Isolation and Structure of Somatostatin from Porcine Hypothalami†

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ABSTRACT: The isolation and structure of somatostatin (GH-RIH) from pig hypothalami are described. This hormone was purified by preparative gel filtration, solvent extraction, countercurrent distribution in two solvent systems, ion-exchange and partition chromatography, and analytical gel filtration. The somatostatin activity was followed by in vitro bioassays and a radioimmunoassay. The isolated product was homogeneous chromatographically and had biological and immunological properties similar to synthetic somatostatin corresponding to the ovine hormone. The primary structure of porcine somatostatin was shown to be H-Ala-Gly-*cyclo*-(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH. Other immunologically and biologically active form(s) of somatostatin were also detected.

Brazeau et al. (1973, 1974) isolated from sheep hypothalami a peptide named somatostatin or GH-RIH,[1] which inhibited the release of GH in vitro and in vivo in rats. After the primary structure of this tetradecapeptide was established (Brazeau et al., 1973; Burgus et al., 1973), it was synthesized by several groups (Rivier et al., 1973; Coy et al., 1973; Yamashiro and Li, 1973; Immer et al., 1974). In clinical studies this substance was shown to suppress not only the release of GH and thyrotropin from the pituitary (Hall et al., 1973; Siler et al., 1973), but also to inhibit the secretion of glucagon and insulin (Yen et al., 1974; Mortimer et al., 1974) as well as gastrin (Bloom et al., 1974). In addition to inhibiting gastrin release, somatostatin also reduced gastric acid and pepsin secretion by a direct effect on both parietal and peptic cells (Gomez-Pan et al., 1975). Recently, we were able to demonstrate a high concentration of somatostatin in the pancreas and stomach of the rat (Arimura et al., 1975b). All of these results suggest that somatostatin could play a role in the regulation not only of the pituitary, but also of the pancreas and the stomach.

In view of the widespread effects and possible clinical significance of this hormone, we thought it would be important to look for it in other species. This report describes the isolation and structure of porcine somatostatin. Our findings have previously appeared in abstract form (Schally et al., 1975).

Experimental Procedures

Purification. A total of 470 000 pig hypothalami was dissected, defatted, and extracted as described previously (Schally et al., 1969, 1971). The extracts were purified by gel filtration on Sephadex G-25 (Schally et al., 1969, 1971). Somatostatin concentrate from the gel filtration was extracted with the upper phase of 0.1% acetic acid–1-butanol–pyridine (11:5:3) (v/v) (system 1) and lyophilized. This material was then further purified by preparative CCD in the same system using the C-2 model apparatus (H. O. Post Co.), followed by another CCD in 1-butanol–acetic acid–water (4:1:5) (system II) in the A-4 apparatus, chromatography on CM-cellulose, and partition chromatography. In all the steps, the separation pattern was followed by the Folin–Lowry reaction (Lowry et al., 1951) or by optical density readings at 278 nm.

Homogeneity and Composition Tests. Thin-layer chromatography was carried out on microcrystalline cellulose. Two solvent systems were used: (a) 1-butanol–acetic acid–water (4:1:5) (v/v), and (b) 1-butanol–pyridine–acetic acid–water (15:10:3:12) (v/v). The developed chromatograms were visualized by spraying with chlorine *o*-toluidine reagents. Synthetic somatostatin in cyclic form made by classical methods (R. Geiger and W. König, Hoechst Pharmaceuticals) or solid-phase methods (Coy et al., 1973) was used as standard. Amino acid analyses were performed on Beckman-Spinco Model 119 automatic amino acid analyzer. Hydrolysates were prepared using 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole at 110 °C for 17 h (Moore, 1972).

Structural Methods. Performic Acid Oxidation. Native porcine and synthetic ovine somatostatin were oxidized separately with performic acid [H_2O_2 (30%)–formic acid (88%), 10:95] for 2 h at 0 °C.

Enzymatic Digestion of Performic Acid Oxidized Porcine and Synthetic Somatostatin: Tryptic Digestion. Performic acid oxidized porcine (10 nmol) and synthetic somatostatin (40 nmol) were digested at 37 °C for 3 h in 200 μl of 0.2 M NH_4HCO_3 (pH 8.2) using 2 μg of trypsin (treated with tosylamino-2-phenylethyl chloromethyl ketone, Worthington Biochemicals) for the former and 8 μg of trypsin for the latter.

