronic subcutaneous infusion of neurotensin during 14 days would affect pancreatic and gastric growth of rats. In another experiment, neurotensin (836 pmol/kg) was injected intraperitoneally three times a day for three days in 12 rats. Thereafter, pancreatic DNA and *in vitro* incorporation of ³H-thymidine into pancreatic DNA was determined. Long term infusion of 282 pmol/kg neurotensin induced an increase of pancreatic weight, DNA, and pancreatic polypeptide, whereas pancreatic protein, RNA, amylase and lipase contents were not increased. In relation to DNA, even these parameters were significantly depressed. Insulin remained unchanged. Neurotensin, therefore, caused hyperplasia of the pancreas. Intraperitoneal injection of neurotensin induced an increase of pancreatic DNA content and stimulated ³H-thymidine incorporation into DNA, whereas caerulein only augmented ³H-thymidine incorporation. Moreover, long term neurotensin infusion led to a rise in protein concentration and an increase in the thickness of the gastric antrum; antral DNA concentration was insignificantly stimulated. Neurotensin, therefore, can act as a trophic factor on pancreas and gastric antrum of the rat.

One of the many actions of neurotensin is its stimulatory effect on exocrine pancreatic secretion.¹⁻³ Because other pancreatic secretagogues, such as cholecystokinin and secretin, also stimulate pancreatic growth, we investigated the effect of chronic infusion of neurotensin on pancreatic growth. As neurotensin also affects gastric secretion and motility, we further examined the effect of long term administration of neurotensin on the stomach.

Methods

ANIMALS

Alzet osmotic minipumps delivering $0.465 \pm 0.023 \mu\text{l/h}$ ($M \pm SD$) for 14 days were prepared to pump 43 or 282 pmol/kg/min neurotensin subcutaneously in 20 male Wistar rats. The control group consisted of 10 Wistar rats from the same litter in which plastic cylinders with the same size and shape as the minipumps had been implanted. Fourteen days after implantation, the rats were killed by neck dislocation, the pancreata and stomachs were removed, weighed, and frozen. Blood taken by cardiac puncture was centrifuged, the plasma was stored at -20°C until assayed. The frozen tissue was homogenized in

phosphate buffer with an Ultraturrax and a Potter instrument. Protein was determined according to Lowry *et al*⁴ using human serum albumin as standard. DNA was measured according to Giles and Myers⁵ using calf thymus DNA as standard. RNA was determined according to the Munro-Fleck⁶ modification of the Schmidt-Thannhauser method. Alpha-amylase and lipase were determined using the Phadebas amylase test or the Boehringer Mannheim Monotest. Pancreatic polypeptide was determined by radioimmunoassay.⁷ Insulin was determined with the PhadesephTM insulin RIA kit (Pharmacia, Uppsala/Sweden). Plasma neurotensin was determined by radioimmunoassay.⁸ One half of the stomachs was frozen for determination of DNA and protein as described above; the other half was fixed with Bouin's solution, embedded in paraffin, cut perpendicularly in $5 \mu\text{m}$ sections and stained with haematoxylin-eosin. The thickness of the mucosal and muscular layers was determined with a caliper lens at 3 points in a defined distance from the ridge between rumen and corpus.

Thirty six male Wistar rats (approx. 100 g) received intraperitoneal injections of neurotensin (836 pmol/kg), caerulein ($148 \text{ pmol} \times \text{kg}^{-1}$), or 0.5 ml saline every eight h for three days. Thereafter and subsequent eight h-fast, the rats were killed, the pancreata were minced with a scalpel, and incubated for 30 min at

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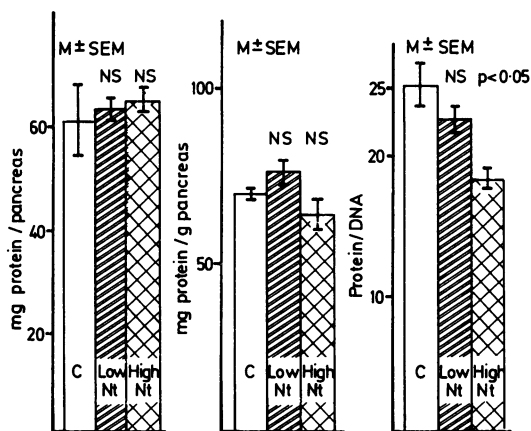


Fig. 1 Protein content, protein concentration, and protein per mg DNA in rat pancreas after 14 day subcutaneous infusion with 43 and 282 pmol/kg neurotensin and in control rats.

