Release of somatostatin-like immunoreactivity from the perfused canine thyroid. Selective stimulatory effect of calcium ions.

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Research Article

It is well accepted that the C cells of the thyroid contain somatostatin, but the role in local endocrine function has not yet been firmly established in this organ, and it has not been proved that thyroidal somatostatin is released into the circulation. We have measured the contents of somatostatin-like immunoreactivity in the effluent of canine thyroid glands perfused without recirculation with a synthetic buffer medium. During basal conditions a definite release was consistently found in the order of 10 pg/ml corresponding to 12 pg/min. The somatostatin-like immunoreactivity was studied in dilution experiments and by gel-filtration chromatography, and found to have properties identical to those of synthetic cyclic somatostatin, which was also recovered quantitatively when added to sampling tubes. Various compounds were infused in concentrations that are highly active in pancreas perfusion experiments. 14-min infusion of arginine, 5 and 11.5 mmol/liter; isoproterenol, 10 and 23.7 nmol/liter and 68.7 mumol/liter; acetylcholine, 5 mumol/liter, carbamylcholine, 10 and 100 mumol/liter; glucagon, 1 and 30 nmol/liter; and porcine calcitonin, 1 and 100 ng/ml did not affect the basal release of somatostatin-like immunoreactivity significantly. Neither did an increase from the control level of 4 mmol/liter glucose of 10 or 20 mmol/liter, nor an increase in the control level of 4.4 mmol/liter K+ to 7.5 or 14.4 mmol/liter. Each of these compounds were tested in three […]

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Release of Somatostatin-like Immunoreactivity from the Perfused Canine Thyroid

SELECTIVE STIMULATORY EFFECT OF CALCIUM IONS

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A B S T R A C T   It is well accepted that the C cells of the thyroid contain somatostatin, but the role in local endocrine function has not yet been firmly established in this organ, and it has not been proved that thyroidal somatostatin is released into the circulation.

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Various compounds were infused in concentrations that are highly active in pancreas perfusion experiments. 14-min infusion of arginine, 5 and 11.5 mmol/liter; isoproterenol, 10 and 23.7 nmol/liter and 68.7 μmol/liter; acetylcholine, 5 μmol/liter, carbamylcholine, 10 and 100 μmol/liter; glucagon, 1 and 30 nmol/liter; and porcine calcitonin, 1 and 100 ng/ml did not affect the basal release of somatostatin-like immunoreactivity significantly. Neither did an increase from the control level of 4 mmol/liter glucose of 10 or 20 mmol/liter, nor an increase in the control level of 4.4 mmol/liter K^+ to 7.5 or 14.4 mmol/liter. Each of these compounds were tested in three or four dogs.

The effect of an increase in Ca^{++} from the control level of 1.5 mmol/liter to 2.25, 3.0, and 4.5 mmol/liter was tested in random order in five thyroid lobes. All three doses elicited an immediate increase in effluent somatostatin-like immunoreactivity. In most experiments the response was biphasic with an early spike, followed by a stable level that was maintained during prolonged Ca^{++} infusion. The secretory response was not diminished through a series of repeated short pulses of calcium infusion. The response to 3.0 mmol/liter Ca^{++} (control period 8.4±1.5, test period 337±110 pg/ml, mean±SE) and 4.5 mmol/liter Ca^{++} (control period 9.5±1.4, test period 386±125) were significantly higher than 2.25 mmol/liter Ca^{++} (control period 7.2±1.0, test period 149±39), while there was no significant difference between responses to the two high doses. Infusion of salmon calcitonin, 10 ng/ml and 1 μg/ml; or porcine calcitonin, 1 μg/ml during calcium stimulation (2.25 mmol/liter of Ca^{++}) did not induce alterations in the release of somatostatin-like immunoreactivity.

The results demonstrate that thyroidal somatostatin is mobilizable, and it appears to be selectively sensitive to calcium stimulation, indicating a possible role in calcitonin release control.

