Somatostatin-like Immunoreactivity in Human Peripheral Plasma Measured by Radioimmunoassay Following Affinity Chromatography

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SUMMARY

Somatostatin-like immunoreactivity (SLI) concentrations were determined in human peripheral plasma using affinity chromatography followed by radioimmunoassay. In normal subjects, fasting SLI ranged from 2.9 to 22.0 pg/ml with a mean ± SE value of 10.2 ± 2.1 pg/ml. In totally pancreatectomized or gastrectomized patients, fasting SLI levels were not different from the values in normal subjects. In patients with medullary thyroid carcinoma, fasting SLI ranged from 11.8 to 71.0 pg/ml with a mean of 29.3 ± 12.3 pg/ml, which was significantly higher than normal values ($P < 0.01$). Following meal ingestion, plasma SLI increased significantly in normal subjects from a basal level of 9.1 ± 2.1 pg/ml to a peak value of 15.4 ± 2.9 pg/ml ($P < 0.02$). These results indicate that radioimmunoassay combined with affinity chromatography provides an accurate method of measuring SLI in human plasma.

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The mechanism of regulation of somatostatin secretion from the pancreas and stomach of animals has recently been studied by in vitro techniques.[1–4] However, little is known about the factors influencing somatostatin secretion in man because of difficulties in the measurement of plasma somatostatin, as pointed out previously.[5,6] Widely differing normal plasma levels[6–9] of somatostatin-like immunoreactivity (SLI) so far reported might result at least in part from the interference of antigen-antibody reaction by substances in plasma. The present study was, therefore, undertaken to measure SLI in human plasma with sensitive radioimmunoassay following partial purification by immunoaffinity chromatography.

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MATERIALS AND METHODS

PLASMA SAMPLES

Fasting SLI levels. Ten normal subjects who were neither obese nor had a family history of diabetes (six men and four women, age 24–40 yr), four totally pancreatectomized patients (two men and two women, age 40–54 yr), and five totally gastrectomized patients (three men and two women, age 40–60 yr) were studied. Five patients with medullary thyroid carcinoma (two men and three women, age 35–58 yr) were also studied.

Meal ingestion test. Seven normal subjects (five men and two women, age 24–40 yr) ingested a 280-cal meal consisting of 33 g carbohydrate, 10 g protein, and 12 g fat. Blood was withdrawn before and then again 10 and 30 min after the ingestion. The statistical analysis was performed by either Student's *t* test or paired *t* test.

All experiments were performed after an overnight fast and at a resting state. Blood (20 ml) was withdrawn into a heparinized disposable plastic syringe from the antecubital vein and transferred into chilled tubes containing 1000 U of Trasylol and 1.2 mg of EDTA per ml of blood. The mixture was immediately centrifuged at 4°C and plasma was separated and stored at −20°C for the measurement of SLI.

AFFINITY CHROMATOGRAPHY

The immunoglobulin G (IgG) fraction of antiserum T316, raised in a rabbit against synthetic cyclic somatostatin coupled to BSA, was immobilized on a cyanogen bromide-activated Sepharose 6B column, according to the recommendations of the manufacturer,[10] and the conjugate was packed in a column with a bed volume of 5 ml. Plasma samples (10 ml) obtained from a peripheral vein were diluted with an equal volume of phosphate-buffered saline (PBS: pH 7.4), and the insoluble material was removed by centrifugation. The supernate was applied at 4°C to the column (1.6 × 2.5 cm) of the immobilized antibodies at a flow rate of 3–4 ml/min. According to the method of Harris et al.,[11] the column was washed with 40 ml of PBS, after which 7.5 ml of 1 M sodium chloride adjusted to pH 10.4 was applied, fol-

lowed by 7.5 ml of water. The SLI was eluted with 15 ml of 1 M acetic acid. All fractions were lyophylized and reconstituted with assay buffer when assayed.

RECOVERY STUDIES

First, 100 or 200 pg of synthetic somatostatin was added to 10 ml plasma and the mixture was applied to the affinity column. The final concentration of the added somatostatin was 10 or 20 pg/ml. The recovery rate of the somatostatin added was calculated by subtracting the amount of endogenous SLI measured in a parallel assay. Second, 100 or 200 pg of synthetic somatostatin was added to 10 ml PBS rather than plasma and the mixture was subjected to affinity chromatography as before. We then examined whether or not SLI is eluted from the affinity column when it is washed with acetic acid in the absence of plasma or synthetic somatostatin.

