Evidence for the Presence of Somatostatin 28 in Plasma

S. SHOELSON, K. POLONSKY, H. DOCHERTY, J. JASPAN, AND A. RUBENSTEIN

SUMMARY

Somatostatin-like immunoreactivity (SLI) from dog and rat plasma eluted from Biogel P-6 columns as three distinct peaks. A large-molecular-weight peak was present in the void volume of the column, an intermediate-sized peak (SLI^{28}) coeluted with synthetic somatostatin 28 (S-28), and a small-molecular-weight peak (SLI^{14}) coeluted with SRIF. Material from the SLI^{28} peak diluted in parallel to the S-28 standard in the radioimmunoassay and behaved identically to S-28 on high pressure liquid chromatography (HPLC). Levels of SLI^{28} in the portal vein were consistently greater than the simultaneously measured peripheral levels (portal peripheral ratio 2.2 ± 0.2). Venous samples drawn from multiple sites suggested that SLI^{28} is secreted by the duodenum and/or pancreas and the intestine. This data is consistent with the possibility that S-28 is a hormone distinct from SRIF. DIABETES *31*: 474–477, May 1982.

A 28-amino-acid form of somatostatin S-28 has recently been isolated from porcine intestine[1] and ovine[2] and porcine hypothalamus.[3] It is also released by isolated pancreatic islets in perifusion experiments.[4] Its structure is that of the tetradecapeptide somatostatin (SRIF) with a 14-amino-acid amino terminal extension. The biologic significance of this peptide is unknown. It has been suggested that S-28 is a precursor of SRIF.[3] However, since S-28 is more active than SRIF in some biologic systems,[5] it is also possible that S-28 may represent a naturally occurring form of somatostatin discrete from SRIF. S-28 has not yet been identified in the circulation. In this study, we describe the presence of a form of somatostatin-like immunoreactivity (SLI) indistinguishable from S-28 by gel filtration chromatography, high pressure liquid chromatography (HPLC), and radioimmunoassay in the portal and systemic circulations of dogs and rats.

From the Department of Medicine, University of Chicago, Chicago, Illinois.
Address reprint requests to Kenneth S. Polonsky, M.D., University of Chicago, Box 435, 950 East 59th Street, Chicago, Illinois 60637.
Received for publication 16 February 1982.

MATERIALS AND METHODS

At laparotomy, end-sampling catheters were placed in the portal vein and femoral artery of five normal mongrel dogs fasted overnight as previously described.[6] Blood samples were drawn into tubes containing Trasylol (500 KIU/ml) and EDTA (1.2 mg/ml). Plasma was separated immediately at 4°C and stored at −20°C until assayed. Blood was drawn from the portal vein and aorta of anesthetized, fasted rats at laparotomy and handled similarly to the dog samples.

The radioimmunoassay of SLI was carried out in 0.1 M sodium citrate buffer containing 0.1% BSA and 0.2% sodium azide, pH 5.0. The rabbit antibody R3b (anti-SRIF thyroglobulin) was used in a final concentration of 1:15000. [^{125}I-Tyr^{1}]SRIF was prepared by the Iodogen method (Pierce Chemical Co., Rockford, Illinois)[7] and was purified on a Sephadex G25 (fine) column (1 × 50 cm). Final assay volume was 900 μl and incubations were carried out for 72 h. Charcoal was used to separate bound from free peptide. Synthetic SRIF (Bachem, Torrance, California) and S-28 (kind gift of Drs. N. Ling and R. Guillemin, Salk Institute, San Diego, California) were used as standards. [des-Ala′]-SRIF was kindly supplied by Dr. B. Petrach of Ciba Geigy Corp. (Ardsley, New York). Under these acid assay conditions, there was no degradation of labeled SRIF as judged by immunoprecipitability and gel filtration chromatography as previously reported.[8] Recovery of S-28 added to plasma was 94.1 ± 6.8% (N = 7). The sensitivity of the assay as assessed by the least amount of peptide causing significant displacement ($P < 0.025$) of [^{125}I-Tyr′]SRIF was 1 pg/tube for SRIF and 5 pg/tube for S-28. Interassay variation was ±9.4% and intra-assay variation was ±6.7%.

Gel chromatography of plasma samples was performed on Biogel P6 columns (1 × 47 cm). Plasma SLI components were eluted under gravity in assay buffer at a flow rate of 0.10 ml/min. Fractions (0.5 ml) were collected, and the entire fraction was assayed for SLI content. Recovery of SRIF and S-28 standards added to the columns was >90%. Re-

covery of total plasma SLI added to the columns was 87.1 ± 2.7%.