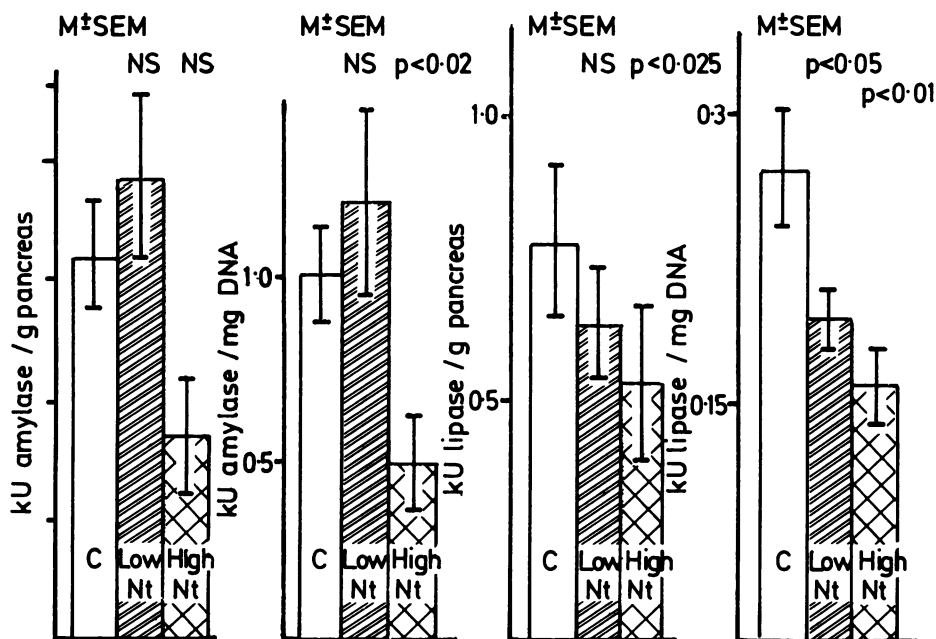


Fig. 2 Alpha-amylase and lipase content per g pancreas and per mg DNA after 14 day subcutaneous infusion of 43 and 282 pmol/kg neurotensin and in control rats.

Table 1 Pancreatic polypeptide (PP), DNA and insulin content in the pancreas of rats stimulated by a 14 day subcutaneous infusion of 2 different doses of neurotensin with minipumps and of control rats.

	PP ng/pancreas	pp ng/g pancreas	DNA mg/pancreas	DNA mg/g pancreas	Insulin mU/pancreas
control	2.40 ± 0.32	2.89 ± 0.38	2.5 ± 0.13	2.88 ± 0.15	1.2 ± 0.3
low NT	3.77 ± 0.43*	4.5 ± 0.55	2.8 ± 0.10	3.4 ± 0.19	1.3 ± 0.4
high NT	7.04 ± 1.72†	6.95 ± 1.72†	3.6 ± 0.26*	3.52 ± 0.21†	1.2 ± 0.4

*p < 0.05; †p < 0.005; M ± SEM.

