Somatostatin levels and binding in duodenal mucosa of rabbits with ligation of the pancreatic duct

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ABSTRACT

Atrophy of the exocrine pancreas was induced in rabbits by pancreatic duct ligation. Somatostatin concentration and binding in cytosol from rabbit duodenal mucosa were studied after 6 and 14 weeks of pancreatic duct ligation. Somatostatin-like immunoreactivity was significantly increased in the duodenal mucosa in both periods. Scatchard analysis showed a parallel increase in the number of binding sites rather than a change in their affinity. The physiological significance of these findings remains to be clarified.

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INTRODUCTION

Reports concerning the effects of pancreatic exocrine secretion on duodenal somatostatin content are very scarce and contradictory. Lee, Wakasugi & Ibayashi (1983) have shown a significant increase of somatostatin content in the duodenum after 3 weeks of ligation of the pancreatic duct compared with that in the sham-operated group, while Ballmann, Fölsch & Conlon (1985) observed no changes of somatostatin levels in the small intestine after 45 days of pancreatic acinar atrophy in the rat.

Somatostatin-containing cells in the small intestine are located in the region of the crypt of Lieberkühn and have microvilli in contact with the gut lumen (Polak, Pearse, Grimelius *et al.* 1975; Penman, Wass, Butler *et al.* 1983). The intestinal mucosa also contains numerous somatostatin-immunoreactive nerve terminals (Costa, Patel, Furness & Arimura, 1977). These branch extensively in the lamina propria mucosa and come into close contact with a great number of epithelial cells, of which enterochromaffin cells and cholecystokinin cells have been identified (Larsson, 1981). We have recently shown the existence of specific binding sites for somatostatin in cytosol isolated from the intestinal mucosa of the rabbit (Lopez-Ruiz, Arilla, Gonzalez-Guijarro & Prieto, 1985). However, nothing is known of their possible involvement in the duodenal changes which follow pancreatic exocrine insufficiency.

Since the ligation of the pancreatic duct induces rapid atrophy of the exocrine parenchyma (Gebhardt & Stolte, 1978), we have used this experimental model to study changes in both somatostatin content in the duodenal mucosa as well as in somatostatin binding to cytosol from duodenal mucosa in the rabbit.

MATERIALS AND METHODS

Chemicals

Somatostatin-14 was kindly provided by Serono (Madrid, Spain), synthetic [Tyr^{11}]-somatostatin was purchased from Universal Biologicals Ltd (Cambridge, U.K.), aprotinin (trasylol) was from Bayer (Leverkusen, F.R.G.), trypsin inhibitor and bovine serum albumin were from Sigma (St Louis, MO, U.S.A.) and carrier-free Na^{125}I (IMS30; 100 mCi/ml) was from the Radiochemical Centre (Amersham, Bucks, U.K.). [Tyr^{11}]-Somatostatin was radioiodinated by a chloramine T method (Greenwood, Hunter & Glover, 1983). The specific radioactivity of the tracer was about 350 Ci/g. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre. This antiserum was raised in rabbits against somatostatin-14 conjugated to bovine serum albumin, and is specific for somatostatin but, as somatostatin-14 constitutes the C-terminal portions of

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both somatostatin-25 and somatostatin-28, the antiserum does not distinguish between these three forms. All other chemicals were reagent grade.

Animals and experimental procedures

Male New Zealand White rabbits weighing between 2 and 3 kg were used. After a 12-h fast with water freely available, the abdomen was opened by a midline incision under light anaesthesia. The terminal zone of the pancreatic duct was doubly ligated and cut, and the main part of the gland, surrounding the duodenum, was dissected from the duodenal adventitia to avoid intestinal contamination by pancreatic juice coming from aberrant or accessory pancreatic ducts. Control groups went through a sham-operation in which the thread was positioned but the pancreatic duct was not ligated. In all experiments, the animals together with their pair-fed control groups, were starved but not deprived of water for 12 h before killing.

