Effect of vasoactive intestinal polypeptide and somatostatin on secretion of epidermal growth factor and bicarbonate from Brunner's glands

P KIRKEGAARD, P S OLSEN, E NEXØ, J J HOLST, AND S S POULSEN

From the Department of Surgery C and Department of Clinical Chemistry ML, Rigshospitalet, Department of Physiology C, and Department of Anatomy B, University of Copenhagen, Denmark

SUMMARY The effect of VIP and somatostatin on secretion of epidermal growth factor and bicarbonate from Brunner's glands was investigated in the rat. Vasoactive intestinal polypeptide infused in doses of 10 and 100 ng/kg/h significantly increased epidermal growth factor and bicarbonate output, but the concentrations did not change. Somatostatin infused at doses of 1, 10, 100 and 1000 ng/kg/h against a background of VIP 100 ng/kg/h inhibited in dose-dependent fashion the stimulated epidermal growth factor and bicarbonate outputs from rat Brunner's gland pouches. Also basal secretion was inhibited by somatostatin. Infusion of antisomatostatin serum stimulated Brunner's gland secretion. By immunohistochemical studies of rat duodena, it was found that epidermal growth factor, is almost exclusively present in the secretory cells of Brunner's glands. It is concluded that VIP stimulates secretion of epidermal growth factor and bicarbonate from Brunner's glands, an effect which is inhibited by somatostatin. A possible role for somatostatin in the control of Brunner's gland secretion is suggested.

Brunner's glands of the duodenum produce a mucous, alkaline secretion, which forms a protective lining on the duodenal mucosa, preventing epithelial damage by mechanical or chemical trauma.¹⁻³ In man, the glands have been recently shown to contain epidermal growth factor,^{4,5} a 53 amino acid residue peptide with a number of biological effects, including inhibition of gastric acid secretion, stimulation of cell growth and differentiation⁶ and cytoprotective effect on the gastroduodenal mucosa.^{7,8}

The mechanism controlling Brunner's gland secretion seems to be complex, and it is not well elucidated. The autonomic nervous system as well as local factors are involved, and also a hormonal mechanism has been rendered probable.⁹⁻¹³ In the rat, Brunner's glands are stimulated by small concentrations of circulating secretin,¹⁴ and probably by neuronal vasoactive intestinal polypeptide (VIP).¹⁵ No peptide has yet been shown to inhibit secretion from the glands.

The present study was performed to investigate the effects of VIP and somatostatin on epidermal

growth factor and bicarbonate output from Brunner's glands in the rat.

Methods

ANIMALS AND OPERATIVE PROCEDURE

A total of 105 male Wistar rats weighing 190-210 g were fasted from 8 am for eight hours in raised mesh bottom cages, but allowed water *ad libitum*. For infusion of peptides one of the jugular veins was cannulated with a soft 0.8 mm polyethylene catheter under ether anaesthesia. A laparotomy was performed with a midline incision. The pylorus was ligated, and a second ligature was placed around the duodenum 8 mm distally to the pylorus, a few millimetres proximal to the common bile and pancreatic ducts. Through a stab wound a 2 mm polyethylene catheter was inserted into the lumen of the distal duodenum, and the tip of the catheter was passed up through the previously mentioned ligature, which was then tightened. Thus an 8 mm long *in situ* pouch of the proximal duodenum was isolated and the secretion drained by the catheter. In order to keep the stomach empty during the experiment, a 5 mm catheter was placed in the stomach through a stab wound in the forestomach

Address for correspondence: Preben Kirkegaard, MD, Department of Surgery C, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Received for publication 24 January 1984

and secured by a purse string suture. Finally, the abdominal wound was closed around the two catheters with sutures. In 10 rats an 8 mm long pouch of the distal duodenum was made in a similar way for control. After having regained consciousness the rats were placed in Bollmann cages. The catheters were connected to glass syringes allowing duodenal secretions to be collected anaerobically. Gravity of the piston in the syringe induced a slight vacuum that continuously drained the pouch.