GEL CHROMATOGRAPHY

A 1-ml aliquot of the fraction excluded from the immunoaffinity column was acidified with 0.2 N acetic acid either directly or after being boiled in a water bath. The supernatant, obtained after centrifugation, was placed on a Sephadex G-25 column (1 × 55 cm), equilibrated, and eluted with 0.2 N acetic acid containing 0.2% bovine serum albumin. All fractions, including those around the elution position of synthetic somatostatin, were lyophylized and reconstituted with assay buffer when assayed.

RADIOIMMUNOASSAY

Immunoreactive somatostatin was determined by the dextran-coated charcoal radioimmunoassay described previously.[12] The minimum detectable quantity of this assay is 10 pg/ml.

ANTISERUM SPECIFICITY

To determine the specificity of antiserum T316 used for radioimmunoassay and affinity chromatography, a wide range of hypothalamic, pituitary, and gastrointestinal hormones were tested. In addition, a number of somatostatin analogues were tested in an attempt to clarify the antigenic determinant of T316.

RESULTS

Elution profiles of affinity chromatography. The elution profiles of plasma SLI subjected to affinity chromatography revealed 2 or 3 peaks of immunoreactivity. Fifteen of 24 cases showed first, second, and last peaks, whereas the others showed only first and last peaks. Representative elution patterns are shown in Figure 1. As shown in the upper panel, three peaks appeared in case 1. The first peak appeared when the column was washed with PBS and was considered to be fractions not bound to the column. The second peak appeared when the column was washed with 1 M NaCl and water. These fractions were thought to be bound to the column nonspecifically. The last peak appeared after the application of 1 M acetic acid. Case 2 showed only two peaks, first and last, as illustrated in the lower panel of Figure 1. In order to detect somatostatin in the first or second peak excluded from the immunoaffinity column, the first or second peak was acidified either directly or after being boiled and then subjected to gel chromatography. However, no peaks of SLI were observed in either experiment.

When synthetic somatostatin was applied to the immunoaffinity column in the absence of plasma, it was eluted as a single peak represented by the shaded area which corresponds to the last peak of the elution profile of plasma.

Synthetic somatostatin (100 or 200 pg) was added to 10 ml plasma and the mixture was applied to the affinity column. The final concentration of added somatostatin was 10 or 20 pg/ml; the recovery rate of somatostatin added with plasma was 90 ± 2% (mean ± SE) and 91 ± 2%, respectively, as calculated by subtracting endogenous SLI measured in a parallel assay. The recovery rate of 10 or 20 pg/ml somatostatin added without plasma was 92 ± 3% and 93 ± 2%, respectively. In the later experiments, therefore, we measured only the fractions that were eluted with 1 M acetic acid as SLI. They accounted for less than 50% of the total immunoreactivity. SLI was not eluted from the affinity column when it was washed with acetic acid in the absence of plasma or synthetic somatostatin.

Fasting SLI levels (Figure 2). Fasting peripheral plasma SLI levels in the 10 normal subjects ranged from 2.9 to 22.0

