# EFFECTS OF SOMATOSTATIN ON PANCREATIC EXOCRINE FUNCTION. INTERACTION WITH SECRETIN.

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Received September 12,1975=

SUMMARY. The release of hydrolases from rat pancreas was stimulated in vivo and in vitro by somatostatin. In vitro this hypersecretion was accompanied by a moderate but significant rise in intracellular cyclic AMP. In addition, the tetradecapeptide inhibited rises of cyclic AMP provoked by secretin. The existence of the same sequence of four amino acid residues in the two peptides suggests that somatostatin's activation of the exocrine pancreas depends on its interaction with secretin receptors.

While the structure and effects of somatostatin are well known, its mode of action remains undefined. Somatostatin was first extracted from ovine hypothalamus and after determination of its tetradecapeptide structure (1), the synthetic form was marketed. Its abilities to inhibit the release of growth hormone (2), TSH (3) and prolactin (4) from the pituitary and to reduce the secretion of insulin (5,6) and glucagon (7) by a direct effect on the islets of Langerhans have both been demonstrated under various conditions. Accompanying concentration changes of cyclic nucleotides (a decrease in cyclic AMP together with an increase in cyclic GMP) have been noted in the hypophysis (8). Reversal of the inhibitory effect on insulin secretion by increased extracellular calcium concentration implicates the possible intervention of calcium fluxes in β cells (9).

Three considerations warranted a study of somatostatin's effects on the exocrine pancreas. Firstly, the sizable presence in the gut and especially in the pancreas of immunoreactive material with properties similar to those of somatostatin (10). Secondly, cyclic GMP (11), calcium (12) and probably cyclic AMP (13) play important roles here in stimulus-secretion coupling. Thirdly,

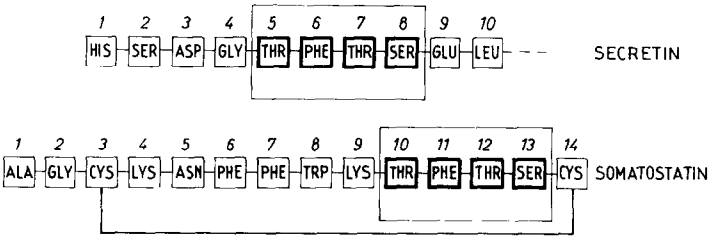


FIGURE 1. Comparison between the primary structure of somatostatin and the N-terminal portion of secretin. The amino acid sequence 10-13 in somatostatin is identical to the 5-8 sequence in secretin.

the presence of a common amino acid sequence (Threonyl-Phenylalanyl-Threonyl-Serinyl) in somatostatin and secretin (Fig. 1) suggests a possibility of hormone interactions with target cells (14).

## MATERIALS AND METHODS.

Wistar rats weighing 150-200 g and bred in our laboratory for 15 years were utilized. Natural secretin and natural pancreozymin (CCK-PZ) were acquired from the GIH Research Unit of the Karolinska Institutet (Stockholm, Sweden). Synthetic secretin and synthetic cyclic somatostatin were generously donated by Dr. Wünsch (Max-Planck Institut für Biochemie, Munich, W. Germany) and Ayerst Laboratories (Montreal, Canada), respectively.

IN VIVO SECRETION OF AMYLASE AND LIPASE. The technique of LEMIRE and IBER was utilized (15). Briefly, a median laparotomy was performed under Nembutal anesthesia. The duodenal loop was exposed and the common pancreato-biliary duct was ligated close to the intestinal wall. A polyethylene catheter with an interior diameter of 1 mm was introduced into the duct and held in position with a second ligature. Pancreato-biliary secretion was collected over ice at 10 min intervals. After 30 min, somatostatin (50 µg/100 g body weight in 0.2 ml 0.9 % NaCl) was administered as a bolus injection in a saphenous vein.

IN VITRO AMYLASE SECRETION AND CYCLIC AMP LEVELS. The pancreases were

carefully dissected out and cut into 20-30 mg fragments. Three or four fragments were selected at random and shaken in 10 ml beakers containing 2 ml of a Krebs-Ringer bicarbonate buffer enriched with 10 mM glucose, Trasylol Bayer (500 Kallikrein inhibitor units/ml) and 10 mM theophylline. A pH of 7.4 was maintained under $O_2$-$CO_2$ (95:5 v/v) at 37° C. After 10 min preincubation, the medium was aspirated and replaced by 2 ml of fresh medium with or without the test hormone(s). The incubation was usually terminated after 10 min by rapidly pipetting the medium for enzyme assays and freezing the fragments over dry ice for the determination of cyclic AMP and proteins.