EXPERIMENTAL PROCEDURE

Natural porcine VIP (GIH Research Laboratory, Karolinska Institutet, Stockholm, Sweden) and synthetic cyclic somatostatin-14 (Beckman Inc, Palo Alto, California, USA) 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). Basal secretion was studied in 10 rats, receiving saline with 2% albumin intravenously. The effect of VIP (10 and 100 ng/kg/h, – that is, 3 and 30 pmol/kg/h) and somatostatin (100 ng/kg/h, – that is, 61 pmol/kg/h) on basal secretion was examined in groups of 10 rats each. On a background infusion of VIP (100 ng/kg/h) four groups of 10 rats were tested with intravenous somatostatin in doses of 1, 10, 100 or 1000 ng/kg/h, – that is, 0.6, 6.1, 61 or 610 pmol/kg/h) respectively. For control two groups of five rats each with pouches of the distal duodenum had either VIP 100 ng/kg/h or somatostatin 100 ng/kg/h.

The effect of rabbit somatostatin antiserum on Brunner's gland secretion was studied in five rats. Somatostatin antiserum (1756) was raised in rabbits against a synthetic somatostatin-bovine serum albumin conjugate.¹⁶ The binding energy of the antiserum was approximately 10^{11} l/mol and the binding capacity 3.0 nmol/l. The binding region was between residues 3 and 10 of somatostatin 1–14, and the antibodies bind somatostatin 1–28 with equal energy.^{16, 17} One millilitre antiserum was injected intravenously as a bolus 10 minutes before the experiment was started, and 1 ml/h was infused intravenously for five hours. In five rats normal rabbit serum was administered similarly for control.

In all groups duodenal secretions were collected for five hours, then the rats were killed by an overdose of ether and the abdomen was opened to make sure that the collection was complete. The volume was measured to the nearest 0.05 ml and bicarbonate concentration was determined. The remaining secretion was mixed with 0.5 ml of a solution of 500 KIU aprotinin (Trasylol^R, Bayer, Leverkusen, FRG) per ml in phthalate buffer 0.1 mmol/l, pH 4.0, and stored at –20°C until assay. In

the groups receiving somatostatin 100 or 1000 ng/kg/h the volume was too small for determination of both bicarbonate and epidermal growth factor.

LABORATORY ANALYSES

Epidermal growth factor concentrations in duodenal secretions were measured radioimmunochemically by use of an antiserum (8136) raised in rabbit against epidermal growth factor purified from rat submaxillary gland. The detection limit of the assay was 0.05 nmol/l and precision was 10%. Further details are described elsewhere.¹⁸ HCO_3^- concentration was calculated from the pH and $p\text{CO}_2$ which were determined anaerobically using pH electrode G 269c and $p\text{CO}_2$ -electrode E 8001 in an ABL 2 (Acid-Base Laboratory 2, Radiometer, Copenhagen, Denmark).

Immunohistochemical studies were performed on duodena obtained from five untreated rats. The tissues were fixed at room temperature in Bouin's fixative by immersion for 24 hours, dehydrated and embedded in paraffin. Epidermal growth factor was localised by the unlabelled antibody peroxidase-antiperoxidase (PAP) technique.¹⁹ Epidermal growth factor antibody was used diluted 1:400. In the control sections epidermal growth factor antibody was preincubated with purified rat epidermal growth factor (10 $\mu\text{mol/l}$) or synthetic human urogastrone (10 $\mu\text{mol/l}$) (supplied by H Gregory, ICI, Macclesfield, UK).

Statistical evaluation of the secretory data was performed by means of Student's *t* test, *p* values <0.05 were considered significant.