Dog portal vein plasma was prepared for high-pressure liquid chromatography by diluting 20 ml of plasma twofold with 1% trifluoroacetic acid (TFA). The mixture was passed through a reverse phase C-18 cartridge (Water's SepPac), to which the SLI adsorbed. The cartridge was rinsed with 5 ml of 1% TFA and the SLI was eluted with a 1.5-ml mixture of acetonitrile, water and TFA (400:99:1). The volume was reduced to 250 μl in a rotary evaporator, and the solution was subjected to reverse phase HPLC. The stationary phase was a 0.39 × 30-cm μBondapac C-18 column and the isocratic mobil phase was 24.6% acetonitrile, 75.4% 0.1 M triethylamine phosphate, pH 3.0, at a flow rate of 1.5 ml/min. Fractions (0.75 ml) were collected and diluted 75-fold with assay buffer and SLI levels determined by radioimmunoassay.

RESULTS

The relative immunoreactivity of SRIF and related peptides is shown in Figure 1. On a molar basis, S-28 is half as reactive with our antibody as SRIF and [des-Ala′]-SRIF reacts equally with SRIF in the assay.

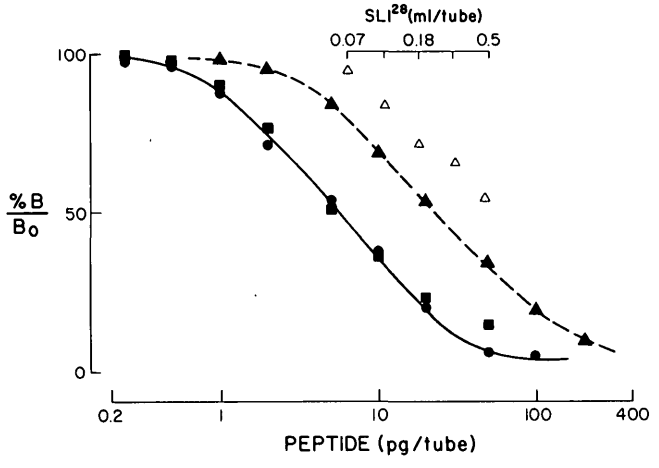
SLI eluted from the columns in three discrete peaks (Figure 2). A high-molecular-weight peak (SLI^{Vo}) eluted in the void volume, an intermediate-molecular-weight peak (SLI^{28}) coeluted with synthetic S-28, and a low-molecular-weight peak coeluted with synthetic SRIF and [des-Ala′]-SRIF. The material from the SLI^{28} peak was compared with the S-28 Standard in the radioimmunoassay. As shown in Figure 1, material from this peak dilutes in parallel to the S-28 Standard.

The levels of SLI present in each peak varied from animal to animal as shown in Table 1, but in the dog, portal levels of both SLI^{14} and SLI^{28} were consistently higher than the corresponding peripheral concentrations. The portal peripheral ratio of SLI^{14} (3.5 ± 0.4) was greater than that of SLI^{28} (2.2 ± 0.2) in all dogs consistent with the greater hepatic extraction of SLI^{14} which we have previously observed.[9]

To determine the source of plasma SLI^{28} samples were drawn from catheters placed in veins draining the gastric fundus (short gastric), gastric antrum (gastroepiploic), pancreas and duodenum (pancreatico-duodenal), and small and large intestine (superior and inferior mesenteric) in three additional dogs. The concentrations of SLI^{28} in the pancreatico-duodenal and superior and inferior mesenteric veins were greater than the arterial concentration (Figure 2). This finding suggests that circulating SLI^{28} is derived from the pancreas and/or duodenum and intestine.

To further elucidate the identity of the intermediate molecular weight SLI fraction, an extract of dog portal vein plasma was analyzed by reverse phase HPLC. Three SLI fractions were obtained (Figure 3). Two of the fractions coeluted with S-28 and SRIF, respectively. The remaining SLI fraction was not adsorbed by the reverse phase packing under these conditions and, although its exact nature is uncertain, it may represent the void volume component seen after gel chromatography.

FIGURE 1. Standard curves of SRIF and related peptides. The antibody R3b reacted equally with SRIF (●-●) and [des-Ala′]-SRIF (■-■) and on a molar basis reacted half as well with S-28 (▲-▲) as with SRIF. Aliquots of the SLI^{28} peak (△-△) obtained after gel chromatography of plasma samples (see Figure 2) were added to the radioimmunoassay in the volumes shown.



