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A high molecular weight form of somatostatin-28 (1-12)-like immunoreactive substance without somatostatin-14 immunoreactivity in the rat pancreas. Evidence that somatostatin-14 synthesis can occur independently of somatostatin-28.

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Research Article

Synthesis of somatostatin-14 (S-14) could occur through direct enzymatic processing of precursor somatostatin (prosomatostatin) or via sequential breakdown of prosomatostatin leads to somatostatin-28 (S-28) leads to S-14. If direct processing is important, it should theoretically generate S-14 and a molecule equivalent to prosomatostatin without the S-14 sequence. In an attempt to identify such a molecule, I characterized the molecular forms of S-28(1-12)-like immunoreactivity (S-28(1-12) LI) in the rat pancreas and compared the relative amounts of these forms with those of S-14-like immunoreactivity (S-14 LI). Pancreatic extracts were chromatographed on Sephadex G-50 and Sephadex G-75 columns (Pharmacia Fine Chemicals Inc., Piscataway, NJ) under denaturing conditions and immunoreactivity in the eluting fractions was analyzed by region-specific radioimmunoassays (RIAs). For RIA of S-28(1-12) LI we used a newly developed rabbit antibody R 21 B, 125I-Tyr12 S-28(1-14), and S-28(1-12) standards. This system detects S-28, S-28(1-12), high molecular weight forms of S-28(1-12), but not S-14. S-14 LI was measured using antibody R149, which detects S-14, S-28, and higher molecular weight S-14-like substances, but not S-28(1-12). Three forms of S-28(1-12) LI were identified: Mr 9,000-11,000, Mr 1,200 (corresponding to S-28(1-12), and Mr less than 1,000, comprising, respectively, 35, 53, and 12% of total immunoreactivity. The relative abundance of the 9,000-11,000 mol wt S-28(1-12) LI material was unchanged following removal of S-14 LI from pancreatic extracts by […]

A High Molecular Weight Form of Somatostatin-28$_{(1-12)}$-like Immunoreactive Substance Without Somatostatin-14 Immunoreactivity in the Rat Pancreas

EVIDENCE THAT SOMATOSTATIN-14 SYNTHESIS CAN OCCUR INDEPENDENTLY OF SOMATOSTATIN-28

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ABSTRACT Synthesis of somatostatin-14 (S-14) could occur through direct enzymatic processing of precursor somatostatin (prosomatostatin) or via sequential breakdown of prosomatostatin → somatostatin-28 (S-28) → S-14. If direct processing is important, it should theoretically generate S-14 and a molecule equivalent to prosomatostatin without the S-14 sequence. In an attempt to identify such a molecule, I characterized the molecular forms of S-28$_{(1-12)}$-like immunoreactivity (S-28$_{(1-12)}$ LI) in the rat pancreas and compared the relative amounts of these forms with those of S-14-like immunoreactivity (S-14 LI). Pancreatic extracts were chromatographed on Sephadex G-50 and Sephadex G-75 columns (Pharmacia Fine Chemicals Inc., Piscataway, NJ) under denaturing conditions and immunoreactivity in the eluting fractions was analyzed by region-specific radioimmunoassays (RIAs). For RIA of S-28$_{(1-12)}$ LI we used a newly developed rabbit antibody R 21 B, ^{125}I-Tyr^{12} S-28$_{(1-14)}$, and S-28$_{(1-12)}$ standards. This system detects S-28, S-28$_{(1-12)}$, high molecular weight forms of S-28$_{(1-12)}$, but not S-14. S-14 LI was measured using antibody R149, which detects S-14, S-28, and higher molecular weight S-14-like substances, but not S-28$_{(1-12)}$. Three forms of S-28$_{(1-12)}$ LI were identified: M_r 9,000–11,000, M_r 1,200 (corresponding to S-28$_{(1-12)}$), and M_r < 1,000, comprising, respectively, 35, 53, and 12% of total immunoreactivity. The relative abundance of the 9,000–11,000 mol wt S-28$_{(1-12)}$ LI material was unchanged following removal of S-14 LI from pancreatic extracts by affinity chromatography before gel filtration. Serial dilutions of fractions containing 9–11,000 and 1,200 mol wt materials exhibited parallelism with synthetic S-28$_{(1-12)}$. The total pancreatic concentration of S-28$_{(1-12)}$ LI was 1.56 pmol/mg protein, of which S-28$_{(1-12)}$ accounted for 0.83 pmol/mg protein and 9–11,000 S-28$_{(1-12)}$ LI comprised 0.55 pmol/mg protein. Pancreatic S-14 LI concentration was 2.07 pmol/mg protein, of which 98% corresponded to S-14. S-28-related peaks accounted for <1% of immunoreactivity in both RIAs. I concluded that (*a*) S-14 is the main form of pancreatic S-14 LI; (*b*) S-28 is present in very small quantities in the pancreas; (*c*) S-28$_{(1-12)}$ LI consists mainly of S-28$_{(1-12)}$ and 9–11,000 mol wt S-28$_{(1-12)}$ LI; (*d*) 9–11,000 mol wt S-28$_{(1-12)}$ LI could represent prosomatostatin without the S-14 sequence; (*e*) the finding of high concentrations of 9–11,000 mol wt S-28$_{(1-12)}$ LI suggests that S-14 synthesis can occur independently of S-28 and that direct processing of prosomatostatin is an important pathway for S-14 synthesis in the pancreas.

