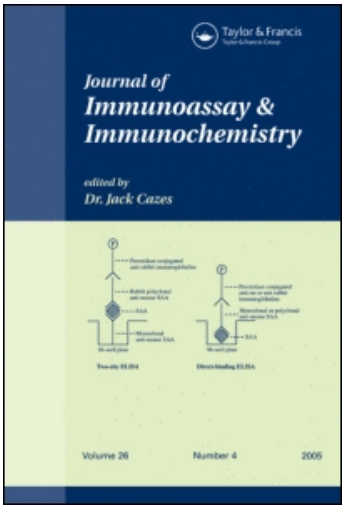

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EFFECTS OF DIFFERENT RADIOLIGANDS ON THE ANTIGEN BINDING SPECIFICITY OF SOMATOSTATIN ANTISERA

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ABSTRACT

The regions of the somatostatin (SRIF) molecule recognized by five antisera were systematically studied using three radioligands (^{125}I-N-Tyr-SRIF, [^{125}I-Tyr^{1}]-SRIF and [^{125}I-Tyr^{11}]- SRIF) and SRIF analogs containing sequential substitutions with alanine or tyrosine. Antisera SA and moreso SB had N-terminal specificity when used with [^{125}I-Tyr^{11}]-SRIF but central molecular specificity when studied with the two N-terminal radiolabelled analogs. The N-terminal and central specific populations of antibodies in antiserum SB were separable by immunoaffinity adsorption using immobilized [Tyr^{1}]-SRIF. It is of practical significance that the same antiserum (SB) could be used with different radioligands to perform N-terminal and central specific radioimmunoassays (RIAs). The central specific RIA detected SRIF-14 and SRIF-28 on an approximately equimolar basis whereas the N-terminal specific RIA was selective for SRIF-14.

INTRODUCTION

Because somatostatin (SRIF) contains no tyrosine or histidine residue that can be radioiodinated, a number of tyrosine-substituted analogs, such as [Tyr^{1}]-SRIF (1-4),

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N-Tyr-SRIF (5-7), [Tyr^{11}]-SRIF (8-10) and [Tyr^{8}]-SRIF (11) have served as radioiodinatable ligands for SRIF radioimmunoassays (RIAs). Radioimmunoassays using either of the two N-terminal radiolabelled analogs have recognized the central region of the SRIF molecule (3-5,8), in cases where this has been studied, whereas RIAs using [^{125}I-Tyr^{11}]-SRIF have recognized either the N-terminal (5,8,9) or central region (10). Given the fact that conventional antisera may contain several antibody species with heterogeneous binding requirements, this study asks the question whether use of different radiolabelled ligands may select for particular subpopulations of antibodies within an antiserum that will recognize different regions of the SRIF molecule.

MATERIALS AND METHODS

Materials

The sources of peptides were: SRIF (lot E1035) and [Tyr^{1}]-SRIF (lot B90424), Beckman Instruments, Inc. (Palo Alto, CA); N-Tyr-SRIF (lot 001179), SRIF-28 (lot 001865) and SRIF-25 (lot 001915), Peninsula Laboratories, Inc. (San Carlos, CA); [Tyr^{11}]-SRIF (lot 9813) and dihydro SRIF (lot R 2264), Bachem, Inc. (Marina Del Ray, CA); alanine-substituted analogs of SRIF, Dr. J. Rivier, Salk Institute (La Jolla, CA). [^{125}I]NaI (100 mCi/ml) was purchased from Amersham/Searle (Arlington Heights, IL). Bovine serum albumin (BSA), RIA grade, was obtained from Sigma Chemical Co. (St. Louis, MO).

Antisera SA and SB were raised in a female and a male sheep, respectively, against synthetic SRIF mixed with methylated BSA (4). Three rabbit antisera were obtained as follows: i. RA was raised against synthetic SRIF conjugated to bovine thyroglobulin by carbodiimide (12); ii. RB, purchased from Immunonuclear, Inc. (Stillwater, MN), was raised against synthetic SRIF conjugated to keyhole limpet hemocyanin by carbodiimide (rabbit 7747, catalog no. 20H2, lot no. 31100); iii. RC was induced against synthetic SRIF mixed with methylated BSA (2).

Methods

[Tyr^1]-SRIF, N-Tyr-SRIF and [Tyr^{11}]-SRIF were radioiodinated by use of chloramine-T and purified by chromatography on Sephadex G-25 (Pharmacia) by the method of Patel and Reichlin (3). The radioiodinated ligands were portioned and stored at -25°C. Peptides were dissolved in 0.01 M acetic acid/0.1% (wt/vol) BSA and stored at -25°C.

The conditions for binding studies were as previously described (4) except that the total incubation volume was 1.0 ml, BSA replaced human serum albumin, and normal carrier serum was added at the same time as precipitating second antibody. Separation of bound and free ligands was achieved using goat antirabbit or horse antisheep precipitating antiserum.

[Tyr^1]-SRIF (100 mg) was conjugated to cyanogen bromide activated Sepharose 4B (Pharmacia) by a previously reported

method (13) with the exception that the conjugation buffer was 0.1 M Na acetate/acetic acid, pH 6.0. An immunoglobulin fraction of antiserum SB was prepared by $(NH_4)_2SO_4$ precipitation as described previously (13). The SB immunoglobulins were adsorbed to the [Tyr^1]-SRIF-Sepharose conjugate by continuously circulating 9 ml of a 1/1000 dilution (in RIA buffer) of SB immunoglobulins through a 6 x 0.7 cm column of the affinity gel overnight at 4°C. The radioligand binding activity of the immunoglobulin solution was studied before and after adsorption to the affinity gel.