INTRODUCTION

Several reports have presented immunohistochemical evidence for the presence of somatostatin in the C cells of the thyroid gland (1-4), and Kronheim et al. (5) measured the somatostatin contents of several organs and tissues in rats by radioimmunoassay and found ~25 ng/mg protein in the thyroid i.e., a value comparable to that of the pancreas and the stomach. In both of these organs somatostatin exerts profound effects on secretory function (6-8), and in vivo and in vitro studies have demonstrated that the somatostatin is released in response to various physiological and pharmacological stimuli (9-12). These findings have contributed to the idea that somatostatin acts as a physiological paracrine mediator.

Has somatostatin such a role also in the thyroid gland? The studies concerned with possible effects on

iodothyronine release have been conflicting (13–16), and we found no such action in the perfused canine thyroid (17). The evidence for an interrelationship with calcitonin release has also been controversial (18–26). These previous investigations have all been directed at studying an effect of administered somatostatin on the thyroid. In the present study we have approached the question in another way, namely by measuring release of somatostatin-like immunoreactivity (SLI)[1] from the thyroid and trying to induce changes in the secretion.

METHODS

Thyroid perfusion was performed in mongrel dogs weighing 18–26 kg. In the dog, the two thyroid lobes are separate and can be perfused simultaneously and independently. The technique has been described in detail elsewhere (27, 28). In brief, the thyroid lobes were isolated *in situ* so that perfusion medium pumped into a segment of each common carotid artery could be collected quantitatively from each lobe through catheters in the internal jugular veins. The dog was exsanguinated after the insertion of the afferent catheters. Once-through perfusions were performed with a Krebs-Ringer bicarbonate buffer modified to the ion concentrations of dog plasma: Na^+ 140.0; K^+ 4.4; Ca^{++} 1.5; Mg^{++} 0.5; Cl^- 121.9; SO_4^{--} 0.5; $H_2PO_4^-$ 1.1; I^- 3.67 × 10⁻⁴; and HCO_3^- 24.4 mmol/liter, with the addition of bovine serum albumin 0.2%, dextran 4% (70,000 M, Pharmacia Fine Chemicals, Uppsala, Sweden) and glucose 4.0 mmol/liter. The flow rate for each lobe was 0.63 ml/min (0.5–1.0 ml/g tissue per min). Perfusion pressure was constant at 30–40 mm Hg. The effluents from 2-min intervals were collected in polyethylene tubes containing 200 μl 30% neutral EDTA.

The compounds to be tested were pumped into the medium through a cannula penetrating a rubber membrane on the afferent catheter at a rate of 6.3 μl/min giving the final concentrations indicated in Table I. Mixing was achieved in a small volume drop-chamber introduced between the thyroid lobes and the rubber membrane (17).

SLI was measured by radioimmunoassay using wick chromatography for separation of free and antibody-bound somatostatin (29). 200 μl standard made up in perfusion medium including EDTA or 200 μl sample, and 50 μl somatostatin antibody 1:10,000 were incubated for 24 h, then 50 μl ¹²⁵I-tyrosine¹¹-somatostatin was added and further incubated for 24 h at 4°C. The detection limit was 2–4 pg/ml, depending on the age of the tracer. The interassay coefficient of variation was 7.4%.

The stability of somatostatin in thyroid effluent and the recovery throughout the period from collection of samples including the radioimmunoassay incubations were tested by adding known amounts (100 pg/ml final concentration) of crystalline somatostatin to every other of a series of sampling tubes receiving effluent during basal conditions in two perfusion experiments. The recovery was 101.7±5.6% (mean±SD, n = 8).

The SLI in the thyroid effluent was studied by dilution experiments, which gave variations in binding percentages parallel to that of standards (Fig. 1). In addition, effluent samples obtained just after start of Ca^{++} infusion, i.e., with

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[1] *Abbreviations used in this paper*: DB-cAMP, N^6, O^2-dibutyryl cyclic adenosine monophosphate; SLI, somatostatin-like immunoreactivity.

very high SLI contents were chromatographed on G-25 Sephadex (fine) columns. The chromatographical properties were identical to those of synthetic cyclic somatostatin (Fig. 2), which is eluted shortly after the total volume of the column. Synthetic somatostatin and SLI was thus adsorbed identically onto Sephadex. The adsorption was much less pronounced than for iodinated tyrosine¹¹-somatostatin (10).