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INTRODUCTION

Tetradecapeptide somatostatin (somatostatin-14, S-14)[1] was the first of the somatostatin-related peptides to be isolated and characterized (1). Subsequent studies from a number of laboratories demonstrated the existence of at least two additional higher molecular weight forms of S-14-like immunoreactive (S-14 LI) peptides of M_r 3,000 (3,000 mol wt S-14 LI), and M_r 12,000–15,000 (12–15,000 mol wt S-14 LI) in mammalian tissues (2–6). A molecule corresponding to 3,000 mol wt S-14 LI has been isolated from the mammalian hypothalamus and intestine as somatostatin-28 (S-28), a 14-amino acid *N*-terminally extended form of S-14 connected to S-14 by dibasic (Arg-Lys) residues (7–9). The 12–15,000 mol wt S-14 LI molecule corresponds to mammalian prosomatostatin, a 10,400 mol wt peptide recently characterized by nucleotide sequencing of cDNAs derived from rat (10, 11) and human (12) somatostatin-producing tumor tissues, which contains the entire S-28 sequence at the COOH-terminus (Fig. 1). 12–15,000 mol wt S-14 LI, or prosomatostatin, appears to be a biosynthetic precursor for S-14 and S-28 (5, 13, 14). Using antisera directed against the NH_2-terminal half of the S-28 molecule, the S-28$_{(1-12)}$ fragment has recently been isolated from the rat pancreas and hypothalamus and shown to be a major molecular species in these and other somatostatin-containing tissues (15, 16).

The relative amounts of the three molecular forms of S-14 LI and of S-28$_{(1-12)}$ vary considerably in different somatostatin-containing tissues, suggesting tissue-specific processing of prosomatostatin (2, 6, 16). However, the precise biosynthetic relationship between prosomatostatin, S-28, and S-14 is not clear, especially in the mammalian pancreas, where S-28 is a very minor species (2). In the rat hypothalamus, we recently demonstrated by pulse-chase experiments that S-14 may be synthesized by both direct processing of prosomatostatin → S-14 and by sequential breakdown of prosomatostatin → S-28 → S-14 (13). If direct processing is important, it should theoretically generate S-14 and a molecule equivalent to prosomatostatin without the S-14 sequence. In an attempt to identify such a molecule, we have employed region-specific antisera to characterize the molecular forms of S-14 LI and S-28$_{(1-12)}$ LI in the rat pancreas. We present evidence for the existence of high concentrations of a 9,000–11,000 mol wt molecule containing the S-28$_{(1-12)}$ sequence, but not the S-14 sequence, suggesting that direct enzymatic processing of prosomatostatin to S-14 is an important pathway for S-14 biosynthesis in the rat pancreas.

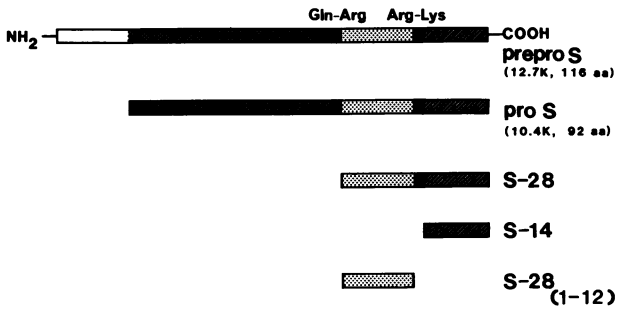

FIGURE 1 Schematic representation of mammalian prepro-somatostatin (prepro S) and its cleavage products, identified in normal tissues which include prosomatostatin (pro S), S-28, S-14, and S-28$_{(1-12)}$.

METHODS

Fed normal male Sprague-Dawley rats (250 g) were killed by decapitation, and the whole pancreas was rapidly removed, washed in ice-cold saline, and extracted in 1 M acetic acid at 0°C by sonication and boiling (17). The homogenate was centrifuged (2,250 *g* for 15 min) to remove insoluble proteins and the supernatant analyzed for S-14 LI and S-28$_{(1-12)}$ LI by separate radioimmunoassays (RIAs) (see below) directly, and after gel chromatography.