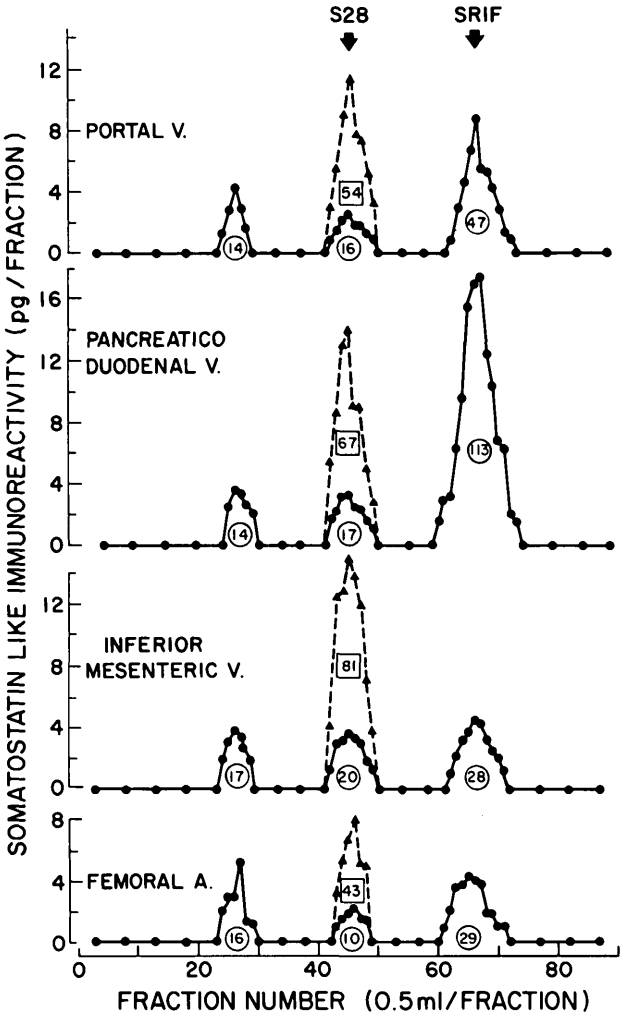
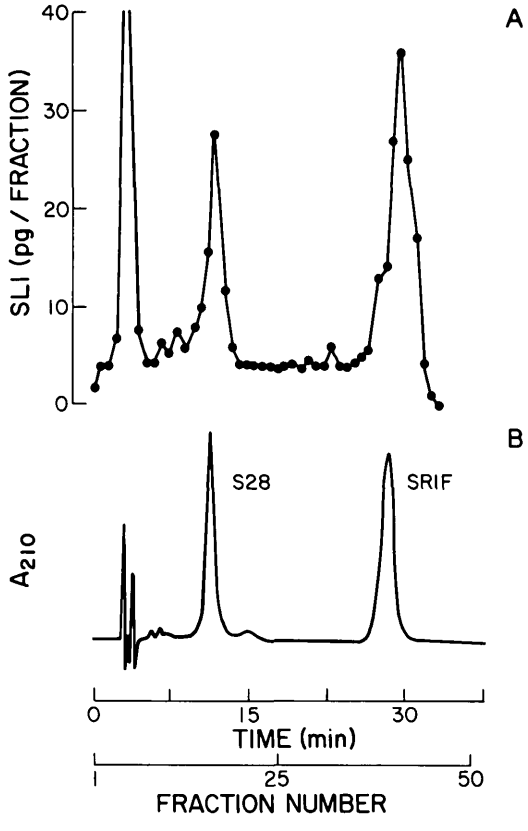


FIGURE 2. Plasma SLI components from four vessels in a representative dog. Plasma samples (1.0 ml) were subjected to gel chromatography on Biogel P-6 columns (1 × 47 cm). Columns were calibrated with synthetic SRIF and S-28 as shown by the arrows (top of figure). Plasma components were eluted with assay buffer at a flow rate of 0.10 ml/min. The entire fraction (0.5 ml) was assayed for SLI content. All three peaks were assayed against a SRIF Standard (●-●) while the intermediate sized peak was also compared with the S-28 standard (▲-▲). Numbers in the circles represent total SLI in that peak compared with the SRIF standard. Numbers in the squares represent total SLI^{28} compared with the S-28 standard.

