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Research Article

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Properties of Endogenous Somatostatin-like Immunoreactivity and Synthetic Somatostatin in Dog Plasma

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ABSTRACT Somatostatin-like immunoreactivity (SLI) in the peripheral venous plasma of dogs and in their pancreatic and gastric venous effluents was characterized and compared with synthetic somatostatin. Both endogenous plasma SLI and somatostatin added to plasma were eluted from Sephadex gels at pH 8.8 in the 150,000–200,000-mol wt region but at pH 2.5 both appeared in the 1,500–2,000-mol wt region. The SLI released from the isolated dog pancreas perfused with plasma-free buffer was eluted entirely as a 1,600-dalton polypeptide, but when the pancreas was perfused with plasma, SLI was eluted in the 150,000–200,000-mol wt zone. Affinity chromatography of plasma samples on immobilized antibodies directed against the central portion of the somatostatin molecule (residues 5–9 and 11) removed $\approx 90\%$ of both endogenous SLI and somatostatin added to plasma, but neither was removed by affinity chromatography on antibodies directed against the NH_2 -terminal region of somatostatin (residues 1–4). The SLI from plasma and from pancreas perfusate isolated by affinity chromatography was identical in molecular size, charge, and immunometric properties to synthetic somatostatin. It is concluded that endogenous SLI is secreted by the pancreas and stomach in a form not distinguishable from synthetic somatostatin, but circulates in plasma bound to large molecular weight components; the NH_2 -terminal residues of somatostatin appear to be important in this binding.

INTRODUCTION

Somatostatin, a tetradecapeptide first isolated from ovine (1) and porcine (2) hypothalamic tissue, has

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been detected in extracts of the pancreas and gastrointestinal tract of several species (3–5), and localized to D-cells in the fundic and antral regions of the stomach (6) and in the islets of Langerhans (7–11). The release of somatostatin-like immunoreactivity (SLI)¹ from the isolated perfused dog pancreas and from isolated rat islets is stimulated by glucose (12, 15), amino acids (13–15) and gut hormones (15–17). SLI has been detected in the plasma of rats, dogs, and humans (18–20), and rises in the peripheral venous plasma of dogs after the ingestion of a fat-protein meal (21). It is likely that the post-prandial rise in peripheral plasma SLI levels is derived largely from the pancreas and stomach (21).

Although the foregoing observations are compatible with some type of physiologic role for somatostatin, the evidence that SLI in plasma resembles biologically active true somatostatin is limited (22). The present study was, therefore, designed to characterize circulating SLI in plasma and to compare it with synthetic hypothalamic somatostatin with respect to certain physicochemical and immunologic properties.

METHODS

Plasma samples. It has been shown that intragastric administration of HCl elicits a rise in pancreaticoduodenal and antral vein SLI and that intraduodenal HCl causes a rise in fundic vein SLI as well (21). Blood samples (30 ml) were obtained from the pancreaticoduodenal and gastroepiploic (antral) veins of dogs during laparotomy after stimulation of SLI release by intragastric administration of 0.1 N HCl and from the short gastric vein (fundic) after the intraduodenal administration of 0.1 N HCl. They were collected in chilled tubes containing 1,000 kallikrein inhibitor units of aprotinin (Trasylol, Delbay Pharmaceuticals, Div. of Schering Corp., Bloomfield, N. J.) and 1.2 mg of EDTA per ml of whole blood

¹ Abbreviation used in this paper: SLI, somatostatin-like immunoreactivity.

and centrifuged at 4°C. The plasma was stored at -20°C until the time of study.

Pancreas perfusate samples. Pancreases from 20- to 30-kg, fasted, male, mongrel dogs were isolated and perfused according to the technique of Iversen and Miles (23), using a nonrecirculating system and a semi-synthetic perfusion buffer as described elsewhere (13). The plasma-free effluent from the pancreas was collected during perfusion with somatostatin secretagogues, arginine (15), vasoactive intestinal peptide (17), and cholecystokinin-pancreozymin (16). Pancreases were also perfused with heparinized plasma collected from the same animals immediately before sacrifice and containing added arginine or cholecystokinin-pancreozymin. Such samples were collected in chilled tubes containing 1,000 kallikrein inhibitor units of aprotinin and 1.2 mg of EDTA per ml of plasma effluent.