Peptides

Synthetic S-14 was obtained from Ayerst, McKenna, and Harrison Ltd., Montreal, Canada, Tyr S-14 was purchased from Bachem Fine Chemicals, CA, and S-28$_{(1-14)}$ and Tyr^{12} S-28$_{(1-14)}$ were prepared by custom synthesis. Synthetic S-28, somatostatin-25, and S-28$_{(1-12)}$ were obtained through courtesy of Dr. N. Ling, Salk Institute, San Diego, CA.

Column chromatography

1 ml of pancreatic extracts was treated with 6 M urea-50 mM PO_4 buffer, pH 7.5. The mixture was applied to 1.1 × 70 cm columns of Sephadex G-50 (superfine) and Sephadex G-75 (Pharmacia Fine Chemicals Inc., Piscataway, NJ) equilibrated with 6 M urea-50 mM PO_4 buffer, pH 7.5, as previously described (2). Immunoreactivity in the eluting fractions was determined by RIA. The nonspecific effects of urea in the RIAs was eliminated by 1:10 dilution of samples and by equalizing the urea content of standard and sample tubes as previously validated (2).

Affinity chromatography

For the separate characterization of pancreatic S-28 LI, affinity chromatography was used to remove S-14 LI from

[1] *Abbreviations used in this paper:* RIA, radioimmunoassay; S-14, somatostatin-14; S-14 LI, somatostatin-14-like immunoreactivity; S-28, somatostatin-28; S-28 LI, somatostatin-28-like immunoreactivity.

pancreatic extracts before Sephadex G-75 gel filtration. An immunosorbent column containing anti-S-14 LI IgG coupled to Affi-Gel 10 (Bio-rad Laboratories, Richmond, CA) was prepared as previously described (column capacity 480 pmol S-14 LI) (18). Acetic acid extracts of pancreas were neutralized with 5 N NaOH, and passed slowly through the column three times. The excluded material (which contained <5% of the initial S-14 LI) was lyophilized, reconstituted in 6 M urea-50 mM PO_4 buffer, pH 7.5, and fractionated for S-$28_{(1-12)}$ LI on Sephadex G-75 columns equilibrated with 6 M urea-50 mM PO_4 buffer, pH 7.5.

RIAS for S-$28_{(1-12)}$ LI and S-14 LI

RIA for S-$28_{(1-12)}$ LI. 6 mg synthetic S-$28_{(1-14)}$ containing a trace quantity of ^{125}I-Tyr^{12} S-$28_{(1-14)}$ (see below for details of preparation) was added to 17 mg hemocyanin (*Limulus polyphemus*, Sigma Chemical Co., St. Louis, MO), dissolved in 2.5 ml 100 mM PO_4 buffer, pH 7.5. 2.5 ml of 2% glutaraldehyde was then added and the resulting solution kept at 4°C for 16 h with constant stirring. The reaction mixture was then dialyzed over 72 h against several changes of distilled water, lyophilized, and stored at −20°C. Under these conditions the percent of S-$28_{(1-14)}$ coupled to hemocyanin was 57% (calculated by expressing the counts remaining in the dialysis bag as a percent of total counts added and assuming equal reactivity of S-$28_{(1-14)}$ and Tyr^{11} S-$28_{(1-14)}$). 4 mg conjugate was mixed with equal volumes of saline and complete Freund's adjuvant and injected into the topeds of four 3-mo-old New Zealand white rabbits. Booster intravenous injections of 0.5–1 mg of the conjugate in saline were administered at 2–4-wk intervals. Antibody production was monitored in heparinized blood obtained from the central ear artery 10–14 d after each injection. Plasma from one of the rabbits obtained 4 mo after the primary immunization was used for RIA.

2 μg Tyr^{12} S-$28_{(1-14)}$ was radioiodinated with Na ^{125}I (New England Nuclear, Boston, MA) by the chloramine-T technique to high-specific activity (1,180 μCi/μg) using the identical procedure that we previously described for radioiodination of tyrosinated analogues of S-14 (17, 18).

