Effect of secretin and glucagon on Brunner's gland secretion in the rat

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SUMMARY Brunner's gland secretion in response to infusion of secretin and glucagon was studied in the rat. Secretin was infused in doses of 15, 150 and 1500 ng/kg/h. All doses significantly increased bicarbonate and protein output and depleted Brunner's glands of PAS-positive mucin. Bicarbonate secretion was related to plasma secretin concentration, and a marked stimulatory effect of secretin was found in very low, probably physiological, plasma concentrations. Maximal bicarbonate output was obtained at a plasma concentration of secretin about 20 pmol/l. Glucagon was infused at a rate of 1.0 µg/kg/h and did not influence secretion rate or cell morphology. Also large doses of 5.0 and 50.0 µg/kg/h had no effect on Brunner's gland secretion. It is concluded that secretin in very low plasma concentrations stimulates secretion of bicarbonate, protein and mucus from Brunner's glands in the rat, while glucagon has no effect, and it is suggested that secretin may be involved in the physiological regulation of Brunner's gland secretion.

Nearly 50 years ago Florey and Harding^{1,2} showed that feeding stimulated secretion from Brunner's glands in the cat after extrinsic denervation. They concluded that the glands were controlled by a hormonal mechanism, and postulated the hormone to be secretin. The effect of feeding on Brunner's gland secretion was later also observed in dog and pig,^{3,4} and several authors have studied secretion in dog and cat after administration of secretin containing material. The results, however, were conflicting as some experiments failed to show an effect of secretin on Brunner's glands,^{4,5} while others showed a marked stimulatory effect.^{6,7} More recently both pure natural and synthetic secretin was shown to stimulate the glands,^{8,9} but the doses used were higher than what is now considered within physiological range. Other members of the secretin family of peptides, glucagon, and vasoactive intestinal polypeptide (VIP), have also been shown to stimulate Brunner's gland secretion,¹⁰⁻¹² but while VIP was effective in very small doses, the glucagon effect was demonstrated only at pharmacological doses.

The present study was performed to investigate whether secretin and glucagon in more physiological plasma concentrations stimulate Brunner's gland secretion.

Methods

ANIMALS AND OPERATIVE PROCEDURE

Female Sprague-Dawley rats weighing 190-210 g were fasted from 8 am for eight hours in raised mesh-bottom cages, but given tap water *ad libitum*. Under ether anesthesia, one of the jugular veins was cannulated with a 0.8 mm polyethylene tube for infusion of peptides. A laparotomy was performed with a midline incision and the pylorus was ligated. Another ligature was placed around the duodenum 8 mm distally, a few millimetres proximal to the common bile and pancreatic duct. Through a stab wound a polyethylene catheter was introduced into the lumen of the distal duodenum and the tip was passed up through the ligature, which was tied so that the catheter was draining the 8 mm duodenal pouch. To drain the stomach during the experiment, another catheter was placed through a stab wound in the lumen and secured by a purse string suture. The abdominal wound was closed around the two

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catheters with interrupted sutures. In five rats an 8 mm long pouch of the jejunum was made in a similar way for control. When the rats had regained consciousness, they were placed in Bollmann cages. The catheters were connected to glass syringes allowing the secretions to be collected under anaerobic conditions.

EXPERIMENTAL PROCEDURE

Either natural porcine secretin (GIH Research Laboratory, Karolinska Institutet, Stockholm, Sweden) or natural porcine glucagon (Novo Research Laboratory, Copenhagen, Denmark) dissolved in saline with 2% human serum albumin (Behringwerke, Marburg, FRG) were infused intravenously at a rate of 2 ml/h for five hours by a constant infusion syringe pump (Braun Melsungen AG, FRG). Groups of 10 rats each were tested with secretin in doses of 0.05, 0.5 and 5.0 clinical units/kg/h or with glucagon in doses of 1.0, 5.0 and 50.0 $\mu\text{g/kg/h}$. A group of 10 rats served as controls and received saline with 2% albumin. After five hours the duodenal secretions were collected for determination of HCO_3^- and protein concentration, and the volume was measured to the nearest 0.05 ml. The rats were anaesthetised with ether and 3 ml of blood was drawn from the inferior caval vein for radioimmunoassay. The blood was collected in ice chilled tubes containing 500 KIU aprotinin (Transylol®, Bayer, Leverkusen, FRG) and 150 IU heparin. The samples were centrifuged at 0°C, and the plasma stored at -20°C until assay. The pouches were removed and prepared for histologic examination, and the animals were killed by an overdose of ether.