Chymotryptic Digestion. Performic acid oxidized porcine (5 nmol) and oxidized synthetic (40 nmol) somatostatin were digested for 3 h at 37 °C in 200 μl of 0.2 M NH_4HCO_3 (pH 8.2) with 5 and 20 μg of α-chymotrypsin (Worthington Biochemicals), respectively.

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[1] Abbreviations used are: CCD, countercurrent distribution; CM-cellulose, carboxymethylcellulose; GH, growth hormone; GH-RIH, growth hormone-release inhibiting hormone; Pth, phenylthiohydantoin; RIA, radioimmunoassay; PhNCS, phenyl isothiocyanate.

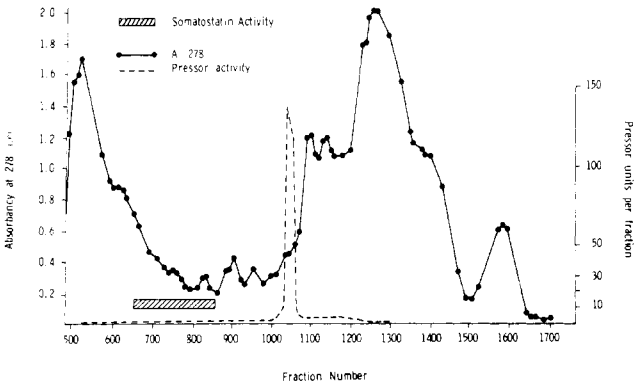

FIGURE 1: Gel filtration of 2 N acetic acid extracts of pig hypothalami (70 g) on a Sephadex G-25 (fine) column (15.5 × 180 cm). Solvent, 1 M acetic acid. Fraction size, 25 ml. Hold-up volume = 11.4 l. = 456 fractions.

Table I: Summary of Purification Scheme of Somatostatin from 470 000 Pig Hypothalami.

Step	Dry Weight (g)	Somatostatin Content[a] (mg)	Recovery as % of total Activity[a]	Recovery of Somatostatin Activity (%) at Each Step[a]
Lyophilized tissue	9900			
Defatted tissue	9200			
Lyophilized 2 N acetic acid extract	3606	18.8	100	
Prep. Sephadex G-25	387	14.1	75	75
Extraction, solvent system I[b]	38	2.43	12.8	17
CCD I	5.39	2.0	10.5	82
CCD II	1.687	1.64	8.7	82
CM-cellulose	0.023	0.425	2.26	26
Partition chromatography	0.003	0.40	2.1	94
Analytical Sephadex G-25	0.001	0.38	2.0	95

[a] As based on the RIA of somatostatin. [b] The amount found in the combined upper phases. The somatostatin remaining in the lower phase was estimated to be 11.8 mg by RIA. The percentage of recovery is based only on the RIA of somatostatin in the extract.

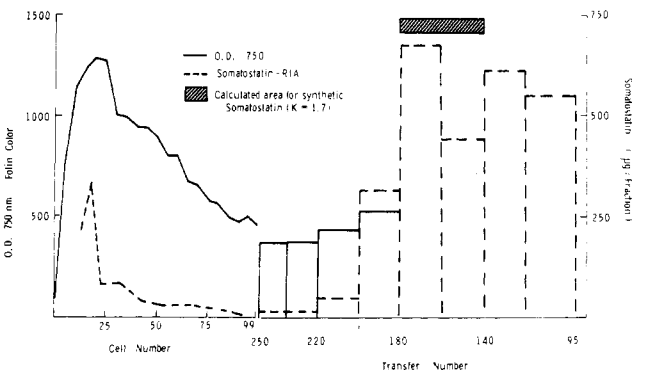

FIGURE 2: Preparative CCD of 38 g of somatostatin extract in a system of 0.1% acetic acid–1-butanol–pyridine (11:5:3) (v/v) by the single withdrawal method. The volume of lower phase and of upper phase was 50 ml; 250 transfers were performed in 100-cell train. Folin–Lowry analyses were carried out using 10 μl of lower phase for cells 0–99, and 10 μl of upper phase for fractions with transfer no. 181–250 removed from the CCD train.