Standard solutions of S-$28_{(1-12)}$ (0–800 fm) or unknown samples were incubated with antibody (final concentration 1:5,000) and RIA buffer (100 mM PO_4-0.2% bovine serum albumin (BSA), pH 7.5) for 24 h, followed by the addition of 5 fm ^{125}I-Tyr^{12} S-$28_{(1-14)}$ for a further 24–48 h. ^{125}I-Tyr^{12} S-$28_{(1-14)}$ not bound to antibody was separated from antibody-bound radioligand by charcoal-BSA as previously reported (17, 18). Under these conditions maximum binding of ^{125}I-Tyr^{12} S-$28_{(1-14)}$ to antibody was 35%. Counts nonspecifically bound in the absence of antibody ranged from 3 to 5% of total counts added. The specificity of the antibody was determined by studying the relative binding potencies of several synthetic analogues (Fig. 2). The antibody recognized S-$28_{(1-12)}$ and S-$28_{(1-14)}$ approximately equally but exhibited somewhat greater reactivity for S-28 (156% of S-$28_{(1-12)}$). It crossreacted <5% with S-25 and <0.001% with S-14. The recognition sites of the antibody were thus directed towards the NH_2-terminal portion of the S-$28_{(1-14)}$ sequence. Mean assay sensitivity was 16 fm S-$28_{(1-12)}$. Intraassay precision, expressed as a coefficient of variation, was < ±5%. The interassay coefficient of variation was ±14% at a level of 200 pg/ml.

RIA for S-14 LI. S-14 LI was measured by RIA using antibody R149, ^{125}I-Tyr S-14 and S-14 standards as described (17, 18). This system detects S-14 and S-28 equally and reacts with higher molecular weight S-14 LI (prosomatostatin).

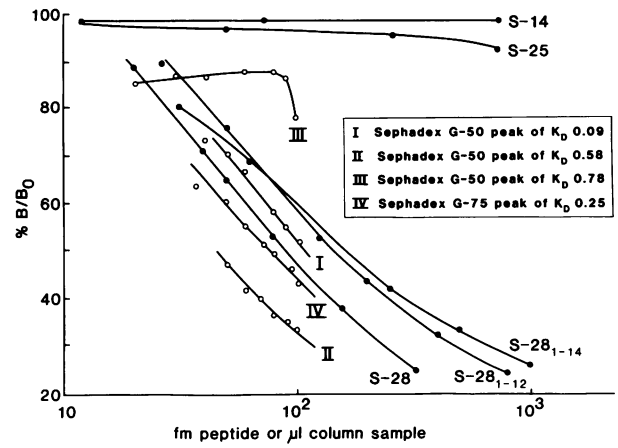

FIGURE 2 Comparison of inhibition curves produced by increasing concentrations of synthetic S-14-, S-28-, and S-28-related peptides with serial dilutions of column samples as indicated.

Protein concentration

The protein concentration in pancreatic extracts was determined by the method of Lowry et al. (19).

RESULTS

Gel chromatography. Sephadex G-50 chromatography of pancreatic extracts revealed three main peaks of S-$28_{(1-12)}$ LI: K_D = 0.09 (corresponding to M_r = 14,000), K_D = 0.58 (coeluting with synthetic S-$28_{(1-12)}$), and K_D = 0.78 (Fig. 3, upper panel). The relative amounts of the three peaks were 35, 53, and 12% of the total S-$28_{(1-12)}$ LI, respectively. Immunoreactivity coeluting with S-28 accounted for <1% of the total S-$28_{(1-12)}$ LI concentration.

To obtain a more accurate estimate of the molecular weight of the largest form of S-$28_{(1-12)}$ LI found on Sephadex G-50 (Fig. 3), pancreatic extracts were separately chromatographed on Sephadex G-75 columns. Under these conditions, the bulk of high molecular weight S-$28_{(1-12)}$ LI eluted at a peak of K_D = 0.25 (M_r = 9,000–11,000), which accounted for 43% of total immunoreactivity (Fig. 4, upper panel). In addition, however, a small quantity (5.3%) of immunoreactivity with K_D < 0.14 ($M_r \geq$ 18,000) was observed. A single peak of K_D = 0.75 made up the remainder (51.3%) of the S-$28_{(1-12)}$ LI. This peak presumably corresponds to the two separate peaks of K_D = 0.58 and K_D = 0.78 found on G-50 chromatography.

Fractionation of pancreatic extracts for S-14 LI on Sephadex G-50 and G-75 columns showed in each case

