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Radioimmunoassay for Somatostatin and Somatostatin-like Immunoreactivity in Human Plasma

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ABSTRACT. A sensitive and specific radioimmunoassay for somatostatin is described. Anti-somatostatin sera was raised in rabbits after immunization with somatostatin-BSA conjugates. Tyr^1-somatostatin was iodinated by the chloramine-T method. In a conventional radioimmunoassay system, the antiserum was used at a final dilution of 1 : 700,000. The sensitivity of the assay was 2 pg/tube and within and between assay precisions as coefficients of variation were 8.2% (range 15-30 pg), and 5.9% (range 20-50 pg) respectively. The recovery of added somatostatin to human plasma was 51 ± 6.8% (Mean ± S. D.), using the acetone extraction method.

The mean plasma immunoreactive somatostatin level among 10 healthy subjects was 10.4 ± 4.8 pg/ml at fasting, and plasma somatostatin-like immunoreactivity(SLI) increased significantly to a peak value of 16.2 ± 7.3 pg/ml 60 minutes after a mixed meal.

Somatostatin (SRIF), a tetradecapeptide originally isolated from the ovine hypothalamus[1], is now known to have widespread effects on endocrine and exocrine secretions. Since the discovery of SRIF, radioimmunoassay (RIA) for SRIF in tissue extracts and plasma-free biological fluids has been accomplished with relative ease in a number of laboratories, but the direct determination of SRIF in plasma has proved difficult because of marked degradation of ^{125}I-Tyr^1-SRIF, the radioiodinated SRIF commonly used in RIA.

Here we describe our RIA system for SRIF, and the plasma SRIF levels after a mixed meal in healthy subjects.

MATERIALS AND METHODS

Chemicals ;

Synthetic cyclic SRIF for antibody production was purchased from Sigma Chemical Company. Synthetic cyclic SRIF (Lot 30016) for assay standard was obtained from Protein Research Foundation, Osaka, Japan. Tyr^1-SRIF, Ser-SRIF

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4-14 (des-Ala^1-Gly^2-Cys^3-[Ser]^{14}-SRIF, NY-NS 11-28), Ser-SRIF ([Ser]^{3,14}-SRIF, NY-NS-11-77) and [Leu]^8-SRIF (NY-NS-14-58) were generous gifts from Dr. N.Yanaihara, Shizuoka College of Pharmacy, Shizuoka, Japan. Mono-component porcine insulin, glucagon, motilin, big gastrin, vasoactive intestinal polypeptide (VIP) and pancreatic polypeptide (PP) were supplied by Dr. Lindholm, Novo Research Institute, and Prof. K. Kaneko and Dr. K.Kobayashi (Yamaguchi University School of Medicine). Secretin (B26BF) was obtained from Eizai, Tokyo, Japan.

Antibody Production ;

Four mg of SRIF was coupled to 4 mg of bovine serum albumin with a water soluble carbodiamide as described[2]. The coupled antigen was desalted on a Bio-Gel P-2 column, lyophilized, and dissolved in saline. After emulsion in an equal volume of complete Freund's adjuvant, it was injected into young rabbits in multiple sub- and intradermal sites (200 μg SRIF/animal). Five booster injections were given every 2-4 weeks after the initial immunization. About a week after each booster injection the rabbits were bled and the antibody titers were examined by measuring binding of ^{125}I-Tyr^1-SRIF.

Iodination of Somatostatin ;

The iodination of Tyr^1-SRIF was carried out by the reported method[3] as follows. One mCi (10 μ1) of Na ^{125}I (Radiochemical Centre) and 10 μg chloramine T (in 10 μl of H_2O) were mixed in 50 μl of 0.5 M phosphate buffer, pH 7.6. After a 45 second reaction at 24°C, Tyr^1-SRIF (4 μg in 8 μl H_2O) was added, and the solution was agitated for 15 sec. Then the reaction was interrupted by addition of 300 μl, 10% (w/v) BSA. To the iodination mixture 3 ml of 0.002 M ammonium acetate buffer pH 4.6 was added and the mixture was immediately chromatographed on a CM-cellulose (CM-52) column (0.9 × 7 cm). After the first radioactive peak eluted, the elution buffer was changed to 0.2 M ammonium acetate buffer, pH 4.6. The collected fractions were 1.5 ml each. The third radioactive peak of this column was collected and diluted to about 200,000 cpm/0.1 ml. Two or 3 ml alliquots of this solution were stored at -20°C until use.

Extraction of Plasma ;

Extraction of plasma was carried out as described by Arimura et al.[5] One ml of plasma was mixed with 2.0 ml of cold acetone, and the supernatant was collected by centrifugation. The ether layer was removed by aspiration after the supernatant was mixed with 4.0 ml of petroleum ether. The remainder was dried by N_2 gas.