TABLE 1
SLI fractions from the portal and systemic circulations of dogs and rats after gel chromatography. Total SLI (pg) in each peak is given. The immunoreactivity in the SLI^{Vo} and SLI^{14} peaks was compared with a SRIF standard, and the SLI^{28} peak was compared with the S-28 standard

Animal	Vessel	SLI^{Vo} (pg)	SLI^{28}	SLI^{14} (pg)
Dog 1	Fem. A.	34	58	30
	Port. V.	43	129	114
Dog 2	Fem. A.	16	76	38
	Port. V.	25	208	153
Dog 3	Fem. A.	21	29	9
	Port. V.	24	59	20
Dog 4	Fem. A.	41	14	19
	Port. V.	52	22	88
Dog 5	Fem. A.	4	10	33
	Port. V.	6	25	97
Rat 1	Aorta	8	<5	36
Rat 2	Aorta	5	<5	23
Rat 3	Aorta	10	<5	16
Rat 4	Aorta	6	31	22
Rat 5	Aorta	6	50	29
Rat 6	Port. V.	2	42	97
Rat 7	Port. V.	5	28	144
Rat 8	Port. V.	4	38	26
Rat 9	Port. V.	7	90	126

FIGURE 3. HPLC of plasma SLI. HPLC was carried out according to the techniques described under METHODS. SLI content in each of the fractions is shown in Panel A. For comparison, standards of S-28, SRIF, and [des-Ala']-SRIF were subjected to HPLC under identical conditions. While SRIF and [des-Ala']-SRIF were not well separated from each other (k' = 10.8 and 11.1, respectively), they were both well separated from S-28 (k' = 3.4) k' = (Vr-Vo)/Vo, where Vo and Vr are the void volume and the sample retention volume, respectively. A chromatograph of a mixture of S-28 and SRIF is shown in Panel B.



DISCUSSION

There has been a great deal of interest in characterizing the circulating forms of somatostatin-like immunoreactivity. Many workers have identified a fraction which coelutes with synthetic SRIF on gel chromatography,[10–12] and this low-molecular-weight SLI fraction is believed to be [des-Ala']-SRIF.[13,14] The nature of the high-molecular-weight SLI fraction is uncertain, although we have confirmed previous observations that its molecular size is greater than 150,000 daltons[15] and that it does not dissociate in 10% acetic acid nor increase when SRIF, [des-Ala']-SRIF, or S-28 are added to plasma (unpublished data).

In this report, we describe the presence of an intermediate-sized SLI peak from the portal and peripheral plasma of dogs and rats. Since this fraction is present in very low concentration, its isolation and complete chemical characterization has not been possible. Nevertheless, the techniques which we have used, namely radioimmunoassay, gel chromatography, and HPLC suggest that this fraction is chemically very similar if not identical to S-28.

Although SRIF has important physiologic functions as a neurotransmitter and regulator of growth hormone and TRH secretion, its status as a hormone is still uncertain.[16] It may regulate the rate of nutrient entry from the intestine[17] and the demonstration that physiologic concentrations of SLI may suppress insulin and glucagon secretion is consistent with a possible hormonal role for the peptide.[18] In the present study, we have demonstrated that the circulating concentrations of SLI^{28} and SLI^{14} are similar. In view of the demonstration that S-28 has greater potency than S-14 in inhibiting both endocrine[5] and exocrine[19] secretions, the plasma levels of SLI^{28} which we have detected may have important physiologic effects.

Many biologic differences between S-28 and SRIF have been described. Thus S-28 has greater potency in some physiologic systems,[5] a more prolonged duration of action in others[9] and an ability to bind to tissue receptors with a specificity different from S-14.[20] The relative rates of secretion of S-28 and S-14 into the circulation may therefore regulate the action of these peptides. Clearly, further studies are needed to define the factors which influence the rate and site of release of S-28 and the contribution of SLI^{28} to total plasma SLI. However, the identification of S-28 as a circulating form of SLI raises the possibility that it may be a true hormone, with physiologic functions and rates and sites of secretion discrete from SRIF.

ACKNOWLEDGMENTS

This work was supported in part by the Diabetes Research and Training Center (AM 20595). Dr. Polonsky is the recipient of the Solomon A. Berson Research and Development Award of the American Diabetes Association and a Research grant from the Juvenile Diabetes Foundation.

REFERENCES

[1] Pradayrol, L., Jornvall, H., Mutt, V., and Ribet, A.: N-terminally extended somatostatin: the primary structure of somatostatin-28. FEBS Lett. *109*: 55–58, 1980.

[2] Esch, F., Bohlen, P., Ling, N., Benoit, R., Brazeau, P., and Guillemin, R.: Primary structure of ovine hypothalamic somatostatin-28 and somatostatin-25. Proc. Natl. Acad. Sci. USA 77:6827–31, 1980.

