

## Effect of Cysteamine on Cytosolic Somatostatin Binding Sites in Rabbit Duodenal Mucosa<sup>1</sup>

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Administration of cysteamine in rabbits elicited a rapid depletion of both duodenal mucosa and plasma somatostatin. A significant reduction was observed within 5 min, returning toward control values by 150 min. The depletion of somatostatin was associated with an increase in the binding capacity and a decrease in the affinity of both high- and low-affinity binding sites present in cytosol of duodenal mucosa. Incubation of cytosolic fraction from control rabbits with 1 mM cysteamine did not modify somatostatin binding. Furthermore, addition of cysteamine at the time of binding assay did not affect the integrity of <sup>125</sup>I-Tyr<sup>11</sup>-somatostatin. It is concluded that *in vivo* administration of cysteamine to rabbits depletes both duodenal mucosa and plasma somatostatin and leads to up-regulation of duodenal somatostatin binding sites. © 1987 Academic Press, Inc.

### INTRODUCTION

Although the precise mechanisms of somatostatin action are still unknown, the initial step appears to be an interaction of the hormone with specific binding sites in target cells (Schonbrunn and Tashjian, 1978; Enjalbert *et al.*, 1982). Binding sites for somatostatin have been characterized in a variety of endocrine and non-endocrine cells (Enjalbert *et al.*, 1982; Bhathena *et al.*, 1981). The control mechanisms which allow somatostatin to act on a tissue or at a particular subcellular site or biochemical pathway must be very finely tuned by specific regulation (Sussman *et al.*, 1982). The magnitude of somatostatin action must be modulated by its ambient concentration, the number and/or the affinity of its binding sites, and the activity of yet unidentified intracellular postbinding events (Draznin, 1985). A central thesis is that the concentration of somatostatin binding sites can undergo rapid change, which may be of paramount importance in determining the degree of somatostatin action in diverse tissues (Srikant and Heisler, 1985). Szabo and Reichlin (1981) have observed that oral administration of the ulcerogen cysteamine rapidly depletes immunoreactive somatostatin in the gastrointestinal tract. This depletion of somatostatin is dose dependent and cannot be accounted for by release of either compound (Szabo and Reichlin, 1985). Furthermore, cysteamine-induced depletion of somatostatin has been shown to affect physiological processes mediated by somatostatin such as duodenal motility (Pihan *et al.*, 1985).

In the present study we have used cysteamine as a tool to deplete both duodenal and plasma somatostatin levels in order to investigate the regulation by somatostatin of its own binding sites in cytosol of rabbit duodenal mucosa. In

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addition, the *in vitro* direct action of cysteamine upon somatostatin binding was determined.

## METHODS

Somatostatin-14 was kindly provided by Serono (Spain); synthetic Tyr<sup>11</sup>-somatostatin was purchased from Universal Biologicals Ltd. (Cambridge, England); trypsin inhibitor, bovine serum albumin (fraction V), and cysteamine-HCl were from Sigma (St. Louis, MO), and carrier-free Na<sup>125</sup>I (IMS 30, 100 mCi/ml) was from the Radiochemical Centre (Amersham, UK). Synthetic Tyr<sup>11</sup>-somatostatin was radioiodinated by a chloramine-T method (Greenwood *et al.*, 1963). The specific radioactivity of <sup>125</sup>I-Tyr<sup>11</sup>-somatostatin was about 350 Ci/g. All other chemicals were reagent grade.

New Zealand male rabbits weighing 2–3 kg were given cysteamine-HCl *per os* in a single dose of 70 mg/100g body wt in distilled water. Controls received distilled water alone. The animals were killed by decapitation at various times after cysteamine administration, and the proximal duodenum was immediately removed. Trunk blood was collected in chilled tubes containing 1000 kIU/ml aprotinin and 1.2 mg/ml EDTA and immediately centrifuged; plasma was stored at -70°C until assay. Somatostatin immunoreactivity in duodenal mucosa was extracted in 1 M acetic acid at 0°C by sonication. Peptide concentration was measured by a radioimmunoassay method (Gerich *et al.*, 1979) with a limit of sensitivity of 10 pg/ml. Cytosol of duodenal mucosa was isolated as previously reported (Reyl-Desmars and Lewin, 1982). Protein concentration was estimated by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Cytosol fraction of fundic mucosa (0.2 mg protein/ml) was incubated at 25°C for 60 min in 0.5 ml of a medium (pH 7.4) of the following composition: 0.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, 80 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1.5 mM MgCl<sub>2</sub>, 50 mM Hepes ((N-2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid), 11 mM glucose, 0.1% bovine serum albumin, 0.1 mg/ml trypsin inhibitor, and 50 pM <sup>125</sup>I-Tyr<sup>11</sup>-somatostatin either alone or together with increasing concentrations of unlabeled somatostatin (up to 2 μM). Unbound peptide was removed by adding 1 ml 0.25% activated charcoal, 0.5% bovine serum albumin, and 0.025% T-70 dextran (Reyl and Lewin, 1982). "Specific" binding was estimated as the difference between "total" binding (i.e., in the presence of tracer alone) and "nonspecific" binding (as measured in the presence of 4 μM unlabeled somatostatin); this nonspecific component represented about 45% of the binding observed in the absence of unlabeled somatostatin and was not modified by addition of 1 mM cysteamine. The integrity of bound <sup>125</sup>I-Tyr<sup>11</sup>-somatostatin was assessed by talc adsorption (Arilla *et al.*, 1984).