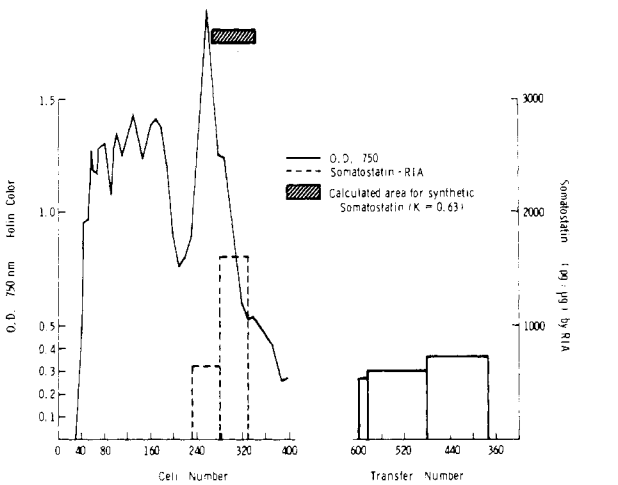

FIGURE 3: CCD II of 5.39 g of somatostatin from CCD I in a system of 1-butanol–acetic acid–water (4:1:5) (v/v). Lower phase was 3 ml and upper phase 5 ml. The number of transfers was 600. Folin–Lowry analyses were done on 10-μl aliquots of lower phase (cells 0–399) and 10 μl of upper phase (no. 400–600).

Sequential Analysis. Phenyl isothiocyanate (PhNCS) degradations were performed by the three-stage method of Edman (1960) as modified by Sauer et al. (1974) using [^{35}S]PhNCS (Amersham Searle Co.).

The identification of Pth-amino acids was carried out by gas chromatography (Pizano and Bronzert, 1969) and by thin-layer chromatography (TLC) (Edman, 1970). Pth-cysteic acid was identified by TLC on silica gel using chloroform–methanol–heptafluorobutyric (70:30:0.5) (D. H. Schlesinger, unpublished data). The thin-layer plates were subjected to autoradiograph development on x-ray film (Jacobs et al., 1973). Some residues were identified as unhydrolyzed free amino acids using the amino acid analyzer.

Assays for Somatostatin Activity. The inhibition of the release of GH in vitro from isolated rat pituitary halves was measured as described previously (Uehara et al., 1973). The concentration of GH in the medium was measured by radioimmunoassay for rat GH using the NIAMDD rat GH kit (Schalch and Reichlin, 1966). The results are expressed as percentage ratio of medium GH in the second hour to medium GH in the first hour and compared statistically to the control using Duncan's new multiple range test. In some experiments, the inhibition of release of GH was measured using dispersed rat pituitary cells in monolayer cultures (Vale et al., 1972). Each sample was assayed in quadruplicate. The concentration of porcine somatostatin activity in various fractions was also followed in most experiments by a radioimmunoassay method for ovine somatostatin (Arimura et al., 1975a).

Results and Comments

Extraction and Gel Filtration. When 470 000 lyophilized pig hypothalami weighing 9.9 kg were defatted, 7% of the total weight was removed (Table I). The hypothalamic powder was extracted five times with 2 N acetic acid and lyophilized. The somatostatin content determined by RIA was 40 ng/hypothalamus.

The extracts were subjected to gel filtration on Sephadex G-25. The pattern of separation can be seen in Figure 1.

