

# Partial enterectomy decreases somatostatin-binding sites in residual intestine of rabbits

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(Received 7 April/28 September 1987; accepted 21 October 1987)

## SUMMARY

1. Three weeks after partial enterectomy in the rabbit there was an increased somatostatin concentration and a decreased number of somatostatin-binding sites (without changes in the corresponding affinity values) in the cytosol of the residual intestinal tissue, except in the terminal ileum and the colon.

2. Five weeks after surgery both the somatostatin concentration and the number of somatostatin-binding sites returned towards control values.

3. These results suggest that an increase in bowel somatostatin content could lead to down-regulation of somatostatin-binding sites in the intestinal mucosa.

**Key words:** cytosol, enterectomy, somatostatin binding.

**Abbreviation:** SLI, somatostatin-like immunoreactivity.

## INTRODUCTION

Somatostatin, originally isolated from the hypothalamus [1], is also distributed throughout the gastrointestinal tract [2] in nerve fibres as well as in endocrine cells (D) [3, 4]. Endocrine D-cells are scattered in the mucosa in close proximity to other peptide-containing endocrine cells [5, 6]. Studies *in vivo* and *in vitro* have shown that somatostatin inhibits a number of gastrointestinal functions [7]. Recent observations suggest that in the gastric antrum a decrease in luminal pH stimulates growth of somatostatin cells [8]. Furthermore, it has been shown that intestinal somatostatin release is directly stimulated by nutrients [9]. Resection of a large segment of small intestine is associated with a low pH of jejunal content [10] as well as an increased quantity of food residue in the lower bowel [11]. However, the effect of partial enterectomy on somato-

statin concentration in the residual intestine is still unknown. On the other hand, an important mechanism for controlling hormonal sensitivity is the regulation of hormone-binding sites by the homologous hormone [12]. In this context, the regulation of its own binding sites has not been demonstrated for somatostatin in the intestinal tract. We have recently shown the existence of specific binding sites for somatostatin in the cytosolic fraction isolated from intestinal mucosa [13, 14]. These somatostatin-binding sites showed a remarkable degree of specificity. High concentrations (1  $\mu\text{mol/l}$ ) in the intestinal tract of neuropeptides such as neurotensin, [Leu]enkephalin, substance P and vasoactive intestinal peptide showed little affinity for somatostatin-binding sites.

The purpose of the present study was to determine the effects of intestinal resection on somatostatin content throughout the residual small intestine and large bowel, as well as the possible regulation by somatostatin of its own binding sites in these tissues.

## MATERIALS AND METHODS

### Chemicals

Somatostatin-14 was kindly provided by Serono (Spain). Synthetic [Tyr<sup>11</sup>]somatostatin was purchased from Universal Biologicals Ltd (Cambridge, U.K.). Trypsin inhibitor and bovine serum albumin were from Sigma (St Louis, MO, U.S.A.), T-70 dextran from Pharmacia Fine Chemicals (Uppsala, Sweden), activated charcoal from Feinbiochemica (Heidelberg, F.R.G.) and carrier-free Na<sup>125</sup>I (IMS 30, 100 mCi/ml) was from the Radiochemical Centre (Amersham, U.K.). [Tyr<sup>11</sup>]Somatostatin was radioiodinated by a chloramine-T method [15] at a specific radioactivity of about 350 Ci/g. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, U.K.). This antiserum was raised in rabbits against somatostatin-14 conjugated to bovine serum albumin and is specific for somatostatin but, as somatostatin-14 consti-

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

## Procedures

**Resection.** Male rabbits (New Zealand) weighing 1–2 kg were fasted and allowed to drink only water for 24 h. After they were anaesthetized with Ketamine hydrochloride, the rabbits were subjected to one of two operative procedures. In one group, the small intestine was resected from 10 cm distal to the ligament of Treitz to 10 cm proximal to the ileocaecal valve. Intestinal continuity was re-established by anastomosis of the proximal jejunal segment to the terminal ileum. In the second group (sham-operated or control group), a laparotomy was performed.