Affinity chromatography. The immunoglobulin (Ig) G fractions of Vale antiserum S-27, raised against Tyr¹-somatostatin coupled to human serum albumin, and of Vale antiserum S-81, raised against Tyr¹¹-somatostatin coupled to human serum albumin (3), were immobilized on cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.) at a concentration of 2 mg of IgG per ml of Sepharose 4B (24). The regional specificity for the somatostatin molecule of antiserum S-27 of Vale is directed towards the central region of the molecule (residues 5-9 and 11) (25), whereas Vale antiserum S-81 is directed towards the NH₂-terminal (residues 1-4) (25). The specificities of the antisera were determined from their ability to bind various analogues of somatostatin under radioimmunoassay conditions. Before affinity chromatography, the columns were irrigated with 4 M guanidinium hydrochloride (1 vol) to insure removal of any endogenous SLI bound to antibody. Plasma samples (15 ml) from pancreatic, antral, and fundic veins, plasma samples (40 ml) from a peripheral vein, and pancreatic perfusate (50 ml) were diluted with an equal volume of 0.1 M sodium phosphate buffer, pH 7.4, and insoluble material was removed by centrifugation. The supernate was applied at 4°C to a column (10 × 1 cm) of immobilized antibodies at a flow rate of 0.5 ml/min. Nonspecifically bound material was removed from the affinity columns by irrigation with 1 M NaCl adjusted to pH 10.4 with ammonia solution (sp gr 0.88), and the SLI bound specifically to the immobilized antibodies was eluted with 1 M formic acid solution.

Gel filtration. Gel filtration was carried out on 60 × 2.5-cm columns of Biogel P-10 (Bio-Rad Laboratories, Richmond, Calif.) and on 110 × 1.0-cm columns of Sephadex G-200, using 0.1 M NH₄HCO₃ buffer (pH 8.8) for elution, and on 60 × 2.5-cm columns of Sephadex G-25, using 2 M CH₃COOH (pH 2.5) for elution. The columns were calibrated with markers of known molecular weight, blue dextran (M₁), human immunoglobulin-G (Ig) (M₂), and synthetic somatostatin (M₃).

Isoelectric focusing. Isoelectric focusing was carried out at 4°C in a LKB model 8100 column (LKB Instruments, Inc., Rockville, Md.) (110 ml) using LKB ampholines in the pH range of 7-11 (26). Cytochrome *c* was employed to provide visual indication of completion of focusing. pH measurements were made using a Beckman Model 3550 pH meter (Beckman Instruments Corp., Fullerton, Calif.).

Thin layer chromatography. Thin layer chromatography of SLI samples, isolated by affinity chromatography, was carried out on cellulose plates (20 × 20 cm) using 1-butanol:pyridine:acetic acid:water (15:10:3:12) (vol/vol) for elution (2). After chromatography, the plates were cut into 0.5-cm strips and each strip was incubated overnight in 0.2 M glycine containing 1% (wt/vol) human serum albumin (2 ml). SLI in the supernate was detected by radioimmunoassay.

Radioimmunoassay. SLI was measured by the radio-

immunoassay procedure of Arimura et al. (19), as modified by Harris et al. (20), using Arimura antisomatostatin serum R101 which is directed against residues in the 5-11 region of the somatostatin molecule (25).

RESULTS

Comparison of molecular size of endogenous SLI and synthetic somatostatin in plasma. At pH 8.8 the endogenous SLI in plasma obtained from peripheral pancreatic and gastric veins was eluted from Sephadex G-200 columns in the 150,000-200,000-mol wt zone (Fig. 1A). When small amounts, (2 ng/ml) of synthetic somatostatin (mol wt 1,636) were added to plasma and incubated at 5°C overnight, all of the SLI was also eluted in this high molecular weight zone (Fig. 1B). However, with the addition of larger amounts of synthetic somatostatin (100 ng/ml) to plasma, the SLI was eluted as two peaks, one in the 150,000-200,000-mol wt zone and one in the low molecular weight zone (Fig. 1C). Similar elution profiles were obtained at pH 7.4. At pH 2.5, however, ≈90% of the endogenous plasma SLI and the synthetic somatostatin added to