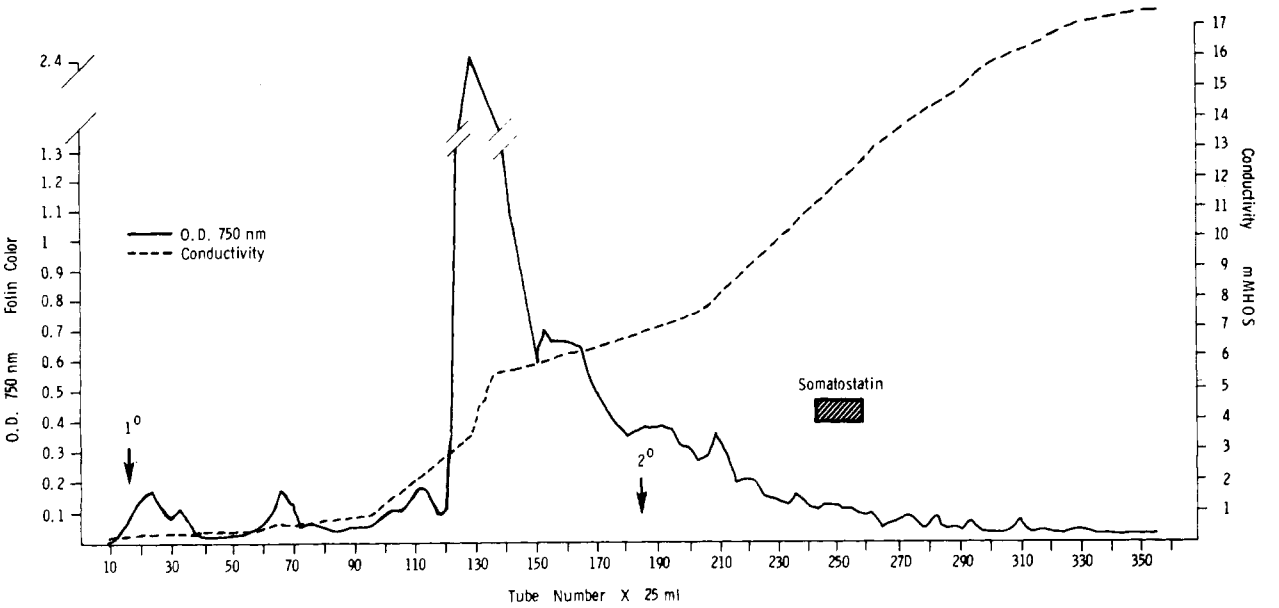

FIGURE 4: Chromatography of 0.687 g of somatostatin concentrate from CCD II on CM-cellulose column, 2.8 × 80 cm, equilibrated with 0.002 M pH 4.5 ammonium acetate buffer. Gradient (1°) to 0.1 M, pH 7.0, buffer through a 2000-ml mixing flask started at fraction no. 17. Gradient (2°) to pH 7, 0.25 M buffer started at no. 186; fraction size, 25 ml.

Fractions 657–856 (R_f 0.7–0.53 (Porath and Schally, 1962) (V_e/V_t = 0.48 – 0.63)) contained 38 pg/μg of immunoreactive somatostatin or about 30 ng/hypothalamus. The recovery of somatostatin activity was about 75%, and the total yield of somatostatin from 44 columns was about 14.1 mg by the RIA (Table I). The extracts also inhibited the release of GH in vitro.

Extraction with Organic Solvents. The Sephadex concentrate (382 g) was dissolved in 2800 ml of the lower phase of solvent I and extracted five times with the upper phase of this system. After lyophilization of the combined upper phases, 38 g of extract was obtained which contained 5.2 ng of somatostatin/hypothalamus by RIA or a total of 2.43 mg. The residue (380 g) had 25 ng of somatostatin/hypothalamus, equivalent to a total of 11.8 mg. Since the K value of synthetic somatostatin in this solvent system was 1.7, five extractions should have removed over 99% somatostatin, if its structure and/or K were similar to those of ovine somatostatin. Since only 17% of immunoreactivity was extracted, the immunoreactive somatostatin in the residue has a different K, most likely due to a partially different molecular structure. This was confirmed by subsequent work (Schally et al., 1975). The extract inhibited GH release in vitro in doses of 40 μg/ml, and the residue in doses of 500 μg/ml.

Countercurrent Distribution I. The somatostatin activity extracted with the upper phase of system I was subjected to CCD in the same solvent system. After 250 transfers, the somatostatin activity with the K = 1.7 was well separated from the second material with somatostatin activity K = 0.07 (Figure 2). The purification of this second somatostatin, presumably due to a different molecular species, will be reported separately.

Fractions 121–200, containing 2.0 mg of somatostatin by RIA and 5.39 g dry weight, were selected for further purification.