**Subcellular fractions.** Control as well as resected animals were studied at 1, 3 and 5 weeks after surgery. Gut segments corresponding to the whole duodenum, proximal jejunum, distal ileum, colon and rectum were immediately removed and incubated at 37°C for 30 min in a 10% suspension (w/w) in ice-cooled 10 mmol/l Tris–250 mmol/l sucrose buffer, pH 7.4, containing trypsin inhibitor (0.1 mg/ml) and then subsequently washed twice in the same suspension in order to remove endogenous somatostatin. The cytosol was isolated according to the method of Reyl-Desmars & Lewin [16]. Homogenates were prepared from intestinal wall using a motor-driven Potter–Elvehjem Teflon-glass homogenizer (1 min at 1800 rev./min) in the suspension described above. The homogenates were then centrifuged for 1 h at 105 000 g. The protein concentration in the cytosol was estimated by the method of Lowry *et al.* [17] using bovine serum albumin as a standard. Results were expressed as somatostatin-like immunoreactivity (SLI) per ng of protein.

**Binding studies.** The cytosolic fractions (0.2 mg of protein/ml) were incubated at 25°C for 90 min in 0.5 ml of a medium (pH 7.4) of the following composition (in mmol/l): NaH<sub>2</sub>PO<sub>4</sub> 0.5, Na<sub>2</sub>HPO<sub>4</sub> 1, NaCl 80, KCl 5, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1.5, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid 50, glucose 11, and containing bovine serum albumin (0.1%, w/v), trypsin inhibitor (0.01 mg/ml) and <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin (50 pmol/l). The fractions were incubated either alone or together with increasing concentrations of unlabelled somatostatin (up to 2 μmol/l). Unbound peptide was removed by adding 1 ml of 0.25% (w/v) activated charcoal, 0.5% (w/v) bovine serum albumin and 0.025% (w/v) T70 dextran [18, 19]. '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 4 μmol/l unlabelled somatostatin); this non-specific component represented about 45% of the binding observed in the absence of unlabelled somatostatin. The integrity of bound <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin was assessed by talc adsorption [13].

**Tissue extraction and radioimmunoassay.** Somatostatin was extracted in 1 mol/l acetic acid at 0°C by soni-

cation. The homogenate was centrifuged at 3000 rev./min for 30 min at 4°C, and the resultant supernatant was stored at –70°C until assay. Just before assay, extracts were neutralized with 1 mol/l NaOH. Somatostatin concentration was determined in tissue extracts by a radioimmunoassay method [20] with a limit of sensitivity of 10 pg/ml. Incubation tubes were prepared in triplicate and the following were added to assay tubes in sequence: 200 μl of sample or standard containing 0–320 pg of cyclic somatostatin, 200 μl of <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin (5000 c.p.m., equivalent to 5–10 pg), and 400 μl of 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 of 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 intra-assay and interassay coefficients of variation were 6.8% and 10.2%, respectively.

## Statistical analysis

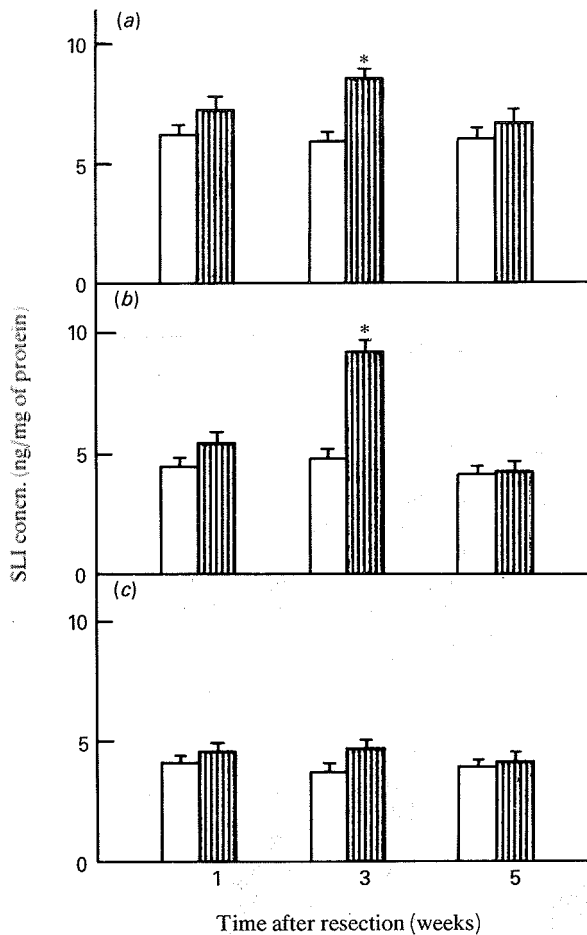
Each individual experiment was performed in triplicate. Results are expressed as mean values ± SEM. The linear equations used to describe the Scatchard plots [21] were determined by standard linear regression analysis. The statistical significance of difference of the means was determined by the Student's *t*-test; differences of *P* < 0.05 were assumed to be significant.