Results

Intravenous infusion of VIP in doses of 10 and 100 ng/kg/h (3 and 30 pmol/kg/h) stimulated both epidermal growth factor and bicarbonate output from Brunner's glands compared with the control group (Fig. 1). The flow rate was significantly increased, while concentrations of epidermal growth factor and bicarbonate in the secretions remained unchanged (Table 1). Infusion of graded doses of somatostatin dose dependently inhibited VIP induced flow rate (Table 2), epidermal growth factor and bicarbonate output from Brunner's glands (Fig. 2), and somatostatin (100 ng/kg/h, – that is, 61 pmol/kg/h) also inhibited basal secretion (Table 1). The secretions obtained from pouches of the distal duodenum were hardly measurable (0–0.10 ml/5 h). Administration of rabbit somatostatin-antiserum to rats induced a marked increase in volume secretion, epidermal growth factor and bicarbonate output compared with infusion of non-immune rabbit serum (Table 3). In no group, irrespective of

Fig. 1 Effect of VIP on Brunner's gland secretion of epidermal growth factor and bicarbonate. Each column indicates mean $\pm$ SEM. Both doses of VIP resulted in values significantly different from basal secretion ($p < 0.05$).

secretory rate, significant changes in concentrations of epidermal growth factor or bicarbonate were seen.

Immunohistochemical investigation of rat duodenum revealed that epidermal growth factor immunoreactivity, as in man,⁵ almost exclusively is present in cells of Brunner's glands (Fig. 3). Control sections were negative.

Discussion

VIP-ergic nerves are present in Brunner's glands, and probably play a role in the control of secretion.¹⁵ Intravenous infusion of VIP in doses of 100 ng/kg/h (30 pmol/kg/h) was previously shown to induce a maximal flow rate and bicarbonate output from Brunner's glands. In this study also epidermal growth factor concentration in the secretion was determined. Although the flow rate increased, epidermal growth factor concentration remained unchanged, and consequently epidermal growth

Table 1 Effect of VIP and somatostatin on basal secretion from Brunner's glands in the rat

	No	Volume (ml/5 h)	HCO ₃ concentration (mmol/l)	Epidermal growth factor concentration (pmol/l)
Basal secretion (saline)	10	1.10 $\pm$ 0.10	64.5 $\pm$ 5.1	260 $\pm$ 30
VIP (10 ng/kg/h)	10	1.60 $\pm$ 0.10†	65.2 $\pm$ 5.8	340 $\pm$ 50
VIP (100 ng/kg/h)	10	2.15 $\pm$ 0.15‡	66.4 $\pm$ 5.6	310 $\pm$ 60
Somatostatin (100 ng/kg/h)	10	0.75 $\pm$ 0.10*	62.1 $\pm$ 4.3	ND

Values given are mean $\pm$ SEM. Statistical significance between experimental and control groups.

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. ND: not determined.

Table 2 Effect of somatostatin on VIP-induced secretion from Brunner's glands in the rat

	No	Volume (ml/5 h)	HCO ₃ concentration (mmol/l)	Epidermal growth factor concentration (pmol/l)
Control (VIP 100 ng/kg/h)	10	2.15 $\pm$ 0.15	66.4 $\pm$ 5.6	310 $\pm$ 60
Somatostatin (1 ng/kg/h)	10	1.20 $\pm$ 0.05*	64.3 $\pm$ 5.2	315 $\pm$ 30
Somatostatin (10 ng/kg/h)	10	1.00 $\pm$ 0.20†	61.4 $\pm$ 5.5	350 $\pm$ 50
Somatostatin (100 ng/kg/h)	10	0.65 $\pm$ 0.10†	ND	315 $\pm$ 35
Somatostatin (1000 ng/kg/h)	10	0.65 $\pm$ 0.05†	61.6 $\pm$ 4.8	ND

Values given are mean $\pm$ SEM. Statistical significance between experimental and control groups.

* $p < 0.01$; † $p < 0.001$. ND: not determined.