Countercurrent Distribution II. The material from CCD I was repurified by the same technique in the solvent system of 1-butanol–acetic acid–water (4:1:5) (Figure 3). The RIA showed the presence of an active fraction in cells 230–329

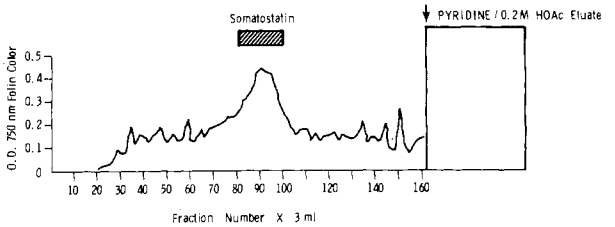

FIGURE 5: Partition chromatography of 23.5 mg of somatostatin from CM-cellulose on column of Sephadex G-25, 1.3 × 145 cm, using 1-butanol–acetic acid–water (4:1:5) (v/v). Fraction size, 3 ml; hold-up volume, 50 ml. Aliquots for Folin–Lowry analyses were 100 μl.

having the same K as that found for synthetic somatostatin (K = 0.6). The total yield of somatostatin by RIA was 1.67 mg. These fractions also powerfully inhibited the release of GH in vitro from rat pituitaries in doses of 50 μg/ml.

CM-Cellulose Chromatography. The somatostatin from CCD II was subjected to chromatography on a CM-cellulose column (Figure 4), content of somatostatin being 18 ng/μg dry weight by RIA. This fraction also showed the highest somatostatin activity in vitro at doses of 2 μg/ml. Other fractions were inactive. The dry weight of fractions no. 243–258 was 23.5 mg and this contained 425 μg of somatostatin.

Partition Chromatography. The material from CM-cellulose was repurified by partition chromatography (Schally et al., 1969). The pattern of separation and the location of somatostatin are shown in Figure 5. The active fraction (no. 81–100) had an R_f 0.18, weighed 3.0 mg, and contained 134 ng of somatostatin/μg.

Analytical Gel Filtration. The final purification step consisted of gel filtration on Sephadex G-25 (Figure 6). Somatostatin emerged in fractions 42–52 with an R_f 0.61, and after lyophilization 1.04 mg of material was obtained. The overall recovery of somatostatin by RIA after seven purification steps was 2.0%.

Biological and Immunological Evaluation of Somatostatin. Porcine somatostatin powerfully inhibited the release of GH from rat pituitary halves in vitro (Table II).

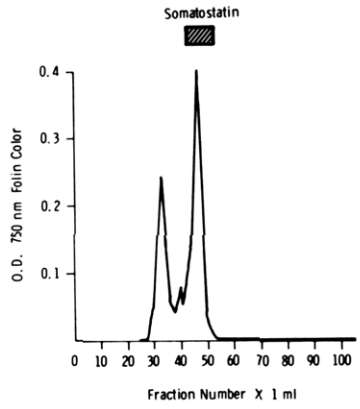

FIGURE 6: Gel filtration of 3.0 mg of somatostatin from partition chromatography on Sephadex G-25, 0.9 × 148 cm. Hold-up volume, 28 ml. Solvent, 2 M acetic acid. Fraction size, 1.0 ml. Folin–Lowry analyses were carried out on 50-μl aliquots.

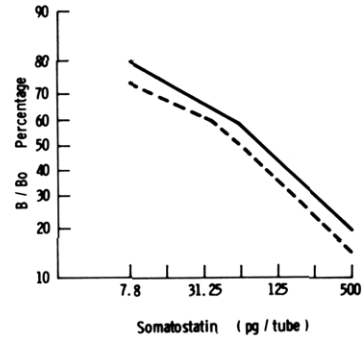

FIGURE 7: Cross-reactivity of pig somatostatin with antiserum to ovine somatostatin. (—) Natural pig somatostatin; (- - - -) synthetic somatostatin. B and B_0 indicate bound radioactivity with and without unlabeled hormone, respectively (for methods, see Arimura et al., 1975a). (The synthetic somatostatin used was the Hoechst preparation W3-52-F6, Geiger and König.)