10 μg/ml of triiodothyronine, thyroxine, reverse triiodothyronine, mono- and diiodotyrosin and thyroglobulin, and 30 μg/ml of porcine and salmon calcitonin, and 30 μg/ml cAMP did not interfere in the somatostatin radioimmunoassay. L-arginine was obtained from Fluka A. G. (Basel, Switzerland), isoproterenol, carbamylcholine, D-glucose, N^6, $O^{2'}$-dibutyryl-cAMP (DB-cAMP) and bovine serum albumin from Sigma Chemical Co. (St. Louis, Mo.) and acetylcholine from Merck A. G. (Darmstadt, W. Germany). Cyclic somatostatin was a gift from Norman Grant (Wyeth Laboratories, Philadelphia, Pa.) and tyrosine¹¹-somatostatin was given to us by Dr. Roger Guillemin (Salk Institute, La Jolla, Calif.).

Purified porcine calcitonin (lot K 700 147D) was a gift from Dr. R. Schleuter, Armour Pharmaceutical Company, Kankakee, Ill.), and purified salmon calcitonin from Sandoz, Basel, Switzerland.

RESULTS

The influence of various concentrations of glucose, arginine, isoproterenol, acetylcholine, carbamylcholine, glucagon, porcine calcitonin, potassium, and calcium on basal SLI release from perfused thyroid lobes was tested by infusing the compounds for 14 min with control intervals of 18 min. The effect of the different calcium concentrations was tested in random order in the same thyroid lobe in five dogs, whereas the other substances were infused in three or four thyroids. Each substance was only tested in one lobe from each dog. In the individual perfusion experiments these compounds were also infused in alternating order, to avoid any possible long acting influence of the preceding exposure to another compound or to a different concentration of the same compound.

During the control periods the basal SLI concentration in the effluent was ~10 pg/ml (Table I). The only compound that exerted any effect on somatostatin release was calcium. 1 mmol/liter DB-cAMP was also infused in three thyroids. The interpretation of the release pattern was somewhat hampered because this compound interferes in the radioimmunoassay, resulting in a spurious elevation in SLI. In these experiments the average apparent SLI value in effluent increased from 14.2±1.9 to 67.0±1.7 pg/ml (±SE). However, similar SLI readings were obtained when 1 mmol/liter DB-cAMP in perfusion medium was added directly in the radioimmunoassay, suggesting that SLI secretion was unaffected. The impression that the apparent effect of DB-cAMP was due solely to assay interference is further supported by the identical SLI readings in the three experiments contrasting the highly variable responses to calcium (Table I) obtained in individual thyroids. None of the other compounds tested interfered in the assay. A representative experi-

TABLE I

Thyroid SLI Release (pg/ml). The Effect of Various Compounds (Mean ±SE).

Compound*	Control period‡	Test period§		No. of experiments
Glucose				
6 mmol/liter	10.2±3.5	11.3±2.9	NS	3
16 mmol/liter	8.2±2.1	7.2±1.6	NS	4
Arginine				
5 mmol/liter	11.5±4.2	11.5±2.8	NS	4
11.5 mmol/liter	17.1±9.2	17.6±5.8	NS	3
Isoproterenol				
10 nmol/liter	7.9±0.9	7.3±2.1	NS	3
23.7 nmol/liter	7.7±2.3	10.4±2.9	NS	4
68.7 μmol/liter	7.8±2.3	10.1±5.5	NS	3
Acetylcholine				
5 μmol/liter	10.2±2.5	11.4±2.3	NS	4
Carbamylcholine				
10 μmol/liter	8.3±2.1	8.5±3.0	NS	3
100 μmol/liter	9.9±4.1	13.9±7.2	NS	3
Glucagon				
1 nmol/liter	10.5±0.9	12.0±2.2	NS	3
30 nmol/liter	8.9±1.9	8.5±1.6	NS	3
Ca^{++}				
0.75 mmol/liter$^{\parallel}$	7.2±1.0	149±39	$P < 0.05$	5
1.5 mmol/liter	8.4±1.5	337±110	$P < 0.05$	5
3.0 mmol/liter	9.5±1.4	386±125	$P < 0.05$	5
K^+				
3.1 mmol/liter	12.1±2.5	8.3±1.2	NS	4
10 mmol/liter	17.2±6.0	21.3±10.3	NS	3
Porcine calcitonin				
1 ng/ml	9.2±0.7	10.8±1.2	NS	3
100 ng/ml	13.7±0.1	10.9±1.9	NS	3