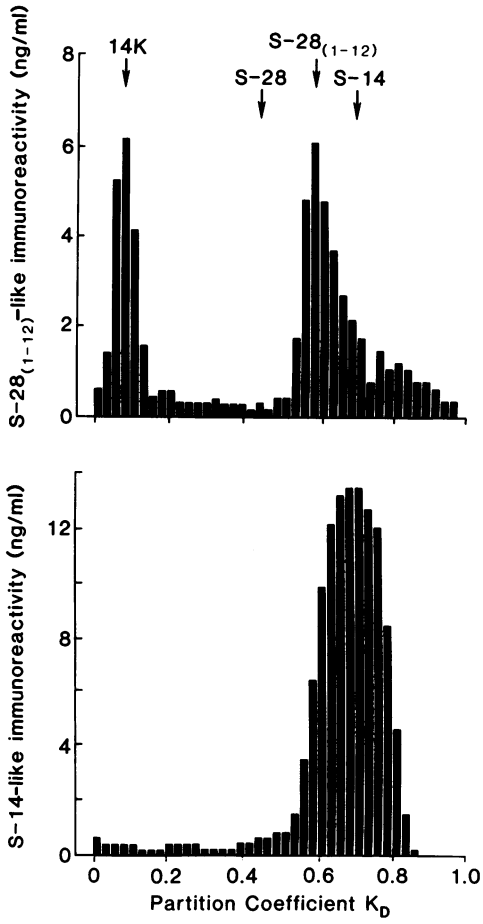

FIGURE 3 Elution profile of S-28$_{(1-12)}$ LI (*upper panel*) and S-14 LI (*lower panel*) extracted from pancreas and chromatographed on a Sephadex G-50 superfine column (1.1 × 70 cm), equilibrated with 6 M urea-50 mM PO_4 buffer, pH 7.5. K_D 0, void volume; K_D 1.0, salt volume. Arrows indicate the elution positions of markers. S-28, synthetic S-28 (mol wt 3,160); S-28$_{(1-12)}$ synthetic S-28$_{(1-12)}$ (mol wt 1,250); S-14, synthetic S-14 (mol wt 1,639). The elution positions of S-28, S-28$_{(1-12)}$, and S-14 were determined by precalibration of the column, with each synthetic peptide applied to the columns as 1–5-ng samples dissolved in 1 ml 6 M urea-50 mM PO_4 buffer, pH 7.5, and detected by appropriate RIA of the effluent. Column recovery was 96% (S-28$_{(1-12)}$ LI) and 105% (S-14 LI). The profiles shown are representative of three chromatographic runs.

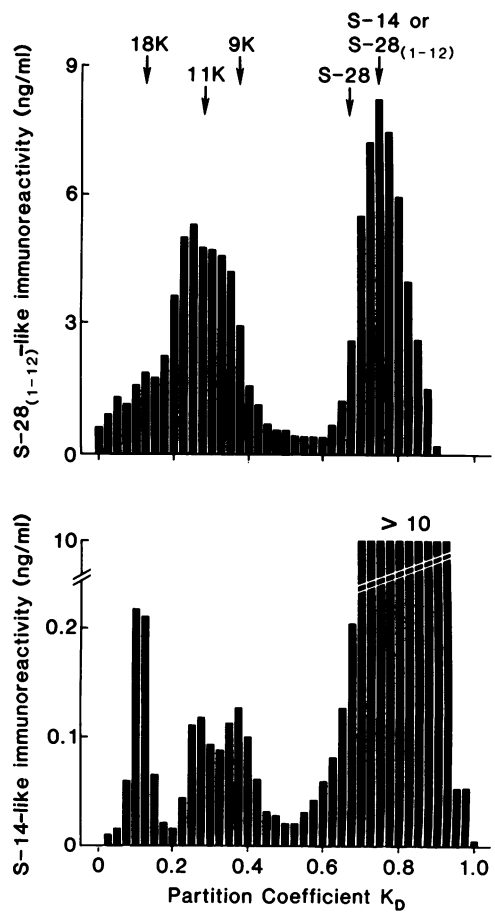

FIGURE 4 Elution profiles of S-28$_{(1-12)}$ LI (*upper panel*) and S-14 LI (*lower panel*) extracted from pancreas and chromatographed on a Sephadex G-75 column (1.1 × 70 cm). Column conditions and markers are as described in Fig. 3. Column recovery was 90% (S-28$_{(1-12)}$ LI) and 89% (S-14 LI). Note the expanded scale in the lower panel. The profiles shown are representative of three chromatographic runs.

a single dominant peak corresponding to S-14 and comprising 98% of total S-14 LI (Figs. 3 and 4, lower panels). Immunoreactivity coeluting with S-28 on Sephadex G-50 (K_D = 0.45) accounted for <1% of S-14 LI. Three higher molecular weight peaks of S-14 LI corresponding to M_r = 18,000 (K_D = 0.11), M_r = 11,000 (K_D = 0.27), and M_r = 9,000 (K_D = 0.38) were found on Sephadex G-75 chromatography. These three peaks together, however, comprised <1% of the total S-14 LI and only 2.4% of the immunoreactivity contained in the high molecular weight peaks of S-28$_{(1-12)}$ LI (K_D = 0.04; Fig. 4, upper panel).

After affinity chromatography was performed to remove S-14 LI, the S-28$_{(1-12)}$ LI in the pancreatic extracts was resolved on Sephadex G-75 columns into two main peaks of K_D = 0.24 (47% of total S-28$_{(1-12)}$ LI) and K_D = 0.74 (48% of total S-28$_{(1-12)}$ LI) (Fig. 5). The relative abundance of the two forms was unaltered as a result of the removal of S-14 LI. This provides additional proof that the large peak of 9,000–11,000 mol wt S-28$_{(1-12)}$ LI material is devoid of S-14 LI.