## RESULTS

The influence of small intestinal resection on SLI concentration in the residual duodenum, jejunum, ileum, caecum, colon and rectum is shown in Figs. 1 and 2. Three weeks, but not 1 week, after surgery, with the exception of the ileum and colon, the intestinal somatostatin concentration was significantly higher in the animals that underwent resection than in the sham-operated animals (*P* < 0.05). Five weeks after resection, the concentration of somatostatin had returned to control values throughout the intestinal tract.

The cytosolic fraction from both sham-operated and resected rabbits bound <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin by a time-dependent process. An apparent equilibrium was observed between 60 and 120 min at 25°C (data not shown). All subsequent binding studies were therefore conducted at 25°C for 90 min.

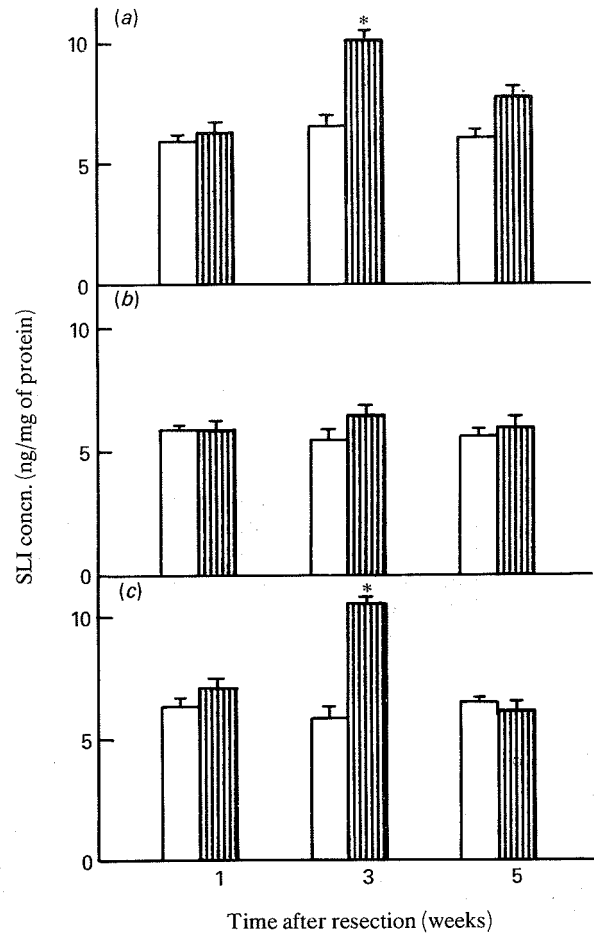
Different somatostatin-degrading activities in the cytosolic preparations studied could affect the interpretation of the results. For this reason, the degradation of the peptide by cytosolic fraction of the six types of extracts were studied. A similar extent of degradation (between 25 and 30%) was observed in all six extracts. Specific binding of <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin was reversible once formed, since the complex labelled peptide–cytosolic fraction could dissociate upon addition of an excess of



**Fig. 1.** Effect of partial enterectomy on SLI concentration in rabbit duodenum (a), proximal jejunum (b) and distal ileum (c). The controls (□) and resected (▨) animals were studied at 1, 3 and 5 weeks after surgery. Values are expressed as means  $\pm$  SEM of four triplicate determinations. Statistical significance: \* $P < 0.05$  vs control value.