RESULTS

All antisera bound the three radiolabelled analogs except RC which failed to bind [^{125}I-Tyr^{11}]-SRIF (Fig. 1 and Table 1). Only in the case of antiserum SB was the titer for binding of [^{125}I-Tyr^{11}]-SRIF greater than the titer for binding of [^{125}I-Tyr^1]-SRIF or ^{125}I-N-Tyr-SRIF.

The region of the SRIF molecule required for binding of antisera were determined using the different radioligands (Fig. 2 and Table 2). Antiserum SB was directed predominantly to the sequence Lys^4-Trp^8 when [^{125}I-Tyr^1]-SRIF or ^{125}I-N-Tyr-SRIF were used whereas the antiserum bound to the N-terminal region when [^{125}I-Tyr^{11}]-SRIF was used. When antiserum SB was used with the N-terminus radiolabelled analogs, only central molecular specificity was evident. SRIF-28 and SRIF-25, which have

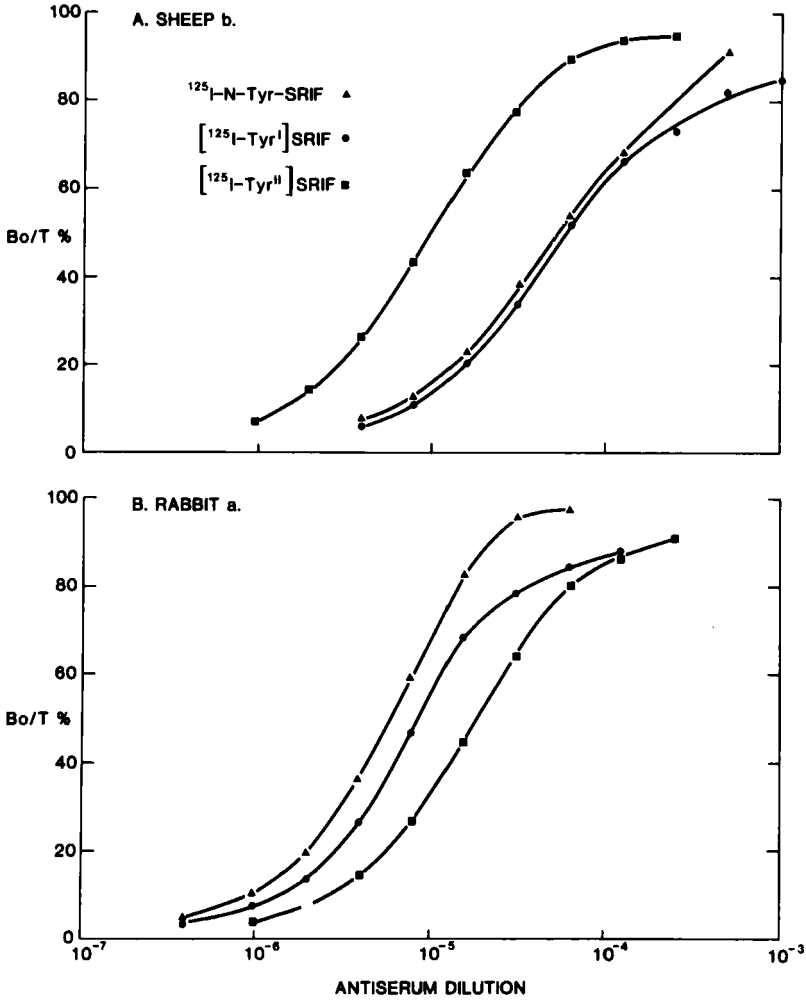

FIGURE 1. Binding of SRIF antisera (A) SB and (B) RA to the three radiolabelled SRIF analogs. Bo/T, ratio of cpm of radiolabelled analog specifically bound to antiserum to total cpm. Each point is the mean of duplicate determinations.

TABLE 1

Titer (x 10^{-3}) of Antiserum Binding to Radioligands.

Antiserum	[^{125}I-Tyr^{1}] SRIF	^{125}I-N-Tyr-SRIF	[^{125}I-Tyr^{11}] SRIF
Sheep A	50	33	2.2
Sheep B	17.2	19.2	100
Rabbit A	116	192	54
Rabbit B	153	160	62.5
Rabbit C	2.2	3.2	<0.1

Titer, reciprocal of dilution of antiserum that specifically bound 50% of radioligand.

extensions from the N-terminus of SRIF, displayed greatly reduced immunoreactivity when antiserum SB or, less so, SA was used with [^{125}I-Tyr^{11}]-SRIF compared to [^{125}I-Tyr^{1}]-SRIF or ^{125}I-N-Tyr-SRIF. Two rabbit antisera, RA and RB, showed similar central molecular specificity (residues Phe^{6} to Phe^{11}) with use of any of the three radioligands (data not shown). Antiserum RC required integrity of amino acids Trp^{8}, Phe^{11}, Thr ^{12} and Ser^{13} for binding using either of the two N-terminal radioiodinated tracers (data not shown). The failure of antiserum RC to bind [^{125}I-Tyr^{11}]-SRIF is explained by the alterations to the binding region present in this radioligand. The site specificity resulting from use of [^{125}I-Tyr^{1}]-SRIF or ^{125}I-N-Tyr-SRIF was similar for any one of the five antisera considered.

Elution of SB immunoglobulin through the [Tyr^{1}]-SRIF-Sepharose affinity gel resulted in loss of the immunoglobulins'