Tissue extraction and radioimmunoassay of somatostatin

Animals were killed by decapitation 6 and 14 weeks after ligation. The abdomen was reopened and the duodenum removed. The duodenal mucosa was then dissected free from the underlying muscle layer, and immediately boiled for 5 min in acetic acid (1 mol/l) in order to destroy the proteolytic enzymes and coagulate the bulk of the proteins, and homogenized (1–2 min) with a motor-driven Teflon pestle. This procedure does not destroy the ability to detect somatostatin, as previously shown by Gerich, Greene, Hara *et al.* (1979). The homogenate was centrifuged at 1160 *g* for 30 min at 4 °C, and the resultant supernatant stored at −70 °C until assay. Just before assay, extracts were neutralized with NaOH (1 mol/l). Somatostatin concentrations were determined in tissue extracts by a radioimmunoassay method (Gerich *et al.* 1979) with a sensitivity limit of 60 pmol/l. A phosphate buffer (0·01 mmol/l, pH 7·4) containing NaCl (0·15 mol/l), EDTA (0·05 mol/l), 0·1% (w/v) bovine serum albumin and 100 kallikrein inhibitor units (KIU) aprotinin (Trasylol)/ml was used in the assay system. The possibility that substances present in the tissue extracts might interfere with antibody–antigen binding, thus giving rise to error, was disproved by performing serial dilutions of selected extracts in the assays, and comparing the resulting changes in hormonal immunoreactivity with those of the diluted standards. In addition, known standard amounts of the hormone were added to various amounts of the extracts, and serial dilutions again assayed in order to determine whether this exogenously added hormonal immunoreactivity could be reliably measured in the presence of tissue extracts. Incubation tubes were prepared in triplicate and the following were added to assay tubes in sequence: 200 µl sample or standard containing 0–320 pg cyclic somatostatin; 200 µl ^{125}I-labelled [Tyr^{11}]-somatostatin (5000 c.p.m.; equivalent to 5–10 pg) and 400 µl appropriately diluted antibody (final dilution usually 1 : 20 000). After brief vortexing, the tubes were incubated at 4 °C for 48 h; separation of bound and free hormone was accomplished by addition of 500 µl dextran-coated charcoal (dextran, 0·025% w/v; Pharmacia T-70, Uppsala, Sweden. Charcoal, Norit A, 0·25% w/v; Serva, Feinbiochemica, Heidelberg, F.R.G.). Dilution curves for rabbit tissue extracts were parallel to the standard curve. The per cent recovery of ^{125}I-labelled [Tyr^{11}]-somatostatin added to tissue extracts was 89 ± 3 (S.E.M.) %. The intra- and interassay coefficients of variation were 7·5 and 9·8% respectively.

Subcellular fractionation

Cytosol was isolated from the duodenal mucosa according to the method of Reyl-Desmars & Lewin (1982). Briefly, homogenates were prepared from duodenal mucosa by using a motor driven Potter–Elvejhem Teflon glass homogenizer (1 min at 1800 r.p.m.) in 10% (w/w) suspension in ice-cooled Tris (10 mmol/l)/sucrose (250 mmol/l) buffer (pH 7·4), containing 0·1 mg trypsin inhibitor/ml. Cytosolic extracts were prepared by centrifuging the homogenates for 1 h at 105 000 *g*. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as a standard.

Binding studies

These studies were carried out as described previously (Lopez-Ruiz *et al.* 1985). Under standard conditions, cytosol of rabbit duodenal mucosa (0·2 mg protein/ml) was incubated in 0·5 ml medium (pH 7·4) with the following composition: NaH_2PO_4 (0·5 mmol/l), Na_2HPO_4 (1 mmol/l), NaCl (80 mmol/l), KCl (5 mmol/l), $CaCl_2$ (1 mmol/l), $MgCl_2$ (1·5 mmol/l), Hepes (50 mmol/l), glucose (11 mmol/l), 0·1% bovine serum albumin, trypsin inhibitor (0·1 mg/ml), and ^{125}I-labelled [Tyr^{11}]-somatostatin (50 pmol/l) either alone or together with increasing concentrations of unlabelled somatostatin (up to 2 µmol/l) or other peptides. Unless otherwise indicated, incubations were performed at 25 °C for 60 min. The amount of ^{125}I-labelled [Tyr^{11}]-somatostatin associated with cytosolic proteins was determined after removal of unbound tracer by activated charcoal, 0·5% bovine serum albumin and 0·025% dextran T-70 (Reyl-Desmars & Lewin, 1982). 'Specific' binding was estimated as the

difference between 'total' binding (i.e. in the presence of tracer alone) and 'non-specific' binding as measured in the presence of unlabelled somatostatin (4 μmol/l). This non-specific component represented about 45% of the binding observed in the absence of unlabelled somatostatin. The integrity of bound ^{125}I-labelled [Tyr^{11}]-somatostatin was assessed by talc adsorption, as described previously (Conlon, Whittaker, Hammond & Alberti, 1981).