ASSAYS. α-Amylase was determined by an automated saccharogenic method (16) and lipase by an automated potentiometric single-time-point assay using a tributyrin emulsion as substrate (17). Protein was measured by the method of LOWRY et al. (18). Cyclic AMP was determined by the protein binding assay of GILMAN (19) as detailed previously (20).

## RESULTS

1. IN VIVO EFFECTS OF AN INTRAVENOUS BOLUS INJECTION OF SOMATOSTATIN ON AMYLASE AND LIPASE SECRETION.

As shown in Fig. 2, a large (50 µg/100 g body weight) bolus injection of somatostatin provoked a rapid and significant 3-4 fold increase in amylase and lipase output. This effect remained high for at least twenty min following the injection. However, this hypersecretion was of shorter duration than that caused by better known stimuli such as pancreozymin and pilocarpine (11). The collection of mixed pancreato-biliary juice prevented the investigation of any variation in the volume of pancreatic juice.

2. IN VITRO EFFECT OF SOMATOSTATIN ON AMYLASE OUTPUT.

Fig. 3A shows the time-course of somatostatin's action on amylase output. A significant two-fold increase was already detectable two min after addition of the hormone. This stimulation persisted for 10 min. Thereafter the rate of

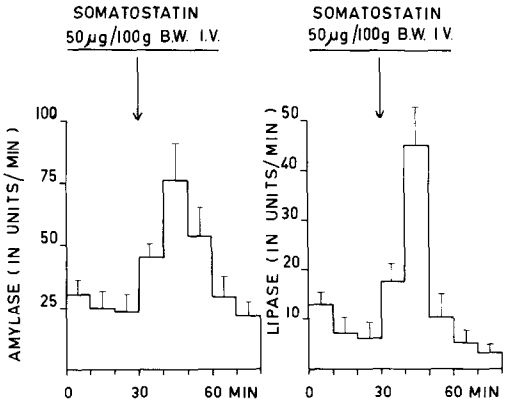


FIGURE 2. Effect of an intravenous injection of somatostatin (50 µg/100 g body weight) on rats. Amylase (left panel) and lipase (right panel) output were measured in the pancreato-biliary secretion. The mean values of 6 experiments are expressed in U/min ± SEM. One unit is defined as that amount of enzyme which liberates one micromole of products per min at 25° C (16,17).

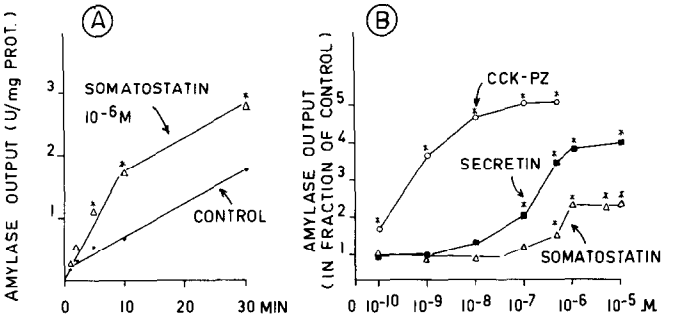


FIGURE 3. In vitro effects of somatostatin on amylase secretion by rat pancrea fragments. Incubation conditions are detailed under Material and Methods.
Fig. 3 A represents a time course in presence (Δ——Δ) or absence (●——●) of $10^{-6}$ M somatostatin. Results are the mean of 4 experiments. Each determination was made in duplicate.
Fig. 3 B represents the dose-effect curve for pancreozymin (CCK-PZ : O——O), natural secretin (■——■), and somatostatin (Δ——Δ). Results are expressed as fraction of control values and are the mean ± SEM of 4 experiments. Each determination was made in duplicate.
In both panels the asterisks indicate values significantly different ($P < 0.05$) from controls, when employing Student's t-test for paired data comparison.

amylase secretion declined to control values, an observation which might be due to the in vitro denaturation of the hormone by the tissue fragments. For this reason, subsequent measurements were performed after only 10 min incubation.