To further characterize the different S-28$_{(1-12)}$-like immunoreactive peaks, column fractions containing the three main peaks on Sephadex G-50 and the peak of K_D = 0.25 on Sephadex G-75 were assayed in serial

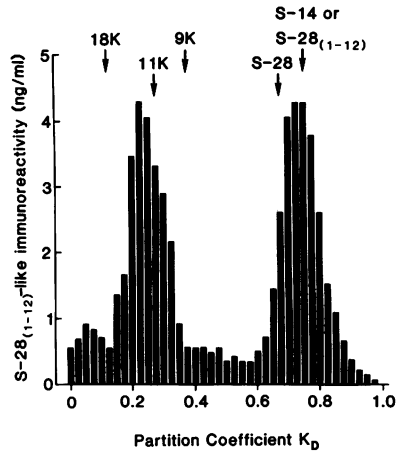

FIGURE 5 Elution profile of S-$28_{(1-12)}$ LI in pancreatic extracts after removal of S-14 LI by affinity chromatography. Column conditions and markers are as described in Figs. 3 and 4. Column recovery was 92%.

dilutions (Fig. 2). The resulting inhibition curves from the first two G-50 peaks (K_D = 0.09, and K_D = 0.58) and the K_D = 0.25 peak on Sephadex G-75 exhibited parallelism, with standard S-$28_{(1-12)}$ indicating immunological identity between the column materials and synthetic S-$28_{(1-12)}$. On the other hand, the very low molecular weight material (K_D = 0.78) eluting after S-$28_{(1-12)}$ on Sephadex G-50 was nonparallel in the RIA (Fig. 2).

Pancreatic content and concentration of S-$28_{(1-12)}$ LI and S-14 LI. The total pancreatic content and concentration of S-$28_{(1-12)}$ LI were, respectively, 213±21 pmol/pancreas and 1.56±0.24 pmol/mg protein. Since 53% of the total S-$28_{(1-12)}$ LI corresponded to S-$28_{(1-12)}$ (Fig. 3), the pancreatic content and concentration of S-$28_{(1-12)}$ were calculated to be 113 pmol and 0.83 pmol/mg protein, respectively. Pancreatic content of S-14 LI was 284±4.6 pmol; the concentration was 2.07±0.18 pmol/mg protein. On the basis of S-14 being 98% of total pancreatic S-14 LI, the pancreatic content and concentration of S-14 was, respectively, 278 pmol and 2.08 pmol/mg protein.

DISCUSSION

In the present study we have confirmed that pancreatic S-14 LI consists almost exclusively of S-14 and that S-28 is a very minor constituent of pancreatic somatostatin (2, 6, 15). With respect to higher molecular weight S-14 LI, two and possibly three forms were identified corresponding to sizes of 18,000, 11,000, and 9,000 daltons, respectively, but each was present in very small concentrations. The 9,000–11,000 mol wt material is of similar size to the 12,500 mol wt precursor identified by pulse labeling of rat islets (5) and also corresponds in size to the recently sequenced rat prosomatostatin molecule (10, 11). The nature of the 18,000 mol wt S-14 LI peak remains to be determined.

With respect to S-$28_{(1-12)}$ LI we have confirmed the observation of Benoit et al. (15) that material equivalent to the S-$28_{(1-12)}$ fragment is present in very high concentrations in the pancreas. Our value of 113 pmol/pancreas (0.83 pmol/mg protein) is comparable to an earlier estimate of pancreatic S-$28_{(1-12)}$ reported by these authors (140–170 pmol/pancreas), but both are somewhat higher than the recent results published by the same authors using a different assay (16) for reasons that are not clear. S-$28_{(1-12)}$ was readily separable from S-14 by the Sephadex G-50 gel filtration technique employed despite the comparable molecular weights of the two peptides. There is no obvious reason for this, especially since the columns were run under denaturing conditions. In practical terms, the different behavior of the two peptides affords an easy means of separating them by gel chromatography.