Statistical analysis

Statistical analysis was performed using Student's *t*-test for unpaired samples to determine significance. Differences with P values lower than 0·05 were considered significant. Each individual experiment was performed in triplicate. All results are expressed as means ± S.E.M.

RESULTS

Development of exocrine pancreatic atrophy and concentration of somatostatin in duodenal mucosa

The body weights of rabbits after 40–42 days of ligation of the pancreatic duct were significantly lower than those of control animals. Examination of pancreatic tissue by light microscopy indicated that the acinar tissue had been almost completely replaced by either adipose or fibrous tissues (data not shown). Six weeks after operation there was an increase in duodenal somatostatin levels and this level was maintained during the first 14 weeks after operation (Fig. 1).

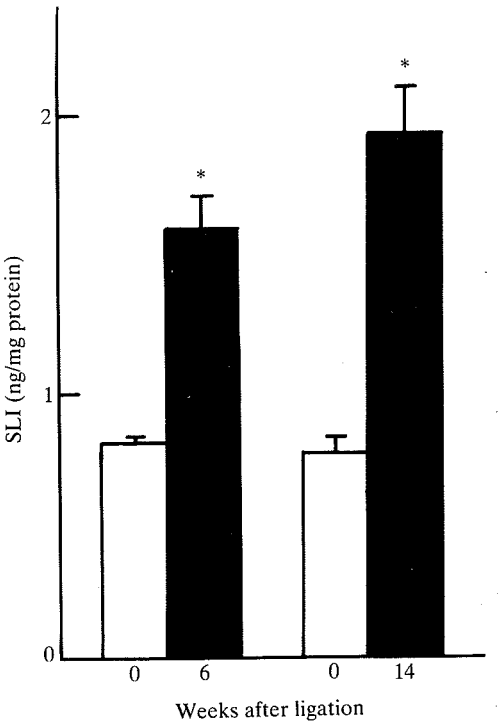

FIGURE 1. Concentration of somatostatin-like immunoreactivity (SLI) in duodenal mucosa of control rabbits (open bars) and animals subjected to pancreatic duct ligation (solid bars), 6 and 14 weeks after surgery. Values are expressed as means ± S.E.M. of five separate experiments. *$P<0·05$ compared with control (Student's *t*-test).

Somatostatin binding to cytosol of duodenal mucosa

The cytosol of the duodenal mucosa from both sham-operated and pancreatic duct-ligated rabbits bound ^{125}I-labelled [Tyr^{11}]-somatostatin by a time-dependent process, an apparent equilibrium being observed between 45 and 120 min at 25 °C (data not shown). All subsequent binding studies were conducted at 25 °C for 60 min.

Somatostatin-degrading activities in the cytosolic preparations studied could affect the interpretation of the results. For this reason, the degradation of the peptide by the cytosol of the duodenal mucosa was studied. The extent of degradation (25–30%) was similar in all cases.

The binding of unlabelled somatostatin was calculated from the displacement of ^{125}I-labelled [Tyr^{11}]-somatostatin at somatostatin concentrations ranging from 1 nmol/l to 2 μmol/l in cytosol of control and pancreatic duct-ligated rabbits (Fig. 2). The binding of somatostatin by cytosolic preparations obtained from pancreatic duct-ligated rabbits was significantly higher than that in the control group, both in the absence and presence of unlabelled somatostatin. Since the observed differences could be due to changes in either affinity or capacity of somatostatin-binding sites, the stoichiometric data were interpreted by the method of Scatchard (1949). The Scatchard plots (Fig. 3) exhibited curvilinear, concave upward curves that were analysed on the basis of two classes of somatostatin-binding sites, by drawing a least-square regression line to fit the low affinity site using the higher somatostatin concentrations; the contribution of this site was subtracted from the binding obtained at the lower somatostatin concentrations to obtain the high-affinity binding. Table 1 shows the corresponding equilibrium parameters for the high affinity–low capacity and for the low affinity–high capacity binding sites. This analysis revealed that there was an increase in the number of both classes of binding sites after 6 and 14 weeks of pancreatic duct ligation, without changes in the corresponding affinities as compared with control animals.