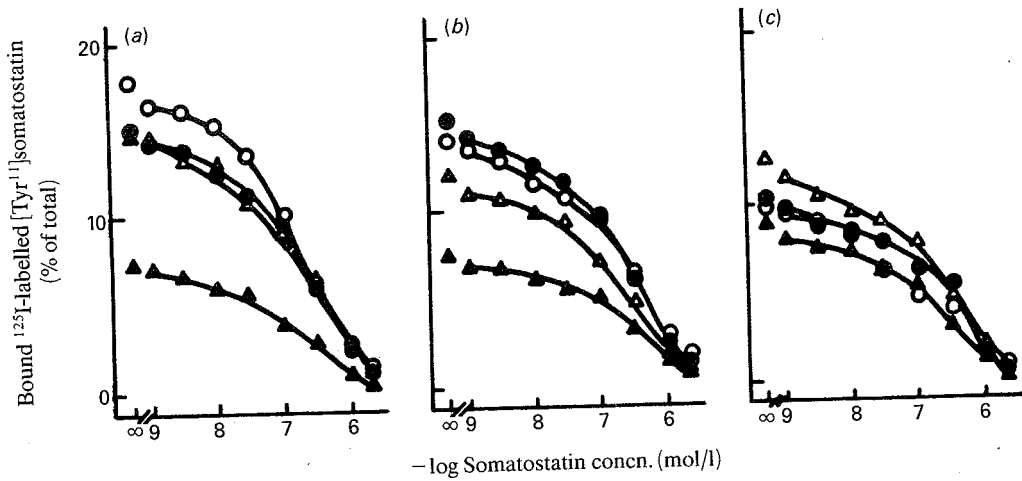
unlabelled somatostatin (4  $\mu$ mol/l). The dissociation patterns from the cytosolic preparation of sham-operated and resected rabbits were not statistically significantly different (data not shown). The time at which 50% of the specifically bound  $^{125}$ I-labelled [Tyr<sup>11</sup>]somatostatin dissociated from the cytosolic fraction varied between 19 and 22 min and no significant differences could be established when comparing all six types of extracts.

Increasing concentrations of unlabelled somatostatin competitively inhibited the specific binding of  $^{125}$ I-labelled [Tyr<sup>11</sup>]somatostatin to the cytosolic fraction of intestinal mucosa (Figs. 3 and 4). Except in the terminal ileum and the colon, specific binding of  $^{125}$ I-labelled [Tyr<sup>11</sup>]somatostatin to the cytosolic fractions 3 weeks after resection was significantly lower than that in the control group, both in the absence or presence of unlabelled somatostatin (1 nmol/l–2  $\mu$ mol/l). However, 5 weeks after resection, binding values were similar in both conditions. When the stoichiometric data of Figs. 3 and 4 were sub-