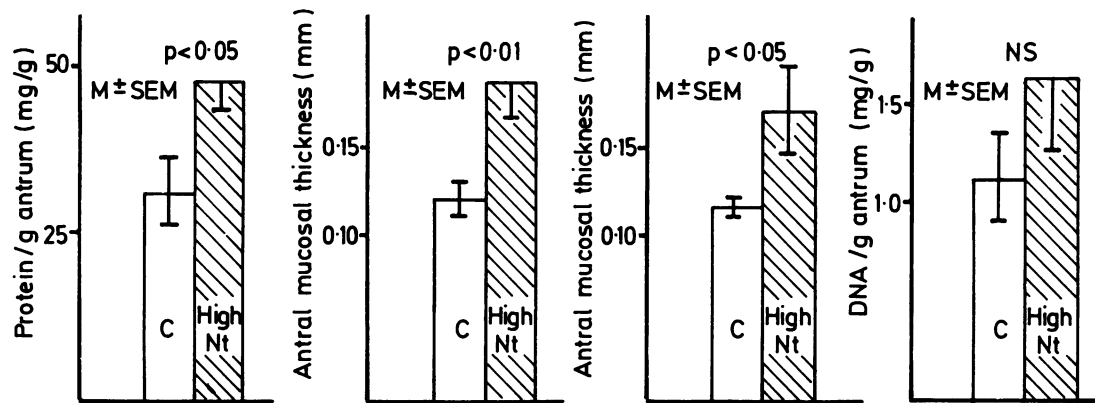


Fig. 3 282 pmol/kg/min neurotensin (high Nt) subcutaneously for 14 days led to a significant rise of protein concentration in the gastric antrum, the thickness of gastric antral muscularis and mucosal layer was increased. There was a tendency for stimulation of antral DNA concentration.

37 °C in medium 99 (Gibco), gassed with 95% O₂, 5% CO₂, with added 50 μCi 5'-³H-thymidine (Amersham). The reaction was stopped by 0.4 M perchloric acid containing 5 mM carrier thymidine. Thereafter, the pancreata were extracted for DNA as described above. ³H-activity in the DNA extracts was determined with a scintillation counter.

Light and electron microscopy of the pancreas was performed with conventional techniques.

Results

Mean neurotensin plasma concentrations in the control group were 137 ± 8 pg/ml (M ± SEM). After the infusion of 43 pmol/kg/min exogenous neurotensin, mean plasma concentrations rose to 170 ± 21 pg/ml, and after infusion of 282 pmol/kg/min to 268 ± 21 pg/ml (p = 0.001). While the weight gain in all rats was similar, pancreatic weight was significantly higher in the group that received the high dose of neurotensin (control 0.87 ± 0.03 g neurotensin 1.02 ± 0.004 g; M ± SEM p < 0.05). Total DNA and DNA concentration per gram pancreas was significantly increased in the high dose group (Table). Pancreatic polypeptide concentration rose significantly in both groups, whereas insulin concentration remained unchanged (Table). Pancreatic protein, enzymes, and RNA were not raised, they decreased significantly in relation to pancreatic DNA (Figs 1, 2). The weight of the stomachs was not affected by neurotensin infusion. The high neurotensin dose, however, led to an increase in the thickness of the muscular and mucosal layers of the antrum (Fig. 3). Moreover, protein/g antrum was significantly, DNA/g antrum insignificantly increased. Thickness of the gastric wall layers, protein, and DNA, however,

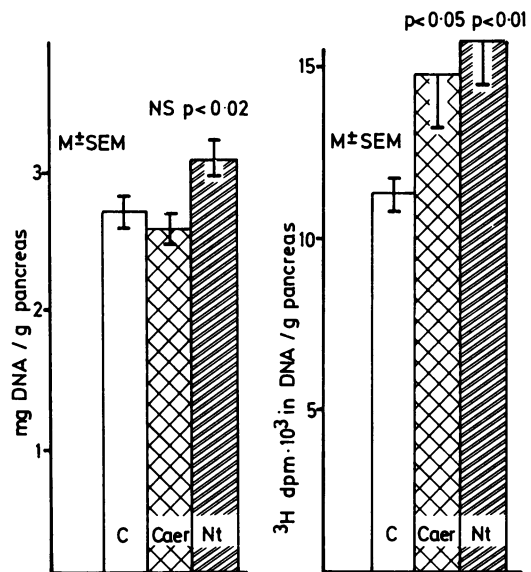


Fig. 4 Three day intraperitoneal injection of neurotensin significantly increased pancreatic DNA concentration. The *in vitro* incorporation of ³H-thymidine into pancreatic DNA was stimulated significantly by the three day treatment with caerulein (Caer) (148 pmol/kg q 8 h), and neurotensin (Nt, 836 pmol/kg q 8 h).

remained unchanged in the body of the stomach (not shown).

In the neurotensin injection study, mean DNA/g pancreas increased significantly from 2.7 ± 0.1 to 3.1 ± 0.1 mg/g pancreas (M ± SEM, p < 0.02). Incorporation of tritiated thymidine into DNA was significantly stimulated by caerulein and neurotensin (Fig. 4).