LABORATORY ANALYSES

Concentrations of immunoreactive secretin and glucagon were measured in plasma as previously described.¹³⁻¹⁵ In duodenal secretions the pH and pCO_2 were determined and the HCO_3^- was calculated by an ABL2 (Acid-Base Laboratory 2, Radiometer, Copenhagen, Denmark). Protein contents in duodenal secretions were measured by the method of Lowry *et al.*¹⁶

Histologic sections of the pouches were stained with PAS-haematoxylin-aurentia.

All data are expressed as median and total range. For statistical evaluation the different groups were compared using the Mann-Whitney U test; p values of <0.05 were considered significant.

Results

Intravenous infusion of secretin in doses of 0.05, 0.5 and 5.0 CU/kg/h (15 ng, 150 ng and 1.5 μg)

increased plasma concentration of secretin from 6.8 (3.2-11) pmol/l in the controls to 9.8 (7.6-12.6), 18.4 (10.0-30.8) and 143 (62.4-194) respectively. All doses significantly and dose dependently increased the flow rate of Brunner's gland secretion (Table). Both bicarbonate and protein output increased in response to infusion of secretin as the concentrations in the secretion remained unchanged (Table). In Figure 1 the semilogarithmic plot of plasma secretin concentration and duodenal bicarbonate secretion is shown. A marked increase of bicarbonate output is seen even at very low plasma secretin levels, and maximal bicarbonate output occurs at about 20 pmol/l. Secretion rate from jejunal pouches was less than 0.1 ml/5 hour.

At histologic examination even the smallest dose of secretin (15 ng/kg/h) had induced a marked depletion of mucus in the secretory cells (Fig. 2), similar changes were seen after the higher doses. In sections from the control groups all cells were tall, columnar and contained large amounts of PAS-positive mucin, the nucleus was often flattened and located in the basal part of the cell (Fig. 3). After secretin infusion the secretory cells were lower, cuboidal and the nuclei were round and localised in the central part of the cells. No pancreatic ducts were found at examination of horizontal sections of the external tunica muscularis of the pouches.

During intravenous infusion of glucagon in doses of 1.0, 5.0 and 50.0 $\mu\text{g/kg/h}$, plasma glucagon increased from 70 (35-150) pmol/l in the controls to 116 (72-397), 375 (150-1178) and 3216 (860-5466) respectively. None of the doses influenced flow rate, concentration of bicarbonate or protein significantly in secretions from Brunner's glands (Table). The

Table Effect of secretin and glucagon on Brunner's gland secretions

	n	Volume (ml/5 hour)	HCO_3^- (mmol/l)	Total protein (g/l)
Control	10	1.05 (0.80-1.50)	66.4 (49.3-75.5)	3.9 (1.7-5.2)
Secretin (15 ng/kg $\times$ h)	10	1.70 (1.40-2.50)*	63.2 (45.2-83.2)	3.1 (1.3-5.4)
Secretin (150 ng/kg $\times$ h)	10	2.15 (1.70-2.40)†	63.5 (48.2-82.5)	3.4 (1.3-5.1)
Secretin (1.5 $\mu\text{g/kg}$ $\times$ h)	10	2.40 (1.90-2.90)†	62.4 (55.8-71.2)	3.6 (1.9-4.9)
Glucagon (1 $\mu\text{g/kg}$ $\times$ h)	10	0.95 (0.70-1.20)	64.1 (46.3-80.4)	3.2 (1.4-5.8)
Glucagon (5 $\mu\text{g/kg}$ $\times$ h)	10	1.00 (0.80-1.20)	61.2 (52.1-73.9)	4.0 (1.2-5.7)
Glucagon (50 $\mu\text{g/kg}$ $\times$ h)	10	1.10 (0.85-1.35)	65.6 (49.5-72.3)	3.4 (1.5-5.2)

Values given are median and total range. Statistical significance between experimental and control groups: * $p < 0.01$, † $p < 0.001$.