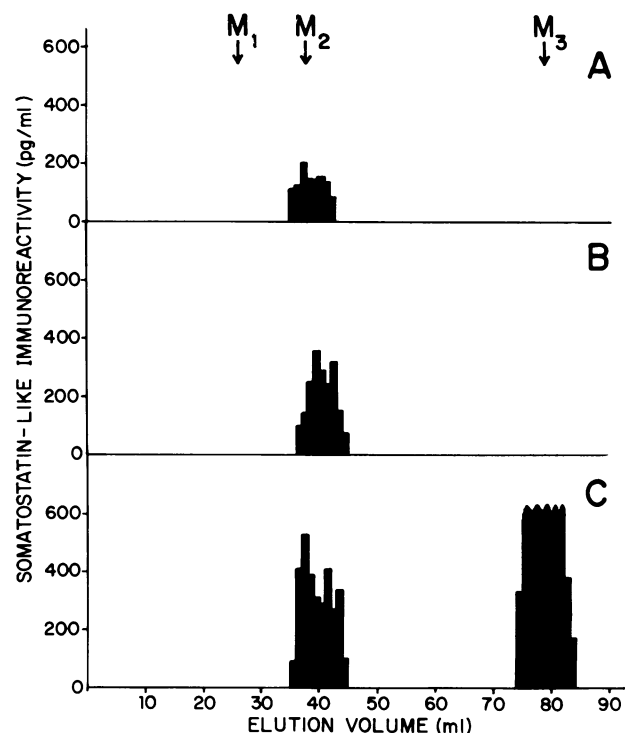


FIGURE 1 Elution profiles of (A) ≈500 pg/ml endogenous pancreatic vein plasma SLI, (B) synthetic somatostatin added to plasma at a concentration of 2 ng/ml, and (C) synthetic somatostatin added to plasma at a concentration of 100 ng/ml on Biogel P-10 columns at pH 8.8. The arrows indicate the elution volumes of blue dextran (M₁), human IgG (M₂), and synthetic somatostatin in the absence of plasma (M₃). The serrated peak in Fig. 1C indicates that the levels of immunoreactivity were >600 pg/ml.

plasma was eluted from Sephadex G-25 columns in the same position as synthetic somatostatin in the absence of plasma (Fig. 2). The minor peak of immunoreactivity observed in both chromatographs in the 3,000-mol wt zone may be indicative of a tendency of somatostatin to dimerize.

To determine if the endogenous SLI was released from the pancreas as a 1,500–2,000-dalton polypeptide, plasma-free perfusate was collected from the effluent of the isolated pancreas during perfusion with D-cell secretagogues and subjected to gel filtration. At pH 8.8 all of the SLI of the plasma-free perfusate appeared in the 1,600-dalton zone of Biogel P-10 columns (Fig. 3A). However, when plasma containing the D-cell secretagogue arginine (10 mM) was perfused through the pancreas, all of the secreted SLI was eluted at pH 8.8 in the void volume of these columns (Fig. 3B). These results suggest that both endogenous SLI and synthetic somatostatin are bound to a high molecular weight component of canine plasma. When the pancreas was perfused with plasma containing the more powerful D-cell secretagogue, cholecystokinin-pancreozymin (0.075 Ivy U/ml), however, the secreted SLI appeared in both the high and low molecular weight zones (Fig. 3C), indicating that the plasma binding components are saturable by endogenous SLI as well as by synthetic somatostatin.

Studies of somatostatin binding in plasma. To determine the region of the somatostatin molecule that

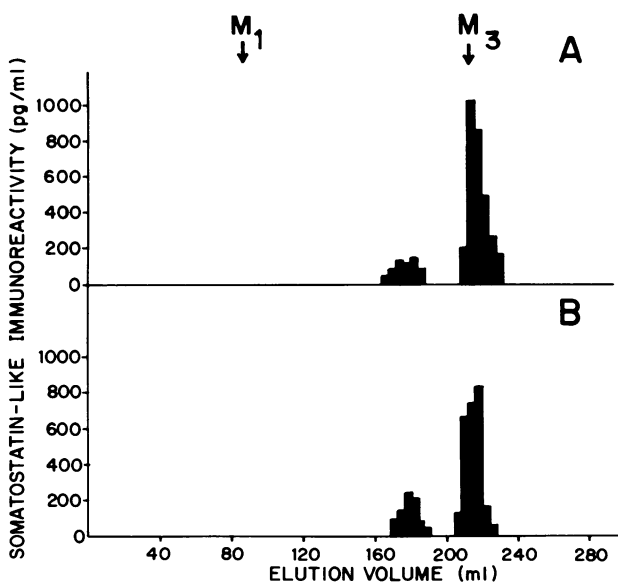


FIGURE 2 Elution profiles of (A) $\approx 1,000$ pg/ml endogenous antral vein plasma SLI (5 ml) and (B) synthetic somatostatin added to plasma (0.5 ml) at a concentration of 10 ng/ml on Sephadex G-25 columns at pH 2.5. The arrows indicate the elution volumes of blue dextran (M_1) and synthetic somatostatin in the absence of plasma (M_3).