[3] Schally A. V., Huang, W. Y., Chang, R. C., Arimura, A., Redding, T., Millar, R. P., Hunkapillar, M. V., and Hood, L. E.: Isolation and structure of prosomatostatin: a putative somatostatin precurser. Biochemistry 77:4489–93, 1980.

[4] Mandarino, L., Reach, G., Itoh, M., Blanchard, W., Stenner, D., and Gerich, J.: Evidence for pancreatic content, release and preferential activity of somatostatin-28: possible mechanism for selective inhibition of insulin and glucagon secretion. Abstr. 329 63rd Annual Meeting of the Endocrine Society, 1981.

[5] Meyers, C. A., Murphy, W. A., Redding, T. W., Coy, D. H., and Schally, A. V.: Synthesis and biological actions of prosomatostatin. Proc. Natl. Acad. Sci. USA 77:6171–74, 1980.

[6] Polonsky, K. S., Jaspan, J. B., Berelowitz, M., Emmanouel, D. S., Dhorajiwala, J., and Moossa, A. R.: The hepatic and renal metabolism of somatostatin-like immunoreactivity: simultaneous assessment in the dog. J. Clin. Invest. *68*:1147–49, 1981.

[7] Salacinski, P., Hope, J., McClean, C., Clement Jones, V., Sykes, J., Price, J., and Lowry, P. J.: A new simple method which allows theoretical incorporation of radio-iodine into proteins and peptides without damage. J. Endocrinol. *81*:131P, 1979.

[8] Vinik, A. I., Levitt, N. S., Pimstone, B. L., and Wagner, L.: Peripheral plasma somatostatin-like immunoreactivity responses to insulin hypoglycemia and a mixed meal in healthy subjects and in non-insulin-dependent maturity-onset diabetics. J. Clin. Endocrinol. Metab. *52*:330–37, 1981.

[9] Polonsky, K., Jaspan, J., Berelowitz, M., Pugh, W., Moossa, A., and Ling, N.: Submitted for publication.

[10] Patel, Y. C., Wheatley, T., Fitz-Patrik, D., and Brock, G.: A sensitive radio-immunoassay for immunoreactive somatostatin in extracted plasma: measurement and characterization of portal and peripheral plasma in the rat. Endocrinology *107*:306–13, 1980.

[11] Utsumi, M., Makimura, M., Morita, S., and Baba, S.: Determination of immunoreactive somatostatin in rat plasma and responses to arginine, glucose and glucagon infusion. Diabetologia *17*:319–323, 1979.

[12] Berelowitz, M., Kronheim, S., Pimstone, B., and Shapiro, B.: Somatostatin-like immunoreactivity in rat blood. J. Clin. Invest. *61*:1410–16, 1978.

[13] McMartin, C., and Purdon, G. E.: Early fate of somatostatin in the circulation of the rat after intravenous injection. J. Endocrinol. *77*:67–74, 1978.

[14] Marki, F., Schenkel, L., Petrack, B., Cyernick, A. J., Ansell, J., Allen, M., Brundish, D. E., Martin, J. R., McMartin, C., Peters, G. E., and Wade, R.: Rapid conversion of somatostatin to active metabolite in human plasma. FEBS. Lett. *127*:22–24, 1981.

[15] Makes, K., Itoh, M., Greene, K., and Gerich, J.: Radioimmunoassay of human plasma somatostatin. Diabetes *30*:728–35, 1981.

[16] Luft, R., Efendic, S., and Hokfelt, T.: Somatostatin—both hormone and neurotransmitter? Diabetologia *14*:1–12, 1978.

[17] Schusdziarra, V., Zyznar, E., Rouiller, D., Boden, G., Brown, J. C., Arimura, A., and Unger, R. H.: Splanchnic somatostatin: a hormonal regulator of nutrient homeostasis. Science *207*:530–32, 1980.

[18] Zyznar, E. S., Pietri, A. O., Harris, V., and Unger, R. H.: Evidence for the hormonal status of somatostatin in man. Diabetes *30*:883–86, 1981.

[19] Susini, C., Esteve, J. P., Vaysse, N., Pradayrol, L., and Ribet, A.: Somatostatin 28: effects on exocrine pancreatic secretion in conscious dogs. Gastroenterology *79*:720–24, 1980.

[20] Srikant, C. B., and Patel, Y. C.: Receptor binding of somatostatin-28 is tissue specific. Nature *294*:259–60, 1981.