Table II: *Effect of Porcine and Synthetic Somatostatin* on the Release of GH from Rat Pituitaries in Vitro.[a]

Sample	Dose Dry Weight (ng/ml)	Medium GH[b] % of Control (2nd hr/1st hr × 100)	P vs. Control[c]
Control		106.9 ± 7.3	
Pig somatostatin	8	89.1 ± 2.8	<0.01
	40	63.8 ± 1.0	<0.01
	200	57.2 ± 3.2	<0.01
Synthetic somatostatin[d]	4	88.7 ± 1.1	<0.01
	20	68.5 ± 5.5	<0.01
	100	52.6 ± 4.3	<0.01

[a] On the basis of responses to the low and intermediate doses, the potency of porcine somatostatin = 52.6% of synthetic somatostatin with 95% confidence limits of 31.6–88.6%. Precision index of assay, $\lambda = 0.15$. [b] Mean ± S.E. of four determinations in each group. [c] Duncan's new multiple range test. [d] Hoechst, lot no. W3-52-F6.

Table III: Effect of Porcine and Synthetic Somatostatin on the Release of GH from Dispersed Rat Pituitary Cells in Monolayer Cultures.[a]

Sample	Dose Dry weight (ng/ml)	Medium GH[b] (μg/ml)	% GH Release vs. Control	P vs. Control[c]
Control		4.83 ± 0.21	100	
Pig somatostatin	10	1.97 ± 0.29	41	<0.01
	50	1.46 ± 0.15	30	<0.01
	250	1.23 ± 0.13	25	<0.01
Synthetic somatostatin[d]	5	2.04 ± 0.11	42	<0.01
	25	1.58 ± 0.19	33	<0.01
	125	1.19 ± 0.20	25	<0.01

[a] The potency of porcine somatostatin = 61.5% of synthetic somatostatin with 95% confidence limits of 25.4–147%. Precision index of assay, $\lambda = 0.042$. [b] Mean ± S.E. of four determinations in each group. [c] Duncan's new multiple range test. [d] Hoechst, lot W3-52-F6.

The potency of porcine somatostatin was 52.6% of that of synthetic Hoechst somatostatin. The Hoechst material contained 63% amino acid content and ours 38.8% (see below), so that on the basis of this test, 1 mg of pig somatostatin would correspond to 332 μg of peptide.

When porcine somatostatin was assayed using dispersed rat pituitary cells in monolayer cultures (Vale et al., 1972), its activity was 61.5% of that of Hoechst material (Table III). This corresponds to 389 μg of somatostatin/mg. The cross-reactivity of porcine somatostatin with antisera to synthetic ovine somatostatin was 60% of that of the Hoechst preparation (Figure 7). This would correspond to 380 μg of peptide/mg (Table I) in agreement with amino acid analyses (see below).

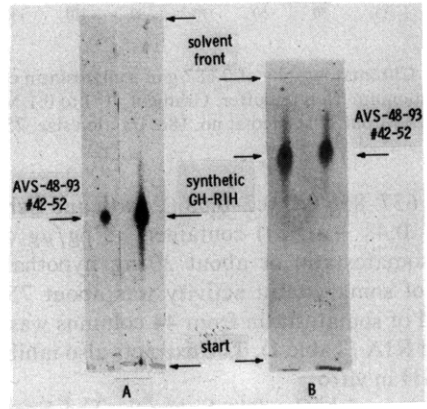

FIGURE 8: (A) Solvent system, 1-BuOH–AcOH–H_2O (4:1:5, v/v); (B) solvent system, 1-BuOH–pyridine–AcOH–H_2O (15:10:3:12, v/v). Thin-layer chromatography of the pig somatostatin (AVS-48-93, no. 42-52) and synthetic cyclic somatostatin (DC-2-37) on microcrystalline cellulose. (A) R_f synthetic = 0.42, natural = 0.42; (B) R_f synthetic = 0.72, natural = 0.73.

Homogeneity Tests and Amino Acid Analyses. TLC of pig somatostatin in two solvent systems (Figure 8) showed that it was homogeneous. Chlorine *o*-tolidine reagent revealed only one spot with the same R_f as synthetic cyclic somatostatin. The amino acid analyses of porcine somatostatin shown in Table IV revealed the same amino acids in the same molar ratios as those previously reported for ovine somatostatin (Brazeau et al., 1973, 1974). The recovery of cystine in this method was similar to that found for other peptides. The amino acid content was 38.8% of dry weight, so that 1 mg would correspond to 388 μg of peptide material, in agreement with the biological and immunological tests.