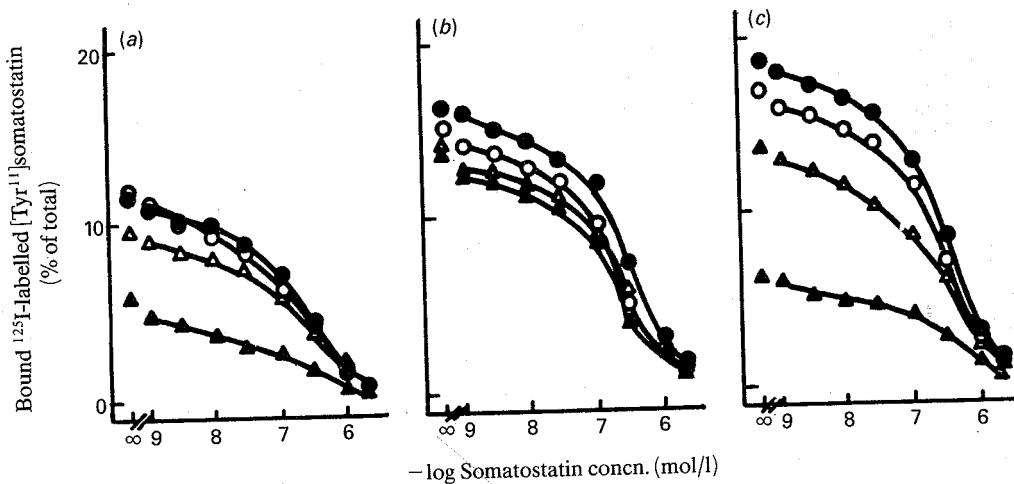


**Fig. 2.** Effect of partial enterectomy on SLI concentration in rabbit caecum (a), colon (b) and rectum (c). The controls (□) and resected (▨) animals were studied at 1, 3 and 5 weeks after surgery. Values are expressed as means  $\pm$  SEM of four triplicate determinations. Statistical significance: \* $P < 0.05$  vs control values.

jected to Scatchard plot [21], the values of somatostatin binding to the cytosolic preparations exhibited curvilinear, concave-upward curves (not shown) that were analysed on the basis of two classes of binding sites. A least-squares regression line was drawn to fit the low-affinity site using the four 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. Tables 1 and 2 show the corresponding equilibrium parameters for the high-affinity, low-capacity and for the low-affinity, high-capacity binding sites. From these results the decrease in somatostatin binding to the cytosolic fraction of intestinal mucosa 3 weeks after surgery must be accounted for by a decreased number of both high- and low-affinity binding sites rather than a change in their corresponding affinities. In order to determine whether in the binding experiments the apparent decrease in somatostatin-binding sites is due to occupancy of sites by endogenous somatostatin, cyto-



**Fig. 3.** Competitive inhibition of specific <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin (50 pmol/l) binding to mucosal cytosol (0.2 mg of protein/ml) from rabbit duodenum (a), proximal jejunum (b) and distal ileum (c) by unlabelled somatostatin. Data correspond to control rabbits (●) and animals subjected to small bowel resection and studied 1 (○), 3 (▲) and 5 (△) weeks after surgery. Each point is the mean of five separate experiments, each performed in triplicate. For the sake of clarity, SEM are not represented but were always below 10% of the mean values.



**Fig. 4.** Competitive inhibition of specific <sup>125</sup>I-labelled [Tyr<sup>11</sup>]somatostatin (50 pmol/l) binding to mucosal cytosol (0.2 mg of protein/ml) from rabbit caecum (a), colon (b) and rectum (c) by unlabelled somatostatin. Data correspond to control rabbits (●) and animals subjected to small bowel resection and studied 1 (○), 3 (▲) and 5 (△) weeks after surgery. Each point is the mean of five separate experiments, each performed in triplicate. For the sake of clarity, SEM are not represented but were always below 10% of the mean values.

solic somatostatin concentration was measured by radio-immunoassay. The somatostatin content was so low as to be mostly undetectable.

## DISCUSSION

Present results provide evidence that, except in the terminal ileum and colon, the somatostatin content of the intestinal wall increased 3 weeks after small bowel resection, whereas the number of somatostatin-binding sites

decreased without any change in their affinity. Five weeks after resection, intestinal somatostatin content as well as the number of somatostatin-binding sites had returned towards control values.

The changes in duodenal and jejunal somatostatin after intestinal resection differ from those observed by Gelinas *et al.* [22] in rats. These workers did not demonstrate any effect of enterectomy on somatostatin concentration in small intestinal mucosa. The discrepancies may be explained in a number of different ways: (a) Gelinas *et al.*

**Table 1. Equilibrium parameters of somatostatin binding in the cytosol of rabbit intestinal mucosa after small bowel resection**

Binding parameters were derived from the experimental data in Fig. 3. Results are means  $\pm$  SEM ( $n=5$ ). Statistical significance: \* $P < 0.05$  vs control value. Abbreviation:  $K_d$ , dissociation constant.