| Hormone additions | Cyclic AMP pmoles/mg protein | Percentages A | B | C |
|---|---|---|---|---|
| Control | 3.43 ± 0.25 | 100 | - | - |
| SOMATOSTATIN $10^{-8}$ M | 4.00 ± 0.30 | 116 | - | - |
| SOMATOSTATIN $10^{-6}$ M | 4.65 ± 0.31* | 135 | - | - |
| SECRETIN $10^{-9}$ M | 5.67 ± 0.40* | 165 | 100 | 100 |
| SECRETIN $10^{-8}$ M | 14.65 ± 0.60* | 427 | 100 | 100 |
| SECRETIN $10^{-7}$ M | 16.06 ± 0.63* | 468 | 100 | 100 |
| SECRETIN $10^{-9}$ M + SOMATOSTATIN $10^{-8}$ M | 5.53 ± 0.33 | 161 | 98 | 68 |
| SECRETIN $10^{-9}$ M + SOMATOSTATIN $10^{-6}$ M | 5.31 ± 0.26 | 154 | 94 | 29 |
| SECRETIN $10^{-8}$ M + SOMATOSTATIN $10^{-8}$ M | 11.89 ± 0.55** | 347 | 81 | 70 |
| SECRETIN $10^{-8}$ M + SOMATOSTATIN $10^{-6}$ M | 8.71 ± 0.43** | 254 | 59 | 36 |
| SECRETIN $10^{-7}$ M + SOMATOSTATIN $10^{-8}$ M | 12.42 ± 0.50** | 362 | 77 | 66 |
| SECRETIN $10^{-7}$ M + SOMATOSTATIN $10^{-6}$ M | 10.39 ± 0.50** | 303 | 64 | 45 |

TABLE I : The effect of in vitro interactions between somatostatin and secretin on cyclic AMP levels in pancreatic fragments. Cyclic AMP was determined after 10 min incubation in the presence of 10 mM theophylline (details under Material and Methods). Values ± SEM are the mean of 5 experiments. Each determination was made in triplicate. Column A gives percentages of control values without hormones. Column B gives percentages of absolute cyclic AMP concentrations obtained with secretin only. Column C gives percentages of cyclic AMP rises due to the presence of secretin only.
One asterisk indicates significant differences ($p < 0.05$) with paired controls; two asterisks indicate significant differences ($p < 0.05$) with the effects of secretin only.

Fig. 3B compares dose-effect curves of somatostatin, secretin, and pancreozymin (CCK-PZ). Somatostatin was a relatively weak stimulant : the 2.3 fold increase in amylase secretion observed at a $10^{-6}$ M concentration was significantly lower than the 3.8 and 5.0 fold increases obtained, respectively, with $10^{-6}$ M secretin and $10^{-6}$ M pancreozymin (Stimulations due to synthetic secretin were as potent as those due to natural secretin : data not shown).

3. IN VITRO EFFECT OF SOMATOSTATIN ON CYCLIC AMP LEVELS.

Somatostatin exerted moderate effects on cyclic AMP levels (Table I). Increases were significant only at a high $10^{-6}$ M concentration (+ 35 %). As previously described (20), marked elevations in cyclic AMP levels were observed using secretin. This effect was already significant at a $10^{-9}$ M concentration (+ 65 %) and maximal (+ 368 %) at $10^{-7}$ M.

In spite of the fact that somatostatin was a weak agonist of secretin on adenylate cyclase activity, it inhibited the rise in cyclic AMP induced by secretin. At high secretin concentrations ($10^{-8}$ M and $10^{-7}$ M), variations caused by $10^{-8}$ M and $10^{-6}$ M somatostatin were evident when total cyclic AMP levels in the presence of both hormones were compared to the levels observed with secretin only (column B in Table I). In the presence of a lower $10^{-9}$ M secretin concentration, the inhibitory effect of $10^{-8}$ M and $10^{-6}$ M somatostatin was apparent only when comparing increases in cyclic AMP levels elicited by secretin, in the presence and in the absence of somatostatin (column C in Table I)