Assay Procedure ;

The dried samples were dissolved in 1.8 ml of the assay buffer (0.05 M phosphate buffer, 0.25 M EDTA, 0.5% BSA, pH 7.8). Incubation tubes contained 0.8 ml sample or standard solution, 0.1 ml antiserum (1 : 70,000), 0.1 ml ^{125}I-Tyr^1-SRIF (5000 cpm), and buffer sufficient to give a final volume of 1.0 ml. Bound and free ^{125}I-Tyr^1-SRIF were separated by the dextran-coated charcoal method (charcoal 1.0 mg - dextran 0.1 mg/tube) after two days incubation at

4°C, and the precipitate was counted.

Gel filtration ;

Five ml of postprandial plasma obtained from a healthy subject was extracted as described before, and the extract was dissolved in 2.5 M propionic acid and gel filtered on a Bio-Gel P-10 column (1.6×25 cm) equilibrated with 2.5 M propionic acid. Each fraction (2.2 ml) was lyophilized and dissolved in 1.8 ml assay buffer, and then 0.8 ml of each solution was assayed for SLI in duplicate.

Meal ingestion test ;

A mixed meal containing 77 g carbohydrate, 56 g lipid, 42 g protein, about 980 cal, was given to ten healthy adult volunteers (age 21–27 y.o., five males and five females) as a breakfast. Blood samples were drawn before, and 30 min, 60 min and 120 min after the beginning of the meal. The blood samples were immediately centrifuged at 4°C, and plasma was separated and stored at −20°C until use.

TABLE 1. Plasma SLI levels after mixed meals in 10 healthy individuals

Subjects	SLI pg/ml, Before and After mixed meal			
	Fasting	30 min	60 min	120 min
1	14.9	14.9	21.6	20.3
2	7.2	6.8	13.7	9.0
3	13.5	18.0	22.5	18.0
4	8.6	9.5	17.7	4.5
5	13.5	18.9	18.9	17.6
6	6.1	13.5	4.7	10.8
7	10.4	16.7	19.4	16.2
8	5.9	13.5	8.4	4.5
9	19.4	21.6	27.0	27.0
10	4.5	5.0	7.9	13.5
Mean ± SD	10.4 ± 4.8	13.8 ± 5.4	16.2 ± 7.3	14.1 ± 7.1

$P < 0.02$

RESULTS

Separation of ^{125}I-Tyr^{1}-SRIF from the iodination mixture was performed by CM-cellulose Chromatography and three main peaks of radioactivity were consistently obtained (data not shown). The first and second peaks were not immunoreactive. The third peak showed 85.3% (mean) immunoreactivity in the presence of excess antibody. Antiserum of sufficient titer for SRIF RIA was obtained five months after the first injection. A typical dose response curve of our RIA system and the specificity of the anti-SRIF antibody are shown in Fig. 1. Displacement of ^{125}I-Tyr^{1}-SRIF by linear somatostatin analogues ([Ser]$^{3.14}$-SS, des-Ala^{1}-Gly^{2}-Cys^{3}-[Ser]^{14}-SS) but not by [Leu]^{8}-somatostatin analogue was noted. From these results, it was suggested that the determinant

of this antibody was present in the middle portion of somatostatin molecule.

Cross-reactivities with porcine insulin, glucagon, big gastrin, VIP, motilin, secretin, and PP were not found.

The mean assay sensitivity was 2 pg/assay tube, and the within assay

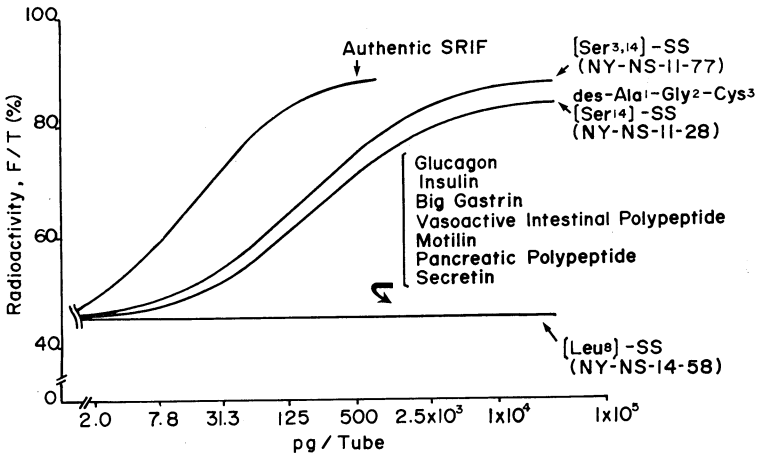

Fig. 1. Typical dose response curve of SRIF RIA and specificity of the antibody. Competition of SRIF analogues and various pancreatic and gastrointestinal hormones with ^{125}I-Tyr^{1}-SRIF is shown.