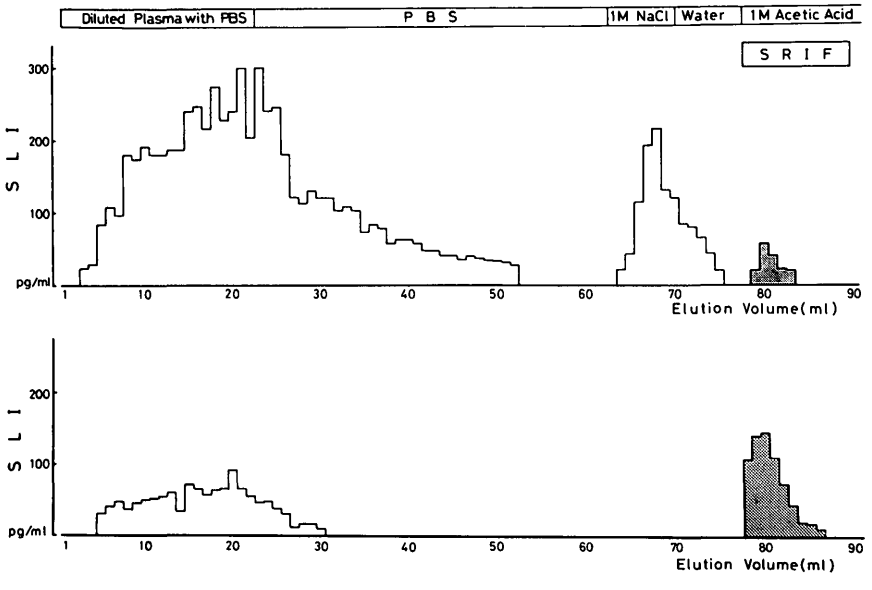

FIGURE 1. Elution patterns of SLI in human peripheral plasma fractionated by the immunoaffinity chromatography. Two representative patterns are shown. Two or three peaks of immunoreactivity appeared. The first peak appeared when the column was washed with PBS. The second peak in the upper panel appeared when the column was washed with 1 M NaCl and water. The last peak appeared after the application of 1 M acetic acid. When synthetic somatostatin was applied to the column without plasma, it was eluted as a single peak represented by the shaded area.

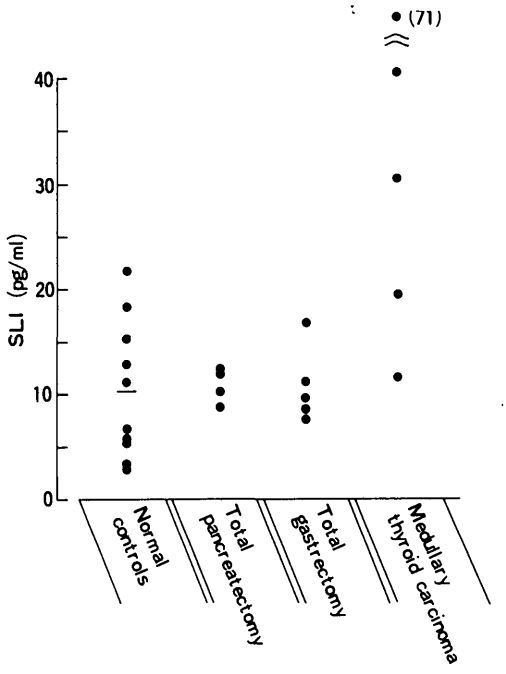

FIGURE 2. Basal plasma SLI levels in normal subjects and patients after either total pancreatectomy or gastrectomy, and patients with medullary thyroid carcinoma.

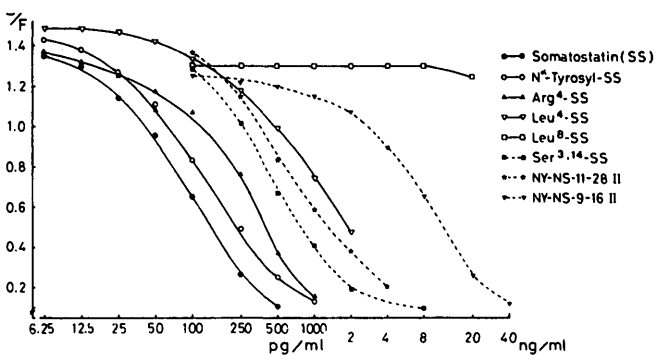

FIGURE 3. Dose-response curves of various somatostatin-related peptides.

pg/ml with a mean ± SE of 10.2 ± 2.1 pg/ml; from 9.0 to 12.0 pg/ml with a mean of 10.3 ± 0.7 pg/ml in the four totally pancreatectomized patients; from 7.0 to 15.3 pg/ml with a mean of 10.1 ± 1.5 pg/ml in the five totally gastrectomized patients; and from 11.8 to 71.0 pg/ml with a mean of 29.3 ± 12.3 pg/ml in the five patients with medullary thyroid carcinoma, significantly higher than the other values ($P < 0.01$). Moreover, the patient with medullary thyroid carcinoma whose fasting SLI level was 71.0 pg/ml had undergone a complete resection of the tumor and his fasting SLI level had decreased to 11.9 pg/ml.