Fig. 1 Dose response curve of bicarbonate output and plasma secretin concentration in fasting controls and during infusion of secretin in doses of 0.05, 0.5 and 5.0 CU/kg/h. The horizontal and vertical bars are total range of plasma secretin concentrations and bicarbonate output from 10 rats. Medians are in the centre.

histologic examination revealed no changes in cell morphology compared with the control group.

Discussion

Brunner's gland secretion is believed to protect the mucosa in the first part of the duodenum from the acid chyme ejected from the stomach.¹⁷⁻¹⁹ Impaired function of the glands may be a pathogenetic factor in experimentally induced duodenal ulcer.^{20,21} The mechanisms regulating Brunner's gland secretion, however are still largely unknown.

The secretion obtained from the duodenal pouches has a characteristic limpid and viscid nature, quite different from secretions from other parts of the small intestine, where Brunner's glands are absent. Although secretions from the glands of Lieberkühn and the villus cells are admixed, these are quantitatively insignificant, as indicated by the lack of measurable secretion from the jejunal pouches. These observations and the histologic evidence of depletion of mucin after stimulation indicate that Brunner's glands actually produce the main part of the secretion obtained from the pouches.

The present study shows that small increments of plasma secretin induce a dose dependent increase in Brunner's gland secretion. The plasma concentrations measured after infusion of secretin in the rat correspond well to the findings in other species,

Fig. 2 Effect of infusion of secretin 15 ng/kg/h and glucagon 1 µg/kg/h on PAS reaction of the secretory cells of Brunner's glands. Significantly less PAS reaction was seen after secretin infusion. Glucagon had no effect ($p < 0.05$, χ^2 test). Grading of PAS reaction: 0 = PAS negative; 1 = 1-2 small glandular areas with PAS positive reaction; 2 = more than two small areas PAS-positive, but not the whole gland; 3 = all secretory cells PAS positive.

including man,^{22,23} and the two lower doses used in this study (0.05 and 0.5 CU/kg/h) raised plasma secretin to concentrations similar to what have been found after endogenous secretin release during duodenal acidification in both pig and rat.^{22,24} Even the lowest dose of secretin induced marked cellular changes and depletion of mucus. Similar morphological changes have earlier been shown after duodenal perfusion with acid.²⁵ These results indicate that secretin may play a role in the physiological regulation of Brunner's gland secretion, probably in concert with other regulatory peptides. The potency of porcine secretin as stimulator of Brunner's gland secretion in the rat is of the same order of magnitude as porcine VIP,¹² and both peptides stimulate volume secretion and output of bicarbonate and protein, but unlike pancreatic secretion, increased secretion rate was not followed by changes in concentration.

Glucagon, which is structurally related to secretin and VIP, was initially tested in a dose of 1 µg/kg/h, but this peptide was without effect on Brunner's

Fig. 3 (A) Brunner's glands in rat. Secretory cells contain large amounts of PAS positive mucus. (B) After infusion of secretin 15 ng/kg/h for five hours. Secretory cells are depleted of mucus. PAS haematoxylin autentia $\times 1000$ (original magnification)

gland secretion, and the histologic examination revealed no changes of the secretory cells. This was also observed after infusion of unphysiologically large doses (5.0 and 50.0 $\mu\text{g/kg/h}$). Infusion of glucagon in high doses (3.0–64.0 $\mu\text{g/kg/h}$) has earlier been shown to stimulate secretion from Brunner's glands in the dog.^{10 11} This discrepancy may be because of species differences, but also contamination with small amounts of potent, stimulating peptides, such as VIP, could explain the results of these early experiments with glucagon.

In conclusion this study has shown that a small rise in plasma secretin concentration, probably within physiological range, induces an increased secretion of bicarbonate, protein, and mucus from Brunner's glands in the rat, while glucagon has no effect.

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