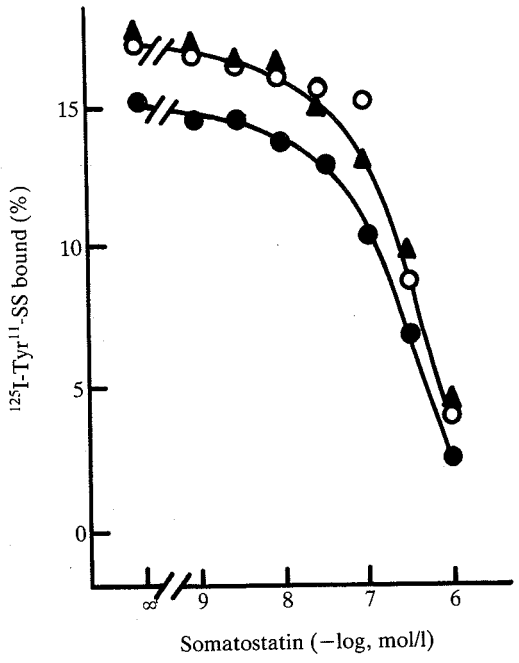

FIGURE 2. Competitive inhibition of specific ^{125}I-labelled [Tyr^{11}]-somatostatin (^{125}I-Tyr^{11}-SS; 50 pmol/l) binding to cytosol (0·2 mg protein/ml) from rabbit duodenal mucosa by unlabelled somatostatin. Points correspond to control rabbits (●) and animals with ligation of the pancreatic duct, studied 6 (○) and 14 (▲) weeks after surgery. Each point is the mean of five triplicate experiments. For the sake of clarity, S.E.M. values are not indicated, but were always less than 10% of the means.

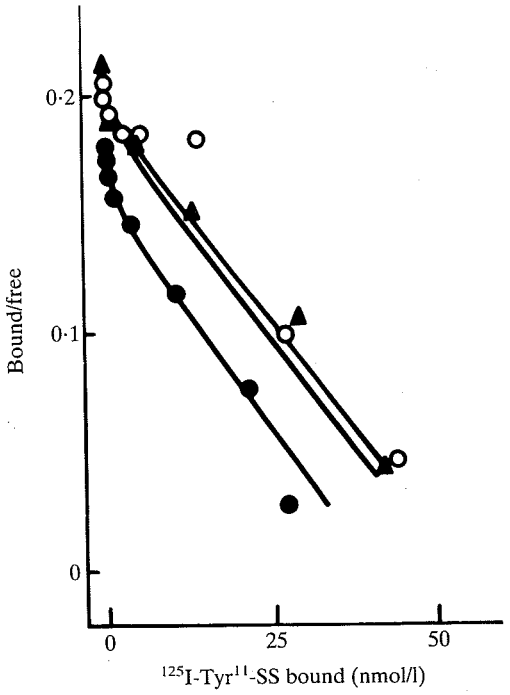

FIGURE 3. Scatchard analysis of ^{125}I-labelled [Tyr^{11}]-somatostatin (^{125}I-Tyr^{11}-SS; 50 pmol/l) binding to cytosol (0·2 mg protein/ml) from rabbit duodenal mucosa by unlabelled somatostatin. Values are means ± S.E.M. ($n=5$) and were derived from the experimental data of Fig. 2. Points correspond to control rabbits (●) and animals with ligation of the pancreatic duct studied 6 (○) and 14 (▲) weeks after surgery. The kinetics constants calculated by Scatchard analysis are given in Table 1.

DISCUSSION

The present data show that ligation of the pancreatic duct increases the somatostatin concentration in duodenal mucosa as well as the number of somatostatin-binding sites of both high and low affinity (without changes in the affinity values) in the cytosol from the same tissue. The study was performed 6 and 14 weeks after ligation of pancreatic duct, because exocrine pancreatic atrophy has been shown to appear between 5 and 6 weeks after ligation (Ballmann *et al.* 1985), which is in agreement with the observed decrease in body weight, as well as with the development of pancreatic acinar atrophy.