Effect of meal ingestion on plasma SLI levels (Table 1). The plasma SLI levels were determined in the seven normal subjects following ingestion of a 280-cal meal. Ingestion of the meal elicited a rise in SLI levels from a baseline of 9.1 ± 2.1 pg/ml to 15.4 ± 2.9 pg/ml within 10 min, significantly higher than the baseline ($P < 0.02$), and reached 18.2 ± 4.6 pg/ml at 30 min.

Antiserum specificity (Figure 3). Antiserum T316, used for radioimmunoassay and affinity chromatography, had no cross-reactivity with insulin, glucagon, pancreatic polypeptide, gastrin, motilin, VIP, or substance-P. Cross-reaction with the somatostatin analogues tested showed that T316 is strongly reactive with the central portion of the somatostatin molecule.

TABLE 1
Effect of meal ingestion on plasma SLI levels in normal subjects

Patient	Plasma SLI levels (pg/ml)		
	Before	10 min	30 min
A.H.	5.4	8.7	10.0
S.S.	2.9	5.9	8.9
K.T.	12.8	15.3	11.6
J.T.	18.6	29.3	36.6
T.O.	5.8	18.6	33.4
T.A.	6.8	12.1	8.0
J.E.	11.2	17.4	19.0
Mean ± SE	9.1 ± 2.1	15.4 ± 2.9*	18.2 ± 4.6

* $P < 0.02$ versus before.

DISCUSSION

The present study demonstrates that the combination of affinity chromatography and sensitive radioimmunoassay permits valid measurement of endogenous SLI in human plasma. Normal plasma SLI levels obtained by this method were lower than those previously reported.[6-9] This difference could not be explained by low recovery of SLI in plasma, because more than 90% of synthetic somatostatin added to plasma was recovered after the affinity chromatography. Because the exact nature of endogenous circulating somatostatin is not known, different antibody may react differently with endogenous hormone.

The elution profile of plasma SLI subjected to affinity chromatography showed two or three peaks of immunoreactivity. The large peak or peaks of SLI (peak I and II) excluded from the column before the acid application might have resulted either from nonspecific interference of antigen-antibody reaction by substances in the plasma[13] or endogenous somatostatin bound to plasma protein.[14] The latter possibility seems unlikely, though it cannot be definitely excluded, because acidification of these peaks did not yield SLI of small molecular size when tested by gel chromatography. More than 50% of total SLI was eluted in peak I and II in our studies. This is in contrast with the observation of Harris et al.[11] that more than 95% of the plasma SLI was bound to the affinity chromatography column. The reason for this discrepancy is not clear, but may be attributed at least partly to the difference in animal species from which the plasma was collected and partly to the difference of antisera used for the affinity chromatography. This is because they produced antisera with synthetic cyclic somatostatin coupled to human serum albumin, whereas ours was produced with cyclic somatostatin conjugated with bovine serum albumin.

The pathophysiologic significance of peripheral somatostatin is unclear. In the present study, fasting plasma somatostatin levels in both totally pancreatectomized and gastrectomized patients were within the normal range. Schusdziarra et al.[15] have demonstrated that basal levels of SLI in depancreatized dogs also are not different from controls. It seems likely, therefore, that at least in the fasting

state neither pancreatic nor gastric SLI affects considerably the circulating SLI. An alternate explanation is that either pancreatectomy or gastrectomy might cause a change in production of SLI in other organs.

In the patients with medullary thyroid carcinoma, mean fasting SLI level was significantly higher than in normal controls. A patient who had extremely high SLI values showed normal values after the complete resection of the tumor. This finding supports the recent report[16] that the measurement of plasma SLI is useful to diagnose somatostatin-producing medullary thyroid carcinoma and the effects of treatment.

Schusdziarra et al.[17] have demonstrated that meal ingestion enhances peripheral venous SLI levels in dogs. In the present study, we have also observed that plasma SLI significantly increases after meal ingestion. Further studies should clarify the factors affecting plasma somatostatin levels not only in normal subjects but also in various disease states.

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