Statistical analysis was done with Student's *t* test for unpaired samples, differences with *P* values lower than 0.05 being considered significant. Each individual experiment was performed in triplicate. All results are expressed as the mean values ± SEM.

## RESULTS

As shown in Fig. 1, cysteamine elicited a significant depletion of both duodenal mucosa and plasma somatostatin levels within 5 min, returning toward control values by 150 min.

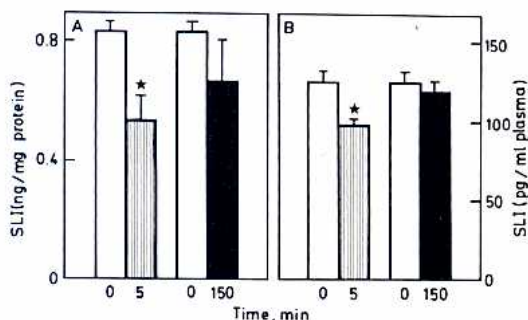


FIG. 1. Time course of cysteamine-induced depletion of somatostatinlike immunoreactivity (SLI) in rabbit duodenal mucosa (A) and plasma (B). Values are expressed as the means  $\pm$  SEM of five triplicate determinations. The significance of the differences with respect to control values is given by  $P < 0.05$  (asterisk).

All cytosolic preparations showed a similar extent (about 30%) of somatostatin degradation at the end of the incubation period, thus ruling out the possibility of misinterpreting the stoichiometric results. Depletion of endogenous somatostatin after *in vivo* cysteamine administration was associated with a time-dependent change in both binding capacity and affinity of duodenal mucosa somatostatin binding sites. Analysis of the competitive inhibition by somatostatin of the specific binding of  $^{125}\text{I}$ -Tyr<sup>11</sup>-somatostatin revealed a cysteamine-induced decrease of affinity, as evidenced by a shift to the right of the inhibition curves (Fig. 2, left panel). When analyzing the stoichiometric data by the method of Scatchard (1949), the values of somatostatin binding to the cytosolic preparations exhibited curvilinear, concave upward curves that were analyzed 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. Table I shows the corresponding equilibrium parameters for the high affinity-low capacity and for the low affinity-high capacity binding sites. The analysis of these results revealed a significant increase in the binding capacity of both high- and low-affinity binding sites at 5 min (but not at 150 min) after cysteamine treatment, as compared to

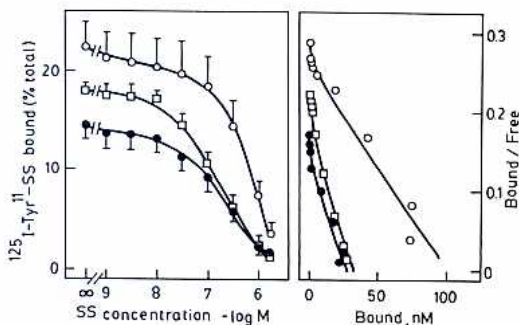


FIG. 2. Left: Competitive inhibition of specific  $^{125}\text{I}$ -Tyr<sup>11</sup>-somatostatin ( $^{125}\text{I}$ -Tyr<sup>11</sup>-SS, 50 pM) binding to cytosolic fraction (0.2 mg protein/ml) from rabbit duodenal mucosa by unlabeled somatostatin. Points correspond to control (●) and cysteamine-treated rabbits 5 (○) and 150 (□) min after drug administration. Values are expressed as the means  $\pm$  SEM of five separate experiments, each performed in triplicate. Right: A Scatchard analysis of the same data.

TABLE I  
Effect of Cysteamine Administration on Equilibrium Parameters of Somatostatin Binding to Cytosol of Duodenal Mucosa<sup>a</sup>

	Somatostatin binding sites			
	High affinity		Low affinity	
	B.C.	$K_d$	B.C.	$K_d$
Control	2.1 ± 0.1	18.4 ± 2.9	129 ± 18	163 ± 17
5 min	3.1 ± 0.1*	35.3 ± 5.1*	500 ± 34*	380 ± 58
150 min	1.9 ± 0.1	15.9 ± 2.4	144 ± 18	148 ± 20

Note. Dissociation constant:  $K_d$ , nM. Binding capacity: B.C., pmole somatostatin/mg protein. Mean values ± SEM ( $n = 5$ ). Significance versus control values, \* $P < 0.05$ .