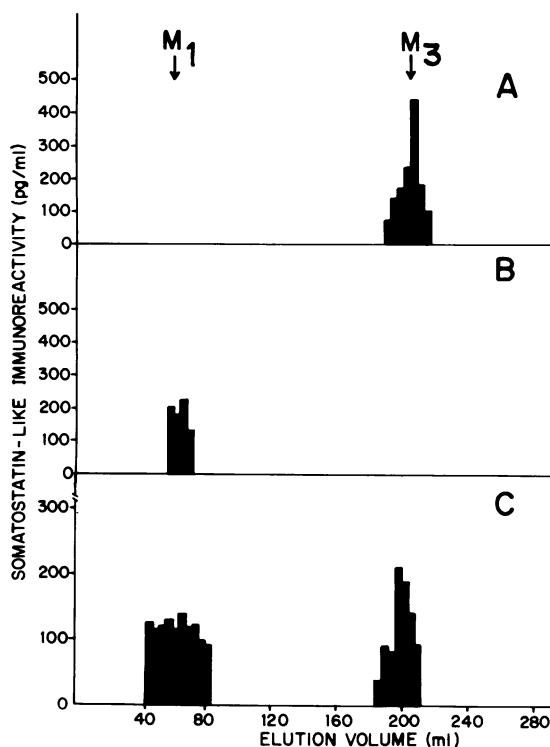


FIGURE 3 Elution profiles of (A) $\approx 1,000$ pg/ml endogenous SLI from the isolated pancreas perfused with plasma-free buffer solution, (B) ≈ 500 pg/ml endogenous SLI from the isolated pancreas perfused with plasma containing arginine, and (C) $\approx 1,500$ pg/ml endogenous SLI from the isolated pancreas perfused with plasma containing cholecystokinin-pancreozymin on Biogel P-10 columns at pH 8.8.

binds to the large molecular weight plasma components, the NH_2 -terminally modified analogue of somatostatin, ^{125}I -labeled Tyr¹-somatostatin, and the analogue, ^{125}I -labeled Tyr¹¹-somatostatin, modified close to the COOH-terminal, were added to plasma and incubated at 5°C overnight. At pH 8.8 the NH_2 -terminally modified analogue was eluted in the 1,500–2,000-dalton zone, but the COOH-terminally modified analogue, ^{125}I -labeled Tyr¹¹-somatostatin, was eluted in the 150,000–200,000-dalton zone.

The region of the somatostatin molecule that binds to the plasma component was further investigated by means of affinity chromatography with both S-27 antiserum (directed against the central portion of the somatostatin molecule) and antiserum S-81 (directed against the NH_2 -terminal region). Affinity chromatography of plasma samples on a column of immobilized antibodies from S-27 removed $\approx 90\%$ of endogenous SLI from plasma collected from the pancreatic and gastric veins and of synthetic somatostatin added to plasma (Fig. 4), whereas chromatography of plasma samples on immobilized antibodies from antiserum S-81 removed neither (Fig. 5). However, when they were in a plasma-

