Immunological properties of novel somatostatin analogs

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ABSTRACT

Cross-reactivity of six novel somatostatin (SRIF) analogs has been examined by SRIF-specific radioimmunoassays using anti-SRIF sera OAL-272 or OAL-283. The analogs examined possessed different chain length in which the S-S linkage was replaced by a methylene bridge of α -amino suberic acid (D-Asu). In the assay using antiserum OAL-272, the labeled SRIF failed to displace any of the six analogs, whereas in the assay with antiserum OAL-283, five of the analogs with the exception of the shortest analog (Phe-D-Trp-Lys-Thr-D-Asu) were bound very weakly (0.01-0.06% vs. SRIF). The results indicate that antisera specific to each of the analogs will be required for assays of these analogs in biological fluids.

Somatostatin (SRIF) suppresses the secretion of various pancreatic (8, 9) and gastric hormones (3, 11, 12). Because of its short biological half life, efforts have been made to develop SRIF analogs with longer half life and selective suppressive effect on hormone secretion. Synthetic SRIF analogs, SMS 201-995, was found to be more potent than SRIF in its specific inhibition of growth hormone (GH) and glucagon secretion (2). Recently, we have reported the synthesis of six novel SRIF analogs and some of their biological activity (10).

In the present study, cross-reactivity of these six analogs has been investigated in SRIF-specific radioimmunoassays using two antisera, OAL-272 and OAL-283; the former is more specific to cyclic SRIF than the latter (7).

SRIF and SRIF analogs shown in Fig. 1 were synthesized by the liquid phase procedure as described (10). Homogeneity of each peptide was confirmed by amino acid analysis, elemental analysis and high performance liquid chromatography (HPLC). The purity of each peptide was over 98% when determined by HPLC. Two SRIF antisera, OAL-272 and OAL-283 (Otsuka Assay Laboratory, Tokushima, Japan), were used.

Antiserum OAL-272 does not cross-react with linear SRIF, (Ser³, Ser¹⁴)SRIF and des-Ala¹, Gly²(Ser³, Ser¹⁴)SRIF, whereas antiserum OAL-283 does not recognize the disulfide linkage and even reacts with the linear form (7). ¹²⁵I-Tyr¹-SRIF (10,000 cpm; New England Nuclear, Boston, U.S.A.) was used as labeled antigen. Two titration curves of anti-SRIF antisera against SRIF were similar in slope.

Radioimmunoassay for SRIF and its analogs was performed using 0.1 ml ¹²⁵I-Tyr¹-SRIF (8,000-10,000 cpm), 0.1 ml of sample or standard, and 0.1 ml of antiserum OAL-272 or OAL-283 at a final dilution of 1:50,000, in 0.4 ml of 0.05 M sodium phosphate buffer, pH 7.5 (6).

Separation of antibody-bound and free labeled hormone was carried out by centrifugation after mixing the above reaction mixture with 0.5 ml of 0.1% charcoal (Norit A) coated with 0.1% dextran T-70 in 0.05 M sodium phosphate buffer. Normal sheep serum (0.1 ml) was added in all tubes. After incubation at 4°C for 40 min, the tubes were centrifuged at 2,500 rpm at 4°C for 20 min, and the charcoal-adsorbed free ¹²⁵I-Tyr¹-SRIF was

D-Asu : D- α -aminosuberic acid

Fig. 1 Chemical structures of somatostatin (SRIF) and its six analogs

counted in an autogamma counter (6).

The relative immunoreactive potency of the SRIF and SRIF analogs is shown in Table 1. Potency of SRIF or analog showing 50% maximal displacement of ^{125}I -Tyr¹-SRIF was expressed in percent of the respective amount of SRIF. All the six analogs were essentially non-immunoreactive in the concentration range of 0–5,000 ng/ml against antiserum OAL-272. With OAL-283, analog 2 was non-reactive, while other five analogs 1, 3, 4, 5 and 6 showed weak cross-reactivity (0.01–0.06%), *i.e.* 4,000–10,000 times less sensitive than SRIF.

The results suggest that replacement of S-S linkage with C-C linkage made the analog to be completely different from native SRIF in an immunological sense.

Although (Ser³,Ser¹⁴)SRIF and des-Ala¹, Gly²(Ser³,Ser¹⁴)SRIF were reported to react with OAL-283 antiserum, analog 1 which cyclized with α -amino suberic acid (5) had no reactivity with this antibody. On the other hand, antiserum OAL-272 has been reported to recognize the N-terminal amino acid residue, Ala¹-Cys³. It is reasonable that the present six analogs which have no Ala¹-Cys³ residue at their N-termini did not react with anti-

Table 1 *Relative Immunoreactivity and Biological Activity of SRIF and Various SRIF Analogs*

	Relative immunoreactivity (%) ^a		Biological activity (%)
	OAL-272	OAL-283	
Cyclic SRIF	100	100	100
Analog 1	—	0.01	88*
Analog 2	—	—	53*
Analog 3	—	0.023	103*
Analog 4	—	0.011	68*
Analog 5	—	0.06	104*
Analog 6	—	0.026	81*

^aAmount of SRIF or analog showing 50% maximal displacement of ¹²⁵I-Tyr¹-SRIF was used for the expression of immunological potency. This dose is inversely proportional to the immunological potency. ^bBiological activity of SRIF was studied at 40 µg/kg/h of SRIF and SRIF analogs for the suppression of GH. The effect of SRIF was defined as 100%. **P* < 0.05 vs. control saline (Student's *t*-test)

serum OAL-272.

Antiserum OAL-283, which recognizes mainly the residue of Asn⁵-Phe¹¹, seems to have properties similar to antiserum A101 (14), R149 (14), R141E (1) and 486 (4). Using the antiserum 486, Gerich and co-workers have reported that residues 5, 8, 11 and 13 may be important as the antigenic determinant, since substitutions at these residues resulted in a complete loss of immunoreactivity (4).

Analog 2 barely reacted with both antisera OAL-272 and OAL-283, and also lacked the biological activity (10). The Phe⁷-Thr¹⁰ sequence is thought to be a binding site for the biological receptor (13).

Although analogs 1 and 5 had broad inhibitory effects and analogs 4 and 6 had specificity for GH or GH and gastrin secretion (10), no apparent difference in the cross-reactivity of these analogs was observed. It is natural that biologically and immunologically active sites are different each other in the peptide hormone. It is however noteworthy that structural change with C-C modification resulted in a complete loss of the cross-reactivity in the present radioimmunoassays. The analogs having C-C linkage may be useful for demonstrating the suppression of endogenous SRIF release by SRIF analog itself, and also antisera specific for the respective analogs will be required for the measurements of the analogs in biological fluids.

The present study indicates that the replacement of the disulfide linkage in SRIF with C-C linkage results in immunologically inert, but biologically active peptide.

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