Sequential Analysis of Performic Acid Oxidized Somatostatin. When somatostatin (28 nmol) oxidized with performic acid was subjected to 13 Edman degradations and the remaining unhydrolyzed material was subjected to

Table IV: Amino Acid Composition of Porcine Somatostatin.[a,b]

Amino Acid	Micromoles per 30 μg[c]	Amino Acid Ratio	Integral Residues
Aspartic acid	0.00836	1.13	1
Threonine	0.01438	1.95	2
Serine	0.00705	0.96	1
1/2-Cystine	0.01142	1.55	2
Glycine	0.00807	1.09	1
Alanine	0.00744	1.01	1
Phenylalanine	0.02126	2.88	3
Lysine	0.02464	1.98	2
Trytophan	0.00548	0.74	1

[a] Amino acid content = 38.8% of dry weight. [b] Hydrolyzed in 4 M methanesulfonic acid, (29) containing 0.2% 3-(2-aminoethyl)-indole under nitrogen at 110 °C for 17 h. [c] AVS 48-93, no. 42–52.

Table V: The Pth-Amino Acids Obtained after Each Cycle of the Edman Degradation on the Unpurified Tryptic and Chymotryptic Peptides of Performic Acid Oxidized Porcine Somatostatin.

Cycle No.	Pth-Amino Acids Identified	
	Tryptic Peptides	Chymotryptic Peptides
1	Ala, Asn, Thr	Ala, Phe, Lys, Thr
2	Gly, Phe (2)[b]	Gly, Thr, Ser
3[a]	$CysSO_3H$, Phe, Thr	
4	Ser	

[a] In cycle 3 of the tryptic peptides, butyl chloride was used to extract Pth-cysteic acid. Benzene was used as the thiazolinone-extracting solvent in all other degradations. [b] Two molar equivalents of phenylalanine with respect to Pth-glycine was obtained.

amino acid analysis, free cysteic acid was found. Every residue in the sequence between 1 and 13 was identified by both thin-layer and gas chromatography [Ala (position 1) = 20.8 nmol; Gly (position 2) = 20.8 nmol; Phe (positions 6, 7, and 11) = 7.9, 7.1, and 2.0 nmol, respectively] except residues 3, 4, 8, and 9. Positions 4 and 9 (lysine) were identified only by TLC. Tryptophan in position 8 and cysteic acid at position 3 were only weakly identified by TLC. Placement of cysteic acid position 3 is strengthened by the observation that no other Pth-amino acid appeared in the organic phase at this cycle with either performic acid oxidized porcine or synthetic somatostatin.

Sequential Analysis of Peptides Produced by Enzymic Digestion of Performic Acid Oxidized Somatostatin. The peptides produced from tryptic and chymotroptic digests of performic acid oxidized somatostatin were subjected to manual Edman degradations without purification. The finding of Asn (position 5) and Thr (position 10, 12) at the end of the first cycle of the degradation on the tryptic peptides indicates that a basic residue must be present at position 4, and 9 or 11 (Table V). Since somatostatin contains two lysine residues and since position 11 was identified as phenylalanine, the two lysine residues must occupy positions 4 and 9.

The placement of tryptophan at position 8 in the sequence of porcine somatostatin is supported by the detection of free tryptophan and Pth-lysine (position 9) after one cycle of degradation, and threonine (position 10) at the end of the second cycle of the degradation on the chymotryptic peptides of both porcine and synthetic ovine somatostatin (Table V).

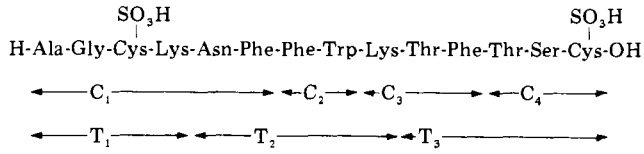

FIGURE 9: Amino acid sequence of performic acid oxidized porcine somatostatin and peptide fragments obtained after tryptic and chymotryptic digest.