* Added to the basal perfusion medium containing 4 mmol/liter glucose, 1.5 mmol/liter Ca^{++}, and 4.4 mmol/liter K^+.

‡ In each experiment the value was calculated as the mean of the three samples before infusion of the compound and the three samples obtained 4–10 min after the infusion.

§ In each experiment the value was calculated as the mean of the seven samples obtained during infusion.

$^{\parallel}$ The various Ca^{++} concentrations were tested in the same thyroid lobes in alternating sequence. The responses to 1.5 and 3.0 mmol/liter Ca^{++} were both significantly higher than the response to 0.75 mmol/liter ($P < 0.05$), while there was no significant difference between 1.5 and 3.0 mmol/liter.

ment with infusion of some of these compounds is shown in Fig. 3 (top). In the other lobe of this dog (Fig. 3, bottom), calcium was infused in various concentrations and during various periods. An increase in perfusate calcium from the basal 1.5 to 2.25 mmol/liter induced a small but clear-cut increase in SLI release, whereas increases to 3 and 4.5 mmol/liter gave large responses. When tested in five experiments (Table I) the release induced by each of the two higher Ca^{++} concentrations was significantly larger than that oc-

curring after 0.75-mmol/liter increase, while there was no significant difference between the response to the increases of 1.5 and 3.0 mmol/liter Ca^{++}. A prolonged (46 min) infusion of 3 mmol/liter calcium induced a high sustained SLI release (Fig. 3). Short-term pulses of 3 mmol/liter calcium (2 min, with 4-min intervals) at the very end of the perfusion experiment were still able to elicit immediate spikes of SLI release without any apparent decline in secretory capacity. Identical results were obtained in another perfused thyroid lobe.
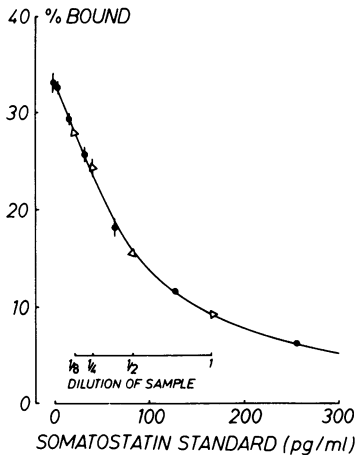
FIGURE 1 Displacement of antibody-bound ^{125}I-tyrosine^{11}-somatostatin by synthetic cyclic somatostatin (●) and by an effluent sample undiluted and dilutions: 1:2, 1:4, 1:8, △, mean of triplicate determinations ±SE.

In most experiments the calcium induced SLI responses were higher initially than later on during each stimulation. In Fig. 4, which illustrates the effect of a Ca^{++} infusion of a sufficient duration to study the release dynamics, this pattern is clearly demonstrated. In other words, the response of the SLI secreting cells in the thyroid is "biphasic" as it is for pancreatic D cells (10).