The most significant finding of our study is the identification of high concentrations of a high molecular weight form of S-$28_{(1-12)}$ LI of M_r = 9,000–11,000. This material comprised 35–47% of total S-$28_{(1-12)}$ LI. Since our columns were run under denaturing conditions the material is likely to represent a single-chain polypeptide rather than an aggregated or noncovalently bound form of S-$28_{(1-12)}$ LI. Parallelism of this molecule with synthetic S-$28_{(1-12)}$ suggests that it has the antigenic determinants present in the S-$28_{(1-12)}$ sequence. Finally, the lack of reactivity of this molecule in the S-14 RIA and its persistence as S-$28_{(1-14)}$ LI following affinity removal of S-14 LI indicates the presence of the S-$28_{(1-12)}$ sequence but not the S-14 sequence. This molecule thus appears to be a portion of prosomatostatin with the S-$28_{(1-12)}$ sequence intact but lacking the S-14 sequence. Such a molecule could conceivably arise from direct enzymatic processing of prosomatostatin to S-14 without prior formation of S-28 (Fig. 1). The size of such a molecule should theoretically be ~1,600 daltons smaller than that of prosomatostatin, although no such difference could be demonstrated in the present study by gel filtration techniques. Recently Benoit et al. (16), also using Sephadex G-75 chromatography, have identified two S-$28_{(1-12)}$-like immunoreactive forms in rat pancreas of M_r 4,400 and M_r 8,000, respectively. The relationship of these molecules to our 9,000–11,000 mol wt S-$28_{(1-12)}$ LI and to prosomatostatin remains to be clarified.

The biosynthesis of somatostatin has been extensively investigated in the fish pancreas and to a limited extent

only in the rat pancreas (5, 20–23). Since fish pancreatic prosomatostatins lack the complete sequence of S-28, and since mammalian pancreas contains very small quantities of S-28, it is not yet possible to determine the precise role of S-28 in the biosynthesis of S-14 and particularly whether S-28 is a precursor for S-14 (2, 5, 23). Synthesis of S-14 could occur by direct processing of prosomatostatin or through sequential breakdown of prosomatostatin → S-28 → S-14, or both. In the case of the rat hypothalamus, studies of the enzymatic processing of prosomatostatin and of S-28, as well as pulse-chase studies, have provided evidence for the existence of both pathways (13, 24, 25). In rat cerebrocortical neurons, however, S-14 appears to be derived exclusively from direct cleavage of prosomatostatin (14). The relative importance of the two pathways in the rat pancreas remains uncertain. Our finding of high concentrations of the 9,000–11,000 mol wt $S\text{-}28_{(1\text{-}12)}$ LI moiety suggests that the direct pathway is important for S-14 synthesis in the pancreas. This is supported by direct studies of biosynthesis in which pulse-chase labeled rat islets have been shown to incorporate radioactive amino acids into a 12,500 mol wt prosomatostatin molecule which undergoes rapid processing to S-14 without the prior accumulation of S-28 (5). Finally, direct cleavage of prosomatostatin to yield S-14 appears to be the main mechanism for S-14 synthesis in the fish pancreas (26).

Evidence for the sequential breakdown of prosomatostatin → S-28 → S-14 in the rat pancreas is less definite. The finding of small but very definite quantities of S-28 in the pancreas suggests that the enzymes necessary for processing prosomatostatin to S-28 do exist in this tissue. It is possible that S-28 is formed in significant quantities and then rapidly processed to S-14 and $S\text{-}28_{(1\text{-}12)}$. The finding of high pancreatic concentrations of $S\text{-}28_{(1\text{-}12)}$ is consistent with this. An alternative explanation, however, is that the $S\text{-}28_{(1\text{-}12))}$ fragment may be produced by cleavage from 9,000–11,000 mol wt $S\text{-}28_{(1\text{-}12)}$ after removal of S-14. Further biosynthetic studies to define the kinetic relationships between the $S\text{-}28_{(1\text{-}12)}$-like molecules and S-14 will be clearly necessary to elucidate the relative physiological significance of these two pathways in the pancreas.