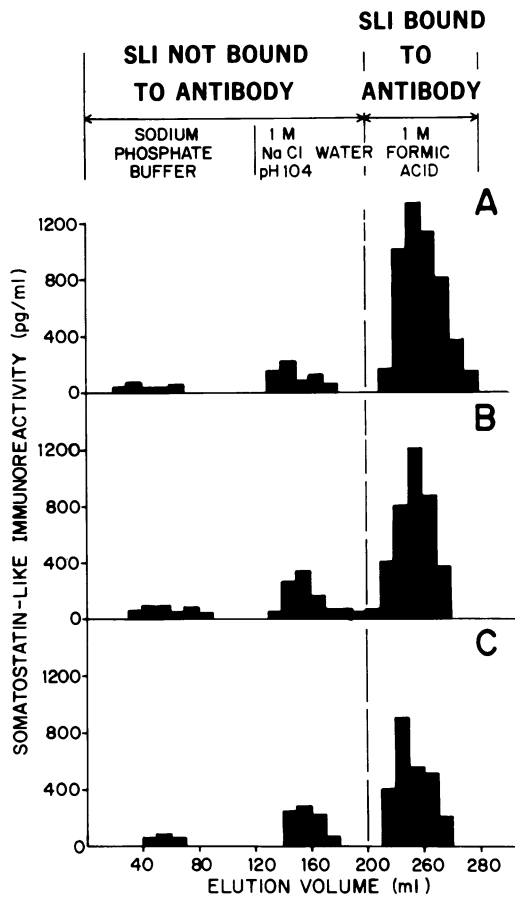


FIGURE 4 Affinity chromatography of (A) $\approx 1,000$ pg/ml of endogenous antral vein plasma SLI, (B) synthetic somatostatin added to plasma at a concentration of 2 ng/ml, and (C) synthetic somatostatin in plasma-free solution at a concentration of 2 ng/ml, on immobilized antibodies from antiserum S-27.

free solution, both synthetic somatostatin and SLI released from the isolated pancreas into plasma-free perfusate were bound to an equivalent degree by both antibody columns (Figs. 4 and 5). (Significant amounts of SLI could be removed from the affinity columns before elution of the SLI bound to immobilized antibody irrigation with a buffer solution of high pH [Figs. 4 and 5] [27]. This material probably represents SLI bound to the antibody columns through nonspecific interactions with the immobilized proteins [28] and with the matrix [29].)

The SLI removed from various plasma samples and from perfusate by the S-27 column was recovered by irrigation with 1 M formic acid. After lyophilization, the SLI recovered from the pancreatic perfusate and from antral, fundic, and pancreatic plasma was eluted from Biogel P-10 columns at pH 8.8 as a single immunoreactive peak in approximately the same position as synthetic somatostatin (Fig. 6). The SLI

recovered from plasma taken from a peripheral vein of a fasted dog was also eluted in the 1,600-dalton region (Fig. 6), but the peak of immunoreactivity was less well resolved than those recovered from the antral and pancreatic venous plasma.

Comparison of isoelectric points of endogenous SLI and synthetic somatostatin. SLI isolated from the pancreatic perfusate by affinity chromatography was focused to a single immunoreactive band in the pH range of 9.9–10.2, and synthetic somatostatin was focused to a single band in the pH range of 10.0–10.4. (A pI of 9.5 for synthetic somatostatin at 10°C has previously been reported by Diel et al. [30].)

Comparison of mobilities on thin layer chromatography. SLI isolated from the plasma-free pancreatic perfusate showed similar mobility to synthetic somatostatin on cellulose thin layer chromatography (SLI R_f 0.75 ± 0.03 ; synthetic somatostatin R_f 0.75 ± 0.03). The