To further study a possible effect of calcitonin on SLI release, salmon and porcine calcitonin was infused during calcium stimulation. Fig. 4 illustrates one of the two experiments in which 10 ng/ml and 1 μg/ml salmon calcitonin and 1 μg/ml porcine calcitonin did not modify the submaximally stimulated SLI release.
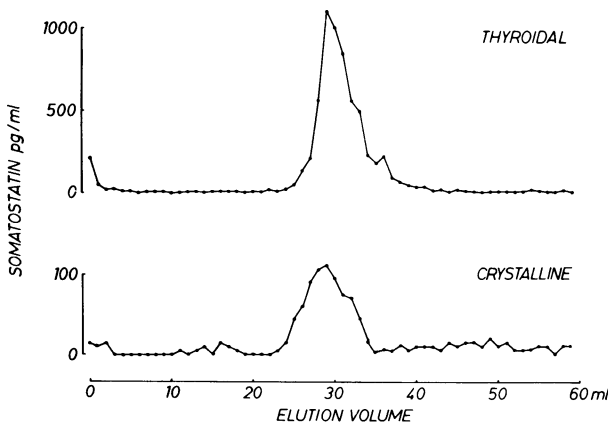

FIGURE 2 Sephadex G-25 elution of somatostatin immunoreactivity. Elution pattern of synthetic cyclic somatostatin and a pool of effluent samples. Void volume was 11 ml and total volume 24 ml. Column: 30 cm Sephadex G-25-fine. Flow rate: 0.08 ml/min. Temperature: 4°C. Elution buffer: phosphate 0.04 mol/liter pH 8, with 0.2% bovine albumin and 10% EDTA, pH 8.

DISCUSSION

The present study demonstrates that thyroidal SLI is mobilizable and may be released into the circulation. The SLI containing cells in the thyroid seems to be perculiarly insensitive to stimulation with compounds and nutrients, which are powerful SLI secretagogues elsewhere (10, 29). Increases in perfusate calcium, however, induce a pronounced release of SLI from the thyroid gland as is the case in the pancreas (10).

Somatostatin has been found in many different organs and tissues including the hypothalamus, pancreas, and intestine (30), where pronounced inhibitory effects on hormonal release, probably physiologically significant, have been demonstrable (30). In the thyroid gland, immunoreactive somatostatin has been found in several species (1–3, 5), including dogs (4). Immunohistological studies have shown that it is situated in the C cells, and it has been suggested that SLI is localized in cells also containing calcitonin (3, 31).

With the clear-cut inhibitory actions of somatostatin in other tissues in mind, it was obvious to speculate that somatostatin present in the thyroid could also elicit local effects on hormonal release. Several reports have appeared, with controversial evidence for a direct inhibitory effect of somatostatin on iodothyronine secretion (13–16). In a recent study we have tested the possible effect of somatostatin infusion under the well-controlled experimental conditions attainable with the isolated perfused canine thyroid preparation, and found that even very high perfusate somatostatin levels did not alter the secretion of thyroxine, triiodothyronine, or reverse triiodothyronine (17).

On the other hand, the close cellular relationship of somatostatin and calcitonin would render a functional dependence between these two hormones more probable. Experimental evidence offered by Linehan et al. have appeared (26), favoring a direct inhibitory role of somatostatin on calcitonin release in pigs. In this study, however, infusion of large amounts of somatostatin in the thyroid artery was required to demonstrate the inhibition. Other reports have also suggested that somatostatin inhibits calcitonin secretion (20, 22, 25), whereas some have not been able to disclose any such action (18, 21, 23, 24).