Table 3 Effect of somatostatin antiserum on Brunner's gland secretion in the rat

	No	Volume (ml/5 h)	HCO ₃ output (µmol/5 h)	Epidermal growth factor output (fmol/5 h)
Normal rabbit serum	5	1.05 $\pm$ 0.05	68.5 $\pm$ 4.5	375 $\pm$ 35
Somatostatin antiserum	5	2.40 $\pm$ 0.15†	161.3 $\pm$ 5.5†	600 $\pm$ 110*

Values given are mean $\pm$ SEM. Statistical significance between the groups: * $p < 0.05$; † $p < 0.01$.

Fig. 2 Effect of somatostatin on VIP-induced secretion of epidermal growth factor and bicarbonate from Brunner's glands. Each column indicates mean $\pm$ SEM. All doses of somatostatin resulted in values significantly different from control ($p < 0.05$). ND: not determined.

Fig. 3 (a) Brunner's glands from rat duodenum with positive immunostaining for epidermal growth factor in all glandular cells. (b) Negative control section. $\times 80$ (original magnification).

factor output increased in response to VIP infusion. As in the duodenum, epidermal growth factor-immunoreactivity is only found in the secretory cells of Brunner's glands, the unchanged epidermal growth factor concentration and the lack of secretion from distal duodenum support the view that the secretion obtained from the pouches is predominantly a product of these glands, and the contribution from the glands of Lieberkuhn is negligible.

Somatostatin is known to inhibit various secretory processes in the gastrointestinal tract,²⁰ and this study shows that the peptide is also a potent inhibitor of Brunner's gland secretion. Furthermore, infusion of anti-somatostatin serum induced a pronounced increase of the secretory rate suggesting that endogenous somatostatin may play a role in the regulatory mechanisms controlling Brunner's gland secretion. Somatostatin immunoreactivity is present in endocrine-like D-cells throughout the gastrointestinal tract, and D-cells have been described to be scattered between the secretory cells of Brunner's glands in man.²¹ In the rat, however, only very few D-cells are seen in these glands, making any physiological significance unlikely. Neither can somatostatin containing nerves be shown in Brunner's glands (L-I Larsson, personal communication), but a rich network of somatostatin-immunoreactive nerves is seen in the lamina propria and submucosa of the duodenum.²² Thus a direct paracrine or nervous inhibitory effect is not very likely, but if somatostatin acts as a circulating hormone,²³ a direct effect on the secretory cells is still possible. An indirect action, hormonal or neuronal, on the secretion is also possible, as somatostatin has been shown to stimulate enteric

inhibitory neurones,²⁴ and inhibition of Brunner's gland secretion by stimulation of splanchnic nerves has previously been shown.¹¹ The localisation of somatostatin in the submucous plexus agrees well with a hypothesis of somatostatin as transmitter of inhibitory interneurones influencing Brunner's gland secretion.

In conclusion this study has shown that, as in man, epidermal growth factor immunoreactivity in rat duodenum is localised to the cells of Brunner's glands, and that VIP stimulates secretion of epidermal growth factor from the glands. Vasoactive intestinal polypeptide induced secretion of epidermal growth factor and bicarbonate is inhibited by small doses of somatostatin, and a possible role for somatostatin in the control of Brunner's gland secretion is suggested.

This study was supported by the Danish Medical Research Council (12-1759 and 12-3088) and the NOVO Foundation.