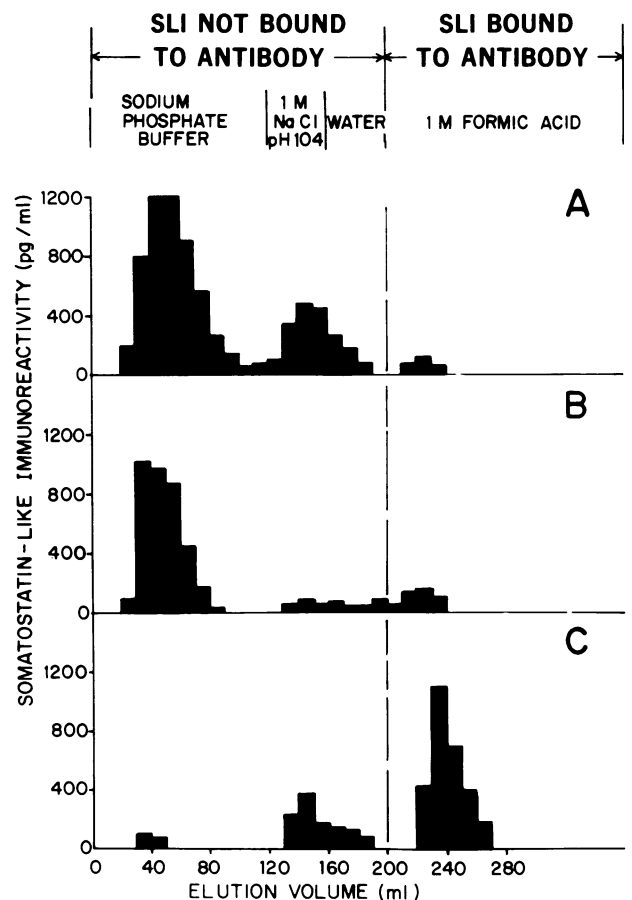


FIGURE 5 Affinity chromatography of (A) $\approx 1,000$ pg/ml of endogenous antral vein plasma SLI, (B) synthetic somatostatin added to plasma at a concentration of 2 ng/ml, and (C) synthetic somatostatin in plasma-free solution at a concentration of 2 ng/ml on immobilized antibodies from antiserum S-81.

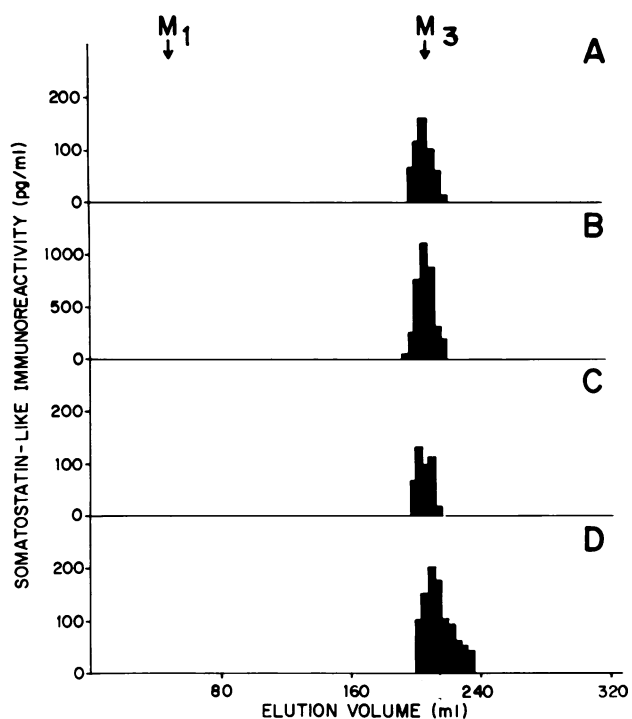


FIGURE 6 Elution profile of (A) endogenous pancreatic vein plasma, (B) endogenous antral vein plasma SLI, (C) fundic vein SLI, and (D) endogenous peripheral venous plasma SLI on Biogel P-10 columns at pH 8.8. The SLI samples were purified by affinity chromatography on immobilized antibodies from antiserum S-27. The arrows indicate the elution volumes of blue dextran (M_1) and synthetic somatostatin (M_3).

R_f values obtained compare well with those reported by Schally et al. (2) under the same experimental conditions (R_f synthetic 0.72, natural 0.73).

Comparison of immunologic properties. Serial dilutions of SLI isolated from the pancreatic vein, the gastroepiploic vein and from pancreatic perfusate were proportional and their dilution slopes were parallel to those of synthetic somatostatin in plasma-free buffer (Fig. 7).

DISCUSSION

The present findings show that plasma SLI released from the dog stomach and pancreas is similar in molecular size, in charge, and in certain immunologic characteristics to synthetic hypothalamic somatostatin. (They do not, however, rule out small structural differences between hypothalamic and extrahypothalamic somatostatin, such as the modification or substitution of certain amino acid residues.) Comparison of the elution profile of plasma SLI isolated by affinity chromatography from a peripheral vein revealed a broader peak of immunoreactivity than those of plasma SLI isolated from veins draining the pancreas and stomach, suggesting that some of the endogenous