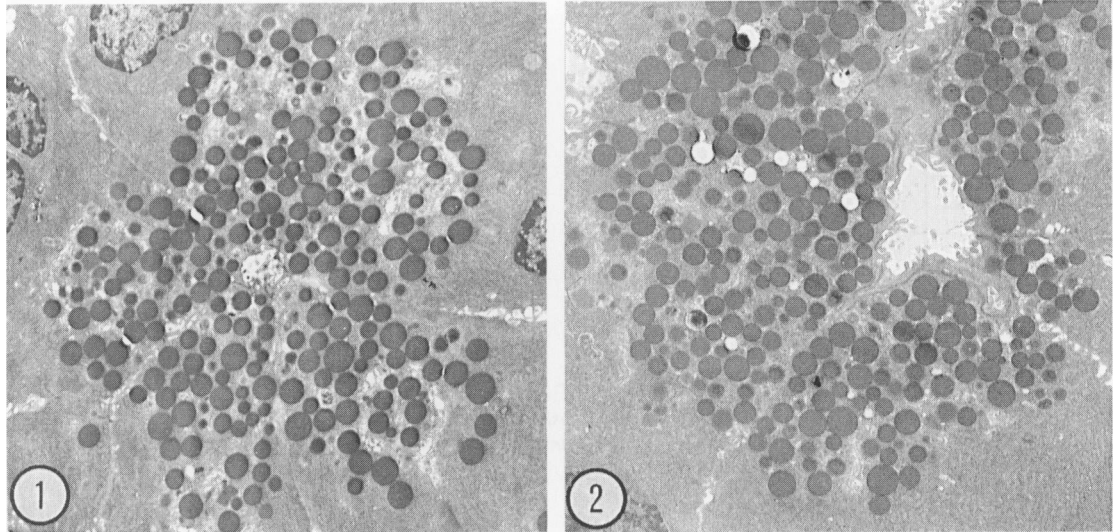


Fig. 5 Electronmicroscopy of a pancreatic acinus of a rat treated with the high dose of neurotensin (2) and of a control animal (1). Number, size, and electron density of the granules apparently are unchanged.

Morphometry on the microscopical level indicated no discernable change of the areas containing enzyme granules within the acinar cells. On electronmicroscopy the size, number, and electron density of the granules were unchanged after the high dose of neurotensin when compared with control pancreas (Fig. 5).

Discussion

This study shows that chronic parenteral neurotensin, unlike CCK, which leads to an increase of pancreatic weight, DNA as well as pancreatic RNA and protein,⁹ selectively increased pancreatic weight and DNA. Enzymes and RNA were reduced in relation to DNA. In addition, neurotensin induced pancreatic cell proliferation shown by incorporation of ³H-thymidine into DNA. Whereas chronic CCK treatment thus leads to pancreatic hypertrophy as well as hyperplasia, neurotensin seemed to induce pancreatic hyperplasia only. An additional hypertrophic effect seems unlikely although the net unchanged pancreatic enzyme content may be the consequence of enzyme depletion induced by a continuing infusion of neurotensin. The RNA content, however, was also unchanged. The unaltered content of enzymes in the pancreas (when not related to DNA) also in accordance with the morphological data indicates that release of enzymes after long term neurotensin infusion is in balance with synthesis of enzymes. The unchanged size and number of enzyme granules in the presence of an increased content of DNA, therefore, supports the

conclusion of a predominantly hyperplasiogenic effect of neurotensin. Hence, pancreatic secretagogues seem to regulate pancreatic secretion as well as pancreatic growth. In view of the presence of neurotensin immunoreactive nerves within the pancreas,¹⁰ a neural action of neurotensin on secretion as well as on growth seems also possible. The action of neurotensin may, however, be indirect. The most sensitive pancreatic parameter after two weeks administration of neurotensin was pancreatic polypeptide which itself has been reported to increase DNA synthesis in the rat pancreas.¹¹ It is, therefore, very possible that neurotensin affects pancreatic proliferation indirectly via pancreatic polypeptide.

Furthermore, neurotensin led to an increase in the thickness and the protein concentration of the gastric antrum. One possible mechanism of this action is that neurotensin reduces gastric emptying¹² probably by inducing retention of gastric contents. Gastric stasis and dilatation, however, led to hypertrophy of the gastric antrum.¹³ An indirect effect of neurotensin on the stomach as well, therefore, would appear possible.

It is unknown whether these effects are pharmacologic or physiologic. At any rate neurotensin should be added to the list of peptides which can induce growth of pancreas and stomach in the rat.

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