## DISCUSSION

Secretin activates adenylate cyclase in the exocrine pancreas (11,20) and somatostatin at high concentration may act similarly. Indeed, our results demonstrate a weak but definite increase in cyclic AMP levels in response to somatostatin (Table I). In addition, somatostatin inhibited increases in cyclic AMP induced by secretin. These findings are compatible with the hypothesis that, when utilized alone, somatostatin occupies secretin binding sites and consequently activates adenylate cyclase which may facilitate hydrolase output (Figs. 2 and 3). When somatostatin and secretin are both present, somatostatin inhibits secretin's effects on cyclic AMP. Two arguments support this hypothesis :

1. The activation of plasma membrane adenylate cyclase by hormones of the secretin family is preceded by hormone binding to specific receptors. Glucagon ressembles secretin in having 14 identical amino acid residues including the 5-8 amino acid sequence. Des-His-glucagon acts as a weak agonist of glucagon on hepatic adenylate cyclase : it binds to the glucagon receptor, inhibits adenylate cyclase activation by glucagon but yields at $10^{-6}$ M 70 % of the activity given by $10^{-8}$ M glucagon (21). Our data suggest that somatostatin might similarly act as a partial agonist of secretin. At a moderate $10^{-8}$ M concentration, the tetradecapeptide inhibited by one-third secretin's effects on cyclic

AMP levels (column C in Table I). This suggests a relatively high affinity of somatostatin for the secretin binding site. On the other hand, somatostatin was only capable of activating adenylate cyclase at a high $10^{-6}$ M concentration (column A in Table I). It might be argued that the apparent affinity with which somatostatin interacts as an inhibitor of secretin should be the same as when the peptide is studied as a partial agonist with the secretin receptor. However the occupation of the secretin receptor by both hormones may have kinetic properties different from the resulting activation. It is therefore difficult to further characterize the nature of the interaction between somatostatin and secretin in terms of cyclic AMP accumulation reflecting receptor occupation. In addition, somatostatin being a significant but weak secretin antagonist at $10^{-8}$ M and a significant agonist at $10^{-6}$ M (Table I), the exploration of antagonism was out of necessity limited to a narrow range of somatostatin concentration.

2. The second indication compatible with our hypothesis is the presence in somatostatin of a sequence of four amino acids identical to that observed in position 5 to 8 in secretin (Fig. 1). These amino acids are directly involved in secretin activity. Indeed, secretin fragment 14-27, lacking the 5-8 sequence, is completely inactive (14). Furthermore, the substitution of phenylalanine by tyrosine in the sixth position of secretin (i.e. the introduction of a fourth hydroxyl group) greatly decreases its efficiency (14). These data suggest that this amino acid sequence, common to both peptides, plays a critical role in their activity. However glucagon which possesses the same Thr-Phe-Thr-Ser sequence in position 5 to 8 does not act as a partial agonist of secretin on fragments of rat pancreas since it increases neither cyclic AMP levels nor amylase secretion and does not counteract the effects of secretin on these two parameters (20). In addition, vasoactive intestinal peptide (VIP) shares only a limited Phe-Thr sequence with secretin in position 5 to 8 and this does not

prevent VIP from exerting secretin-like effects (20). These observations are not necessarily in disagreement with the present hypothesis but they clearly indicate that the biological activity of these hormones and their sharing of common receptors in the exocrine pancreas depend also on the decisive function of amino acid residues not considered in the present study. It is therefore recognized that the proposed mechanism is speculative.

An alternative interpretation for our observations is to consider that somatostatin exerts in fact pancreozymin-like effects including stimulation of enzyme secretion, moderate cyclic AMP elevation and inhibition of secretin-stimulated cyclic AMP accumulation (20). We find this line of speculation less attractive. Indeed there is good evidence that pancreozymin stimulates secretion by acting mostly on intracellular calcium movements (22,23,24) whereas somatostatin, at variance with pancreozymin, does not modify $^{45}Ca$ fluxes and is unable to stimulate amylase secretion in cells partially depleted of calcium, suggesting a greater dependence on calcium availability (unpublished observations).

In conclusion, whatever its physiological role, somatostatin is probably an analog of secretin on the exocrine pancreas. It works as an inhibitor or an agonist of secretin depending on peptide concentrations.

ACKNOWLEDGMENTS

This work was supported by grant 20,403 from the Fonds de la Recherche Scientifique Médicale (Belgium) and grant RO1-AM-17010 from the National Institutes of Health (U.S.A.).

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