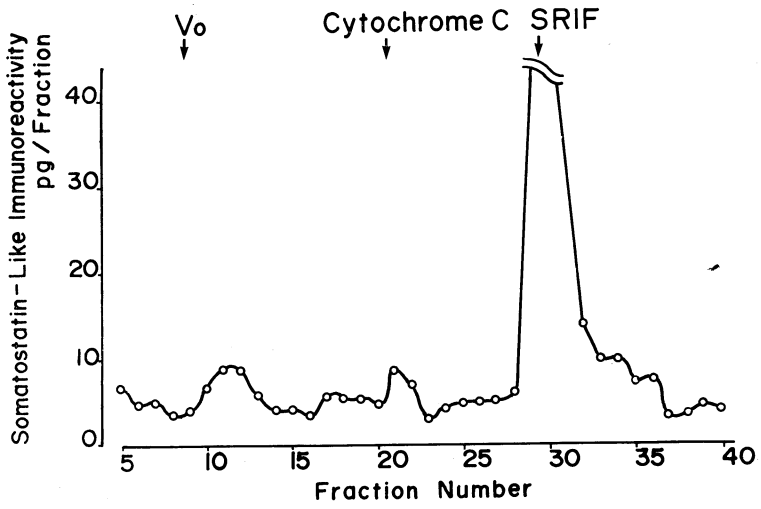

Fig. 2. Gel-filtration of SLI extracted from human plasma.
Postprandial plasma extracted from a healthy subject was gelfiltered on a Bio-Gel P-10 column (1.6×25 cm, 2.5 M propionic acid). Each fraction was assayed for SLI. Void volume (Vo) and the elution volumes of chytochrome C (MW 12327) and SRIF are indicated by arrows.

precision as a coefficient of variation was 8.2% within a range of 15–30 pg (N=5).
The interassay coefficient of variation was 5.9% within a range of 20–50 pg (N=6).

Synthetic somatostatin standard (60 pg) was added to one ml of plasma sample, then extracted and assayed. The mean recovery was 51±8.6% (N=6).

The gel filtration patterns of plasma extracts on Bio-Gel P-10 showed that most of the Somatostatin-like immunoreactivity (SLI) coeluted with synthetic Somatostatin, and other small peaks of SLI were found near the void volume and the elution fraction of cytochrome C (Fig. 2). Table 1 shows the SLI levels after the mixed meal in ten healthy volunteers. In most subjects, SLI levels after the mixed meal were higher than the fasting level. The mean SLI values at fasting (10.4±4.8 pg/ml) peaked 60 minutes after the test meal (16.2±7.3 pg/ml).

DISCUSSION

A sensitive and specific RIA for SRIF was developed. Tyr^1-SRIF was used for preparation of ^{125}I-labeled SRIF according to a modification of the method of Patel et al.[3] A CM-52 column instead of "Sephadex G-25" column was employed for ion exchange chromatographic purification of labeled SRIF, since in a preliminary experiment better separation of labeled SRIF was obtained by "CM-52". The elution pattern of ^{125}I-Tyr^1-SRIF on the CMC column was identical to the previously reported result[4]. Labeled SRIF obtained by this method was stable for about 8 weeks when stored at under −20°C.

It was suggested that our antiserum is directed to the middle portion of the SRIF molecule, and is less immunoreactive to linear SRIF analogues than to authentic SRIF.

To overcome degradation of ^{125}I-Tyr^1-SRIF, we preferred extraction of plasma by acetone as described by Arimura et al.[5] We have also confirmed that acetone extraction of SLI-free plasma did not interfere with the antigen-antibody reaction in the assay system (data is not shown).

It has been shown that plasma SLI are heterogenous, consisting of a tetradecapeptide SRIF and some larger molecular components (Kronheim, Maches, Tsuda). The exact nature of these larger components is not yet clear. The acetone extract, however, contained few larger molecular componets. Most of SLI extracted by acetone from human plasma after the mixed meal coeluted the with tetradecapeptide SRIF, providing that the RIA after acetone extraction will permit the determination of tetradecapeptide somatostatin in plasma.

In normal subjects, fasting SLI levels were 10.4±4.8 pg/ml (Mean±SD) in this study. Similar values were reported by Tsuda et al.[7] However, higher plasma SLI levels were reported by other investigators (Kronheim, Gerich, Epstein). A possible explanation is differences in the extraction methods employed.

After the mixed meal, plasma SLI increased significantly to a peak value of 16.2±7.2 pg/ml in 60 minutes. These values are also in good agreement with the results of Tsuda et al.[6] and Zyznar et al.[7] Postprandial rise of plasma SLI is generally recognized (Tsuda, Zyznar), but its physiological role remains to be verified.

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