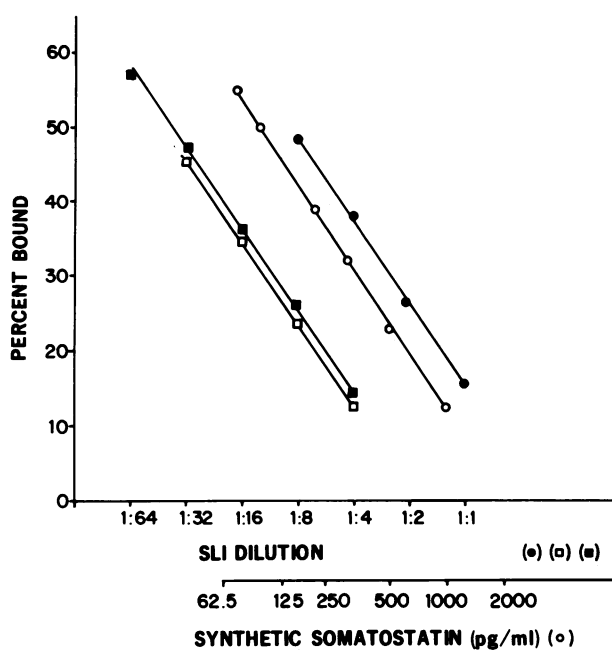


FIGURE 7 Dilution slopes of pancreatic vein plasma SLI (●), antral vein plasma SLI (□), and SLI from the plasma-free perfusate of the isolated pancreas (■), in comparison to serial dilutions of synthetic somatostatin in plasma-free buffer (○). The endogenous SLI samples were purified by affinity chromatography and gel filtration on Biogel P-10 columns.

SLI in the peripheral circulation might have been subjected to enzymatic degradation (Fig. 6). The SLI released by the perfused pancreas appears entirely as a somatostatin-sized species. Although it is possible that larger molecular weight species such as those reported in extracts of the hypothalamus (2, 31) and pancreas (4, 31) are also secreted into the circulation in quantities insufficient for detection by the techniques employed in this study, it seems unlikely that they are released in significant quantities in response to the secretagogues used.

It was previously reported that both endogenous SLI and synthetic somatostatin injected or infused in vivo circulate in plasma bound to molecules comparable in size to IgG (20), and therefore, different from the 80,000-mol wt soluble, somatostatin-binding protein detected by Ogawa et al. (32) in the cytosol fraction of various rat, human, and bovine tissues. The binding of somatostatin to the protein must occur subsequent to secretion inasmuch as it is secreted by the perfused pancreas in vitro as a single small molecular weight moiety. Its binding is disrupted by low pH, confirming the report of Kronheim et al. in human plasma (22) and that of Arimura (33) who has reported that extraction of rat plasma with acetone liberates the 1,600-mol wt SLI from binding components. It appears that the binding proteins are readily saturable and that

the binding capacity of dog plasma for exogenous somatostatin is <5 ng of somatostatin per ml of plasma, suggesting that infusion of somatostatin at pharmacological doses may saturate the binding proteins and result in circulation of unbound somatostatin. The behavior of somatostatin in plasma is analogous to that of the somatomedins, which also show pH-dependent binding to proteins in human plasma (34).

The inability of immobilized antibodies directed against the NH₂-terminal antigenic site of the somatostatin molecule to remove from plasma either endogenous SLI or synthetic somatostatin added to plasma suggests that they both circulate bound to plasma components in a manner that renders the NH₂-terminal residues of the molecule inaccessible to such antibodies. This conclusion is supported by the lack of binding to plasma components of the NH₂-terminally modified somatostatin analogue, ¹²⁵I-labeled Tyr¹-somatostatin, in contrast to COOH-terminally modified analogue Tyr¹¹-somatostatin. It has been proposed (35) that the somatostatin molecule consists of an elongated "hairpin" loop containing a hydrophobic domain at one end of the molecule, and a hydrophilic domain, associated with the disulfide link and the NH₂-terminal residues, at the other end. The present study suggests that the NH₂-terminal region of somatostatin is involved in the binding to plasma components. The contribution of the disulfide linkage of somatostatin to the binding to plasma components was not assessed in this study. Similarly, the study indicates that in radioimmunoassays of canine plasma there will not be competition for the radio-labeled tracer, ¹²⁵I-Tyr¹-somatostatin, between the binding components and an anti-somatostatin serum directed against the central portion of the somatostatin molecule, whereas the binding components may significantly interfere in the reaction of somatostatin and radio-labeled tracer with an NH₂-terminally directed antisomatostatin serum.

Finally, it should be emphasized that these studies do not resolve the question of the physiologic role or roles, if any, of circulating SLI. Similarity in the molecular size, charge, and immunologic characteristics of plasma SLI and biologically active synthetic somatostatin does not constitute proof of their identity. But even if they should be identical, proof that circulating SLI necessarily has a physiological role would require demonstration that biologically active somatostatin bound to plasma components is available to target receptors.

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