	Duodenum		Proximal jejunum		Distal ileum	
	High-affinity binding sites	Low-affinity binding sites	High-affinity binding sites	Low-affinity binding sites	High-affinity binding sites	Low-affinity binding sites
<b>Control</b>						
$K_d$ (nmol/l)	22.3 $\pm$ 2.3	169 $\pm$ 17	20.1 $\pm$ 1.6	209 $\pm$ 14	13.0 $\pm$ 0.4	195 $\pm$ 4
Binding capacity (pmol/mg of protein)	3.3 $\pm$ 0.2	134 $\pm$ 11	3.2 $\pm$ 0.5	159 $\pm$ 10	1.6 $\pm$ 0.1	103 $\pm$ 3
<b>1 week after resection</b>						
$K_d$ (nmol/l)	23.2 $\pm$ 2.4	156 $\pm$ 18	20.0 $\pm$ 2.0	226 $\pm$ 32	9.0 $\pm$ 3.2	195 $\pm$ 15
Binding capacity (pmol/mg of protein)	3.7 $\pm$ 0.6	149 $\pm$ 14	3.0 $\pm$ 0.4	167 $\pm$ 25	1.6 $\pm$ 0.3	98 $\pm$ 10
<b>3 weeks after resection</b>						
$K_d$ (nmol/l)	21.8 $\pm$ 3.4	156 $\pm$ 19	24.0 $\pm$ 4.2	269 $\pm$ 30	12.5 $\pm$ 1.3	183 $\pm$ 10
Binding capacity (pmol/mg of protein)	1.7 $\pm$ 0.4*	63 $\pm$ 10	1.3 $\pm$ 0.5*	87 $\pm$ 18	1.5 $\pm$ 0.2	81 $\pm$ 10
<b>5 weeks after resection</b>						
$K_d$ (nmol/l)	24.1 $\pm$ 0.2	184 $\pm$ 12	20.4 $\pm$ 0.5	213 $\pm$ 30	10.2 $\pm$ 0.2	204 $\pm$ 28
Binding capacity (pmol/mg of protein)	2.6 $\pm$ 0.3	148 $\pm$ 23	3.2 $\pm$ 0.5	122 $\pm$ 12	2.3 $\pm$ 0.5	123 $\pm$ 16

**Table 2. Equilibrium parameters of somatostatin binding in the cytosol of rabbit intestinal mucosa after small bowel resection**

Binding parameters were derived from the experimental data in Fig. 4. Results are means  $\pm$  SEM ( $n=5$ ). Statistical significance: \* $P < 0.05$  vs control value. Abbreviation:  $K_d$ , dissociation constant.

	Caecum		Colon		Rectum	
	High-affinity binding sites	Low-affinity binding sites	High-affinity binding sites	Low-affinity binding sites	High-affinity binding sites	Low-affinity binding sites
<b>Control</b>						
$K_d$ (nmol/l)	22.7 $\pm$ 4.2	172 $\pm$ 18	20.8 $\pm$ 8.0	224 $\pm$ 25	12.6 $\pm$ 0.2	173 $\pm$ 18
Binding capacity (pmol/mg of protein)	3.0 $\pm$ 0.6	102 $\pm$ 7	2.6 $\pm$ 0.2	192 $\pm$ 14	1.9 $\pm$ 0.1	185 $\pm$ 19
<b>1 week after resection</b>						
$K_d$ (nmol/l)	23.0 $\pm$ 4.4	174 $\pm$ 30	22.5 $\pm$ 5.0	190 $\pm$ 68	10.6 $\pm$ 0.4	184 $\pm$ 10
Binding capacity (pmol/mg of protein)	3.2 $\pm$ 0.4	97 $\pm$ 7	2.5 $\pm$ 1.3	158 $\pm$ 3	1.6 $\pm$ 0.2	178 $\pm$ 17
<b>3 weeks after resection</b>						
$K_d$ (nmol/l)	20.0 $\pm$ 4.0	186 $\pm$ 7	23.4 $\pm$ 2.5	219 $\pm$ 47	9.8 $\pm$ 0.5	200 $\pm$ 48
Binding capacity (pmol/mg of protein)	1.5 $\pm$ 0.2*	40 $\pm$ 1*	2.2 $\pm$ 0.4	146 $\pm$ 13	1.0 $\pm$ 0.3*	61 $\pm$ 6*
<b>5 weeks after resection</b>						
$K_d$ (nmol/l)	21.7 $\pm$ 4.2	185 $\pm$ 8	10.6 $\pm$ 0.4	209 $\pm$ 67	10.6 $\pm$ 0.4	206 $\pm$ 7
Binding capacity (pmol/mg of protein)	2.3 $\pm$ 0.2	90 $\pm$ 3	1.6 $\pm$ 0.2	158 $\pm$ 17	1.6 $\pm$ 0.2	153 $\pm$ 17