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REFERENCES

1. Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive growth hormone. *Science (Wash. DC).* 179:77–79.
2. Patel, Y. C., T. Wheatley, and C. Ning. 1981. Multiple forms of immunoreactive somatostatin: comparison of distribution in neural and nonneural tissues and portal plasma of the rat. *Endocrinology.* 109:1943–1949.
3. Spiess, J., and W. Vale. 1980. Multiple forms of somatostatin-like activity in the rat hypothalamus. *Biochemistry.* 19:2861–2866.
4. Zyznar, E. S., J. M. Conlon; V. Schusdziarra, and R. H. Unger. 1979. Properties of somatostatin-like immunoreactive polypeptides in the canine extrahypothalamic brain and stomach. *Endocrinology.* 105:1426–1431.
5. Patzelt, C., H. S. Tager, R. J. Carroll, and D. F. Steiner. 1980. Identification of prosomatostatin in pancreatic islets. *Proc. Natl. Acad. Sci. USA.* 77:2410–2414.
6. Trent, D. F., and G. C. Weir. 1981. Heterogeneity of somatostatin-like peptides in rat brain, pancreas and gastrointestinal tract. *Endocrinology.* 108:2033–2038.
7. Pradayrol, L., H. Jørnvall, V. Mutt, and A. Ribet. 1980. *N*-terminally extended somatostatin: the primary structure of somatostatin-28. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 109:55–58.
8. Schally, A. V., W.-Y. Huang, R. C. C. Chang, A. Arimura, T. W. Redding, R. P. Millar, M. W. Hunkapiller, and L. E. Hood. 1980. Isolation and structure of prosomatostatin: a putative somatostatin precursor from pig hypothalamus. *Proc. Natl. Acad. Sci. USA.* 77:4489–4493.
9. Esch, F., P. Bohlen, N. Ling, R. Benoit, P. Brazeau, and R. Guillemin. 1980. Primary structure of ovine hypothalamic somatostatin-28 and somatostatin-25. *Proc. Natl. Acad. Sci. USA.* 77:6827–6831.
10. Goodman, R. H., D. C. Aron, and B. A. Roos. 1983. Rat pre-prosomatostatin. *J. Biol. Chem.* 258:5570–5573.
11. Funckes, C. L., C. D. Smith, R. Deschenes, M. Magazin, M. A. Tavianini, M. Sheets, K. Collier, H. L. Weith, D. C. Aron, B. A. Roos, and J. E. Dixon. 1983. Cloning and characterization of a mRNA-encoding rat preprosomatostatin. *J. Biol. Chem.* 258:8781–8787.
12. Shen, L.-P., R. L. Pictet, and W. J. Rutter. 1982. Human somatostatin I: sequence of the cDNA. *Proc. Natl. Acad. Sci. USA.* 79:4575–4579.
13. Zingg, H. H., and Y. C. Patel. 1982. Biosynthesis of immunoreactive somatostatin by hypothalamic neurons in culture. *J. Clin. Invest.* 70:1101–1109.
14. Robbins, R. J., S. Mothon, and S. Reichlin. 1983. Somatostatin-28 is not the precursor of somatostatin-14 in cerebral cortical cells. 65th Annu. Mtg. U. S. Endocrine Soc., San Antonio. 85. (Abstr.)
15. Benoit, R., P. Böhlen, N. Ling, A. Briskin, F. Esch, P. Brazeau, S.-Y. Ying, and R. Guillemin. 1982. Presence of somatostatin-$28_{(1\text{-}12)}$ in hypothalamus and pancreas. *Proc. Natl. Acad. Sci. USA.* 79:917–921.
16. Benoit, R., N. Ling, C. Bakhit, J. H. Morrison, B. Alford, and R. Guillemin. 1982. Somatostatin-$28_{(1\text{-}12)}$ like immunoreactivity in the rat. *Endocrinology.* 111:2149–2151.
17. Patel, Y. C., and S. Reichlin. 1978. Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. *Endocrinology.* 102:523–530.
18. Patel, Y. C., T. Wheatley, D. Fitz-Patrick, and G. Brock. 1980. A sensitive radioimmunoassay for somatostatin-

like immunoreactivity in extracted plasma: measurement and characterization of portal and peripheral plasma in the rat. *Endocrinology*. 107:306–313.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
20. Noe, B. D., D. J. Fletcher, G. E. Bauer, G. C. Weir, and Y. C. Patel. 1978. Somatostatin biosynthesis occurs in pancreatic islets. *Endocrinology*. 102:1675–1685.
21. Goodman, R. H., P. K. Lund, J. W. Jacobs, and J. F. Habener. 1980. Preprosomatostatins: products of cell-free translations of messenger RNAs from anglerfish islets. *J. Biol. Chem.* 255:6549–6552.
22. Shields, D. 1980. In vitro biosynthesis of somatostatin: evidence for two distinct prosomatostatin molecules. *J. Biol. Chem.* 255:11625–11628.
23. Hobart, P., R. Crawford, L. Shen, R. Pictet, and W. J. Rutter. 1980. Cloning and sequence analysis of cDNAs encoding two distinct somatostatin precursors found in the endocrine pancreas of anglerfish. *Nature (Lond.)*. 288:137–141.
24. Zingg, H. H., and Y. C. Patel. 1980. Processing of somatostatin precursors: evidence for enzymatic cleavage by hypothalamic extract. *Biochem. Biophys. Res. Commun.* 93:1274–1279.
25. Zingg, H. H., and Y. C. Patel. 1982. Processing of synthetic somatostatin-28 and a related endogenous rat hypothalamic somatostatin-like molecule by hypothalamic enzymes. *Life Sci.* 30:525–533.
26. Fletcher, D. J., B. D. Noe, G. E. Bauer, and J. P. Quigley. 1980. Characterization of the conversion of a somatostatin precursor to somatostatin by islet secretory granules. *Diabetes*. 29:583–599.