The data on duodenal somatostatin content are in keeping with the findings of Lee *et al.* (1983), but not with those of Ballman *et al.* (1985) who were unable to demonstrate any effect of pancreatic acinar atrophy on somatostatin levels in the small intestine. The discrepancy may be due to the fact that the atrophy of the exocrine pancreas was induced by feeding a copper-deficient diet combined with penicillamine. The reason for the increase of duodenal somatostatin concen-

TABLE 1. Effect of ligation of pancreatic duct on equilibrium parameters of somatostatin binding to cytosol of rabbit duodenal mucosa. Values are the means ± S.E.M. ($n=5$) and were derived from the experimental data of Fig. 3

	Somatostatin binding sites	
	High affinity	Low affinity
Control		
K_d (nmol/l)	46·3 ± 8·0	255·0 ± 36·4
BC (pmol/mg protein)	2·7 ± 0·3	204·0 ± 11·8
Pancreatic duct ligation (6 weeks)		
K_d (nmol/l)	49·0 ± 3·8	281·3 ± 17·2
BC (pmol/mg protein)	3·7 ± 0·1*	271·5 ± 21·9*
Pancreatic duct ligation (14 weeks)		
K_d (nmol/l)	45·3 ± 5·5	282·0 ± 28·4
BC (pmol/mg protein)	3·4 ± 0·5*	275·0 ± 26·4*

*$P<0·05$ compared with control (Student's t-test).
K_d, dissociation constant; BC, binding capacity.

tration observed after pancreatic duct ligation remains unclear. Results on somatostatin content may be related to a greater density of somatostatin-containing cells and neurones per unit length of duodenal mucosa in rabbits with pancreatic duct ligation. In the normal mucosa, somatostatin-containing cells are located mainly in the region of the crypt of Lieberkühn (Polak *et al.* 1977; Raptis, Schlegel, Lehmann *et al.* 1978). Somatostatin nerves have been detected immediately beneath the epithelium of the crypts rather than that of the villus (Schultberg, Hökfelt, Nilsson *et al.* 1980). Samples of duodenal mucosa obtained from control rabbits contain villi that are practically devoid of somatostatin, as well as crypts. This is in contrast to the samples from animals with pancreatic duct ligation, which have villous atrophy and contain only crypts of Lieberkühn (Altman, 1971). In addition, it has been shown that pancreatic exocrine impairment is associated with a low pH of duodenal content (Regan, Malagelada, Dimagno & Go, 1979). As a low pH stimulates the growth of D-cells (Arnold, Hülst, Heuhof *et al.* 1982), it is conceivable that chronically low intraduodenal pH could lead to D-cell hyperplasia and, therefore, an increase in duodenal somatostatin content.

It is possible that the increase in the capacity of somatostatin-binding sites in this condition could be a consequence of an increase of the number of target cells for somatostatin. In this regard, pancreatic duct ligation leads to hypertrophy and hyperplasia of Paneth cells, and an increase of the number of both goblet cells and 'intermediate granular cells' (Balas, Senegas-Balas, Bertrand *et al.* 1980). However, it is unknown whether somatostatin binds to these cells. If the increase in the somatostatin concentration of the duodenal mucosa reflects decreased release of peptide, one might expect an increase of the number of somatostatin-binding sites at this level. This hypothesis could explain the present findings.

The physiological or pathophysiological significance of these findings remains unclear since the role of altered binding site capacity in modulating intestinal somatostatin effects has not been evaluated. However, different studies have shown that somatostatin exerts a wide spectrum of effects on different intestinal functions. In fact, the peptide inhibits the release of duodenal hormones (Raptis *et al.* 1978) and decreases the number of goblet cells (Senegas-Balas, Balas, Pradayrol *et al.* 1985). On the other hand, the plasma levels of cholecystokinin (Fölsch, Schafmayer, Ebert *et al.* 1984; Slaff, Wolfe & Toskes, 1985), motilin and enteroglucagon (Besterman, Adrian, Christofides *et al.* 1978) are increased in exocrine pancreatic atrophy, whereas there is also an increase in the number of goblet cells (Balas *et al.* 1980). These previous findings, together with the present results, raise the possibility of a compensatory increase in the capacity of somatostatin-binding sites as a response to the excessive secretion of duodenal hormones as well as to the increase of the number of goblet cells after pancreatic duct ligation. However, the increase in the capacity of somatostatin-binding sites after pancreatic duct ligation reflects a generally unsuccessful effort to correct duodenal hormonal hypersecretion and hyperplasia of goblet cells.

To date, it is not possible to state whether the variations observed are the consequences of a direct mechanism through loss of pancreatic juice poured into the intestinal lumen or a cause of the adaptative response of the duodenal mucosa that follows ligation of the pancreatic duct. The second possibility would support an involvement of somatostatin in the adaptative mechanisms of the intestinal mucosa.

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