<sup>a</sup> Values were derived from the experimental data of Fig. 2 (right panel).

control values. The corresponding dissociation constant increased at the same time interval. To assess whether cysteamine exerts a direct action on somatostatin binding sites, it was included in the incubation medium at the time of the binding assay. The addition of 1 mM of cysteamine to the incubation medium did not change either binding capacity or affinity somatostatin binding sites (data not shown). To determine if cysteamine affects <sup>125</sup>I-Tyr<sup>11</sup>-somatostatin during the binding assay, the integrity of the ligand remaining free at the time of equilibrium binding in the presence and absence of 1 mM cysteamine was assessed. More than 70% of the ligand remained intact.

## DISCUSSION

Present results show that cysteamine-induced depletion of immunoreactive somatostatin in both rabbit duodenal mucosa and plasma results in an increase in the number and a decrease in the affinity of both high- and low-affinity binding sites in the corresponding cytosolic preparation. A significant decrease (about 30%) of somatostatin occurred as early as 5 min and returned toward control values by 150 min after cysteamine administration. That intestinal somatostatin depletion was not due to discharge into the blood was shown by the falling plasma levels of the peptide. Srikant and Patel (1984) have observed that cysteamine administration in rats led to a decrease of cerebrocortical somatostatin by a similar time-dependent pattern. However, Szabo and Reichlin (1981) have reported that duodenal somatostatin reached minimal levels 4 hr after cysteamine intake. This discrepancy may be a result of differences in cysteamine doses and/or the animal species considered.

Despite the growing interest in cysteamine as an investigative tool for producing somatostatin deficiency, the mechanism of action of this agent remains unclear. It has been suggested by others that cysteamine might act by accelerating the intracellular degradation of immunoreactive somatostatin (Szabo and Reichlin, 1981) or, more likely, through a chemical modification of the disulfide bond which would render the molecule unreactive (Rivier *et al.*, 1975). In any case, at the present time the role of somatostatin in experimental duodenal ulcer remains to be further investigated. Present results show that the cysteamine-induced depletion of immunoreactive somatostatin in both duodenal mucosa and plasma was associated with both an increase in the number and a decrease in the

affinity of the two classes of cytosolic somatostatin binding sites. These data suggest that a decrease in somatostatin levels leads to sensitization or up-regulation of somatostatin binding sites in the duodenum and are consistent with a recent report showing that prolonged exposure of pituitary tumor cells to somatostatin resulted in a marked decrease in the density of somatostatin binding sites (Srikant and Heisler, 1985). Indeed, other authors (Srikant and Patel, 1984) have shown that cysteamine-induced depletion of brain somatostatin is associated with up-regulation of the corresponding somatostatin binding sites. Furthermore the increase of hypothalamic somatostatin release after growth hormone administration caused a significant reduction in the interaction of the peptide hormone with pituitary membranes (Katakami *et al.*, 1985). Thus, regulation of somatostatin binding sites appears to be similar to that of other hormone-binding sites systems (Kahn *et al.*, 1973; Srikant *et al.*, 1977; Lesniak and Roth, 1976). In another context, increasing site occupancy has been shown to be accompanied by decreased affinity in a variety of hormone-receptor systems (DeMeyts *et al.*, 1976), as was found in the present work.

Interestingly, when added at the time of binding, cysteamine (1 mM) did not affect either affinity or capacity of somatostatin binding sites. The reason for the difference between *in vivo* and *in vitro* effects of cysteamine on somatostatin binding sites is unclear. It is possible that normal regulation of somatostatin binding sites requires the intact cell. It should be noted that cysteamine has been shown to elicit lysosome activation (King, 1982). If lysosomal enzymes react with somatostatin binding sites it would lead to the observed decrease in the affinity values but it remains to be further investigated.

Somatostatin is an inhibitor of duodenal gastrin secretion (Bloom *et al.*, 1974) and spontaneous intestinal contractions (Tansy *et al.*, 1979) as well as a modulator of gastrointestinal mucus secretion (Johansson and Aly, 1982), processes that can be reversed by cysteamine administration (Pihan *et al.*, 1985; Lichtenberger *et al.*, 1977). Cysteamine-induced increases in the concentration of duodenal somatostatin binding sites could be a mechanism of adaptation, but it is possible that the corresponding low concentrations of somatostatin around target cells are below the threshold level.

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