assayed isolated mucosa, whereas we used the whole intestinal wall, (b) different operative techniques were used and different amounts of gut were resected, (c) there is a species difference, and (d) studies were made at different time intervals after enterectomy. The lack of any significant difference in somatostatin content of the colonic mucosa in resected and control animals is con-

sistent with the previous results of Gregor *et al.* [23]. Murphy *et al.* [24] have shown that other peptides such as secretin and vasoactive intestinal peptide are increased in the duodenum and ileum, respectively, after intestinal resection. The reason for the increase in intestinal somatostatin concentration observed 3 weeks after enterectomy remains unclear. It is not known whether the

increase in somatostatin after small bowel resection is a secondary adaptation to the loss of the intestinal D-cells which are numerous in the rabbit [25]. Recent data suggest that a decrease in the intraluminal pH could be a stimulant for D-cell growth [8] and partial enterectomy is associated with a low pH of small bowel content [10].

Previous results, together with previous studies showing that somatostatin inhibits intestinal mucosal growth and differentiation [26], suggest that somatostatin could play a role in intestinal adaptation after small bowel resection. However, whether these modifications are a consequence or a cause of the intestinal adaptive response remains to be elucidated.

There is strong evidence that rat anterior pituitary somatostatin is internalized by its target cells [27] and that at least some of the effects of somatostatin in the intestinal mucosa are mediated through the intervention of intracellular binding sites coupled with phosphoprotein phosphatase units [19, 28].

Although the affinity values for the intestine are not compatible with the low circulating levels of the peptide, it should be borne in mind that somatostatin is present in both paracrine cells and nerve endings [2-4] within the intestinal wall. This neuroparacrine condition may permit sufficiently large local concentrations of somatostatin to facilitate interaction with the reported binding sites.

The observed high levels of intestinal somatostatin could result in a much increased local somatostatin release. In this context, a low pH of the small bowel contents [29] as well as an increased presence of food residue in lower gut [9] stimulate somatostatin release, both factors being present after small bowel resection [10, 11]. Furthermore, involvement of other factors such as elevated circulating levels of cholecystokinin [30] and secretin [31] observed after small bowel resection cannot be ruled out, since both hormones are known to stimulate somatostatin release [32, 33]. Our results are consistent with the recent data of Srikant & Heisler [34], showing that prolonged exposure of pituitary tumour cells (which contain somatostatin-binding sites) to somatostatin resulted in a marked decrease of the number of somatostatin-binding sites. Our results are also consistent with the evidence that hypothalamic somatostatin release causes a reduction in the capacity (but not in the affinity) of somatostatin-binding sites in rat pituitary membranes [35]. Furthermore, up-regulation of somatostatin-binding sites has been demonstrated to be associated with depletion of endogenous somatostatin after cysteamine administration *in vivo* [36].

This report describes for the first time the effect of extensive enterectomy on somatostatin in intestinal tissue. The physiological meaning of these findings remains unclear, since the role of altered binding site capacity in modulating the effects of intestinal somatostatin has not been evaluated.

#### ACKNOWLEDGMENTS

We are grateful to Mrs P. Zurita for valuable technical assistance. This work was supported by a grant from the

Fondo de Investigaciones Sanitarias de la Seguridad Social (86/911).

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