Placement of cysteic acid at position 3 was confirmed by its identification in the sequential analysis of the tryptic peptides by extraction of the polar thiazolinone derivative with two aliquots of butyl chloride. The presence of cysteic acid at position 14 is supported by the detection of free cysteic acid after two degradation cycles on chymotryptic peptides and after four cycles on the tryptic peptides. The fragments obtained after chymotryptic and tryptic digest of performic acid oxidized somatostatin and the structure of the oxidized hormone are shown in Figure 9.

Since no cysteic acid was detected after hydrolysis of intact material, and because TLC of pig somatostatin in the solvent systems described gave distinct spots with R_f's identical with those of the cyclic form of synthetic somatostatin, rather than the streaks characteristic of the linear SH form of somatostatin, the porcine somatostatin was isolated in the cyclic disulfide form. Its primary structure is therefore: H-Ala-Gly-*cyclo*-(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH.

Discussion

This paper reports the isolation and structure of porcine somatostatin. Since the primary structures of native porcine and ovine somatostatin are identical, this represents the first confirmation that a molecule with the sequence reported for the ovine hormone (Brazeau et al., 1973, 1974) also exists in another species.

It is now well established that somatostatin inhibits the secretion of GH and thyrotropin in several species of mammals including humans (Hall et al., 1973; Siler et al., 1973; Yen et al., 1974), monkeys (Brazeau et al., 1973; Koerker et al., 1974), dogs (Lovinger et al., 1974), and rats (Brazeau et al., 1973, 1974; Coy et al., 1973).

During the isolation, the biological activity of pig somatostatin was followed by bioassays and a radioimmunoassay for ovine somatostatin (Arimura et al., 1975a). The isolated somatostatin contained only 38.8% of amino acid material. A similar content was previously noted for some preparations of thryotropin-releasing hormone and luteinizing hormone-releasing hormone (Schally et al., 1969, 1971) and is due to contamination of the minute quantity of peptide isolated with the inert materials from columns and solvents, or with non-peptide materials from the extracts, as well as to its acetate content and water of hydration. The sequence of purification described led to a slightly higher yield of porcine somatostatin than that described for the ovine hormone, 2 and 0.98%, respectively (Brazeau et al., 1974). All the procedures used, with the exception of CM chromatography, led to high recoveries of activity. In our hands, as well as those of Brazeau et al. (1974), the recovery after CM-cellulose chromatography was low, 26 and 10%, respectively.

The fact that only 17% of somatostatin activity was extracted with 0.1% acetic acid–1-butanol–pyridine (11:5:3) appears to be due to the presence of two distinct forms of

somatostatin, active both biologically and immunologically, which have different partition coefficients (1.7 and about 0.07). The second somatostatin seems to possess different physicochemical properties than the tetradecapeptide isolated from ovine (Brazeau et al., 1973, 1974) and porcine hypothalami. From the behavior on CM-cellulose, this second somatostatin molecule is distinctly more basic than the tetradecapeptide. It may have a higher molecular weight (Schally et al., 1975) and might represent a precursor of somatostatin. Similar findings were reported previously for other hormones: insulin, corticotropin, growth hormone, parathyroid hormone, and gastrin. In extracts of the pancreas and stomach of the rat we have also found a high concentration of somatostatin as well as two immunoreactive types of it (Arimura et al., 1975b). Since somatostatin inhibits the secretion of glucagon, insulin, and gastrin in animals and humans (Yen et al., 1974; Mortimer et al., 1974; Sakurai et al., 1974; Koerker et al., 1974; Bloom et al., 1974), the presence of somatostatin or a somatostatin-like substance in the pancreas and stomach suggests that it could play a role in the regulation of secretory activity of these two organs in addition to controlling the pituitary GH secretion. Histoimmunological studies on hypothalamus and pancreas support this view (Arimura et al., 1975b; Luft et al., 1974). We have also recently concluded that somatostatin has direct antisecretory effect on parietal and peptic cells, since it inhibited gastric acid and pepsin secretion stimulated by the exogenous pentagastrin in cats (Gomez-Pan et al., 1975). Thus somatostatin affects exocrine as well as endocrine secretion and may be a local inhibitory agent, at least in the pituitary, pancreas, and stomach. Somatostatin has a short half-life in vivo (Redding and Coy, 1974), but the synthesis of long-acting analogues of somatostatin may create useful therapeutic agents.

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