In the present study we have demonstrated an unequivocal, but relatively low basal SLI release of ~12 pg/min from the two perfused thyroid lobes. This rate of release is low compared with that from e.g., the pancreas (9, 10) and the stomach (11, 12) and it is unlikely that thyroidal SLI contributes significantly to the circulating SLI, which in the normal dog is 100–150 pg/ml (32). However, the very dispercity of SLI cells in the thyroid suggests that although the venous concentration is low it indicates a high concentration at the cellular level. The close approximation to calcitonin cells, somatostatin possibly being even present
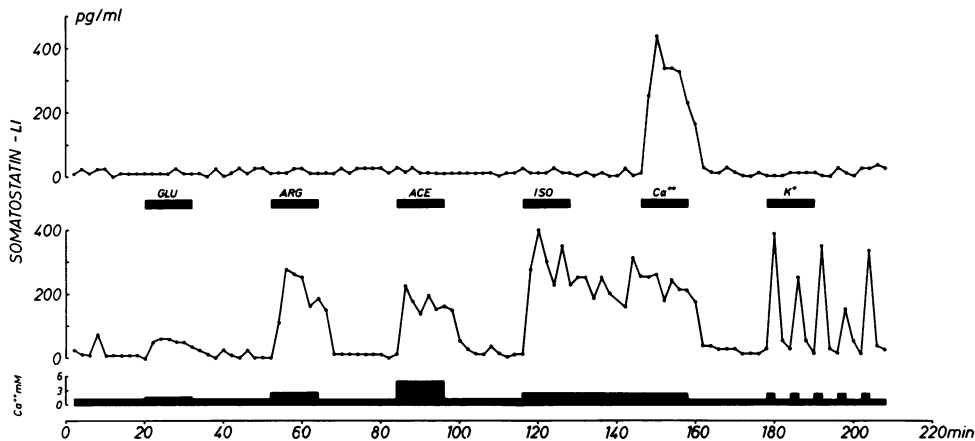
FIGURE 3 Effluent somatostatin-like immunoreactivity from the two canine thyroid lobes perfused independently. Upper part: bars indicate 14 min infusion of glucose (GLU) 16 mmol/liter; arginine (ARG) 5 mmol/liter; acetylcholine (ACE) 5 μmol/liter; isoproterenol (ISO) 23.7 nmol/liter; calcium (Ca^{++}) 1.5 mmol/liter and potassium (K^+) 10 mmol/liter. Lower part: 14 min infusion of Ca^{++}: 0.75; 1.5; and 3 mmol/liter followed by 46 min infusion of 1.5 mmol/liter and 2-min pulses of 1.5 mmol/liter Ca^{++}.

The basal Krebs-Ringer medium contained glucose 4.0 mmol/liter, calcium 1.5 mmol/liter, and potassium 4.4 mmol/liter.

in common C cells, also favors the idea of a "paracrine" system.

The concept of a mutual dependence between the two hormones was recently supported by the report of Chiba et al. (33) demonstrating a stimulatory effect of $(Asu^{1,7})$-eel calcitonin on somatostatin release from the isolated perfused rat stomach (33). However, even high concentrations of salmon and porcine calcitonin had no effect on basal or submaximally stimulated thyroidal SLI release in the present study. The discrepancy may

be due to the species differences between animals, or between calcitonin preparations used. On the other hand, it might be another example of the differences in responsiveness of somatostatin secreting cells in different tissues to certain compounds. Evidence for such a variability is available for the pancreas vs. the stomach (34, 35, 9, 36) and vs. the hypothalamus (37, 38). The heterogeneity in responsiveness is further substantiated by the selective stimulatory effect of calcium on SLI release from the thyroid, found in the present study.

ACKNOWLEDGMENTS

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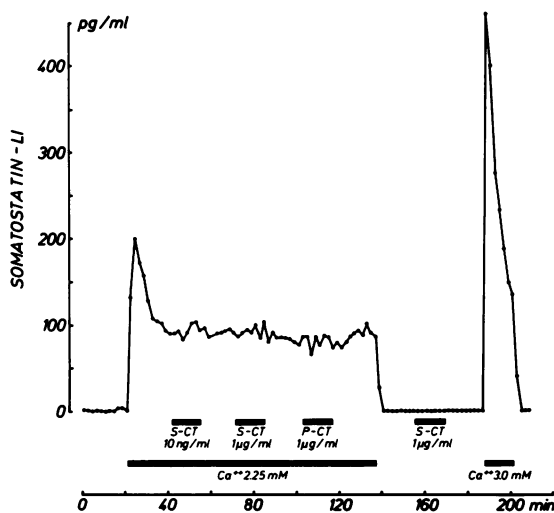
FIGURE 4 Somatostatin-like immunoreactivity in effluent from a perfused dog thyroid. Increases in the Ca^{++} concentration from the control level of 1.5 mmol/liter to 2.25 mmol/liter and 3.0 mmol/liter are indicated by horizontal bars. So are periods of infusion of salmon calcitonin (S-CT) 10 ng/ml and 1 μg/ml and porcine calcitonin (P-CT) 1 μg/ml.

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