References

- 1 Florey HW, Harding HE. Further observations on the secretion of Brunner's glands. *J Pathol Bacteriol* 1934; **39**: 255-76.
- 2 Florey HW, Jennings MA, Jennings DA, O'Connor RC. The reactions of the intestine of the pig to gastric juice. *J Pathol Bacteriol* 1939; **49**: 105-23.
- 3 Griffith CA, Harkins HN. The role of Brunner's glands in the intrinsic resistance of the duodenum to acid-peptic digestion. *Ann Surg* 1956; **143**: 160-72.
- 4 Elder JB, Williams G, Lacey E, Gregory H. Cellular localisation of human urogastrone/epidermal growth factor. *Nature* 1978; **271**: 466-7.
- 5 Heitz PhU, Kasper M, Van Noorden S, Polak JM, Gregory H, Pearse AGE. Immunohistochemical localisation of urogastrone to human duodenal and submandibular glands. *Gut* 1978; **19**: 408-13.
- 6 Hollenberg MD. Epidermal growth factor-urogastrone, a polypeptide acquiring hormonal status. *Vitam Horm* 1979; **37**: 69-110.
- 7 Konturek SJ, Radecki T, Brzozowski T *et al*. Gastric cyto-protection by epidermal growth factor. *Gastroenterology* 1981; **81**: 438-43.
- 8 Kirkegaard P, Olsen PS, Poulsen SS, Nexø E. Epidermal growth factor inhibits cysteamine-induced duodenal ulcer. *Gastroenterology* 1983; **85**: 1277-83.
- 9 Florey HW, Harding HE. A humoral control of the secretion of Brunner's glands. *Proc Roy Soc Lond* 1935; **117**: 68-77.
- 10 Florey HW, Harding HE. The nature of the hormone controlling Brunner's glands. *Q J Exp Physiol* 1935; **25**: 329-40.
- 11 Wright RD, Jennings MA, Florey HW, Lium R. The influence of nerves and drugs on secretions by the small intestine and an investigation of the enzymes in intestinal juice. *Q J Exp Physiol* 1940; **30**: 73-120.
- 12 Preshaw RM, Knauf RS. The effect of sham feeding on the secretion and motility of canine duodenal pouches. *Gastroenterology* 1966; **51**: 193-9.
- 13 Blickenstaff D, Grossman MI, Ivy AC. Stimulating effect of sugar, fat, and meat meals on duodenal secretion in the dog. *Am J Physiol* 1949; **158**: 122-8.
- 14 Kirkegaard P, Olsen PS, Poulsen SS, Holst J, Schaffalitzky de Muckadell OB, Christiansen J. The effect of secretin and glucagon on Brunner's gland secretion in the rat. *Gut* 1984; **25**: 264-8.
- 15 Kirkegaard P, Lundberg JM, Poulsen SS, *et al*. Vasoactive intestinal polypeptidergic nerves and Brunner's gland secretion in the rat. *Gastroenterology* 1981; **81**: 872-8.
- 16 Baldiserra FGA, Munoz-Perez MA, Holst JJ. Somatostatin 1-28 circulates in human plasma. *Regul Pept* 1983; **6**: 63-9.
- 17 Hilsted L, Holst JJ. On the accuracy of radio-immunological determination of somatostatin in plasma. *Regul Pept* 1982; **4**: 13-31.
- 18 Skov Olsen P, Nexø E. Quantitation of epidermal growth factor in the rat. Identification and partial characterization of duodenal EGF. *Scand J Gastroenterol* 1983; **18**: 771-6.
- 19 Sternberger L. *Immunocytochemistry*. Englewood Cliffs, New York: Prentice Hall, 1974: 129-71.
- 20 Patel YC, Zingg HH, Fitz-Patrick D, Srikant CB. Somatostatin: some aspects of its physiology and pathophysiology. In: Bloom SR, Polak JM, eds. *Gut hormones*. London: Churchill Livingstone, 1981.
- 21 Capella C, Solcia E, Frigerio B, Buffa R. Endocrine cells of the human intestine. An ultrastructural study. In: Fujita T, ed. *Endocrine gut and pancreas*. Amsterdam: Elsevier, 1976.
- 22 Schultzberg M, Hökfelt T, Nilsson G *et al*. Distribution of peptide and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig. *Neuroscience* 1980; **5**: 689-744.
- 23 Schusdziarra V, Unger RH. Physiology and pathophysiology of circulating somatostatin in dogs. In: Bloom SR, Polak JM, eds. *Gut hormones*. London: Churchill Livingstone, 1981.
- 24 Furness JB, Costa M. Actions of somatostatin on excitatory and inhibitory nerves in the intestine. *Eur J Pharmacol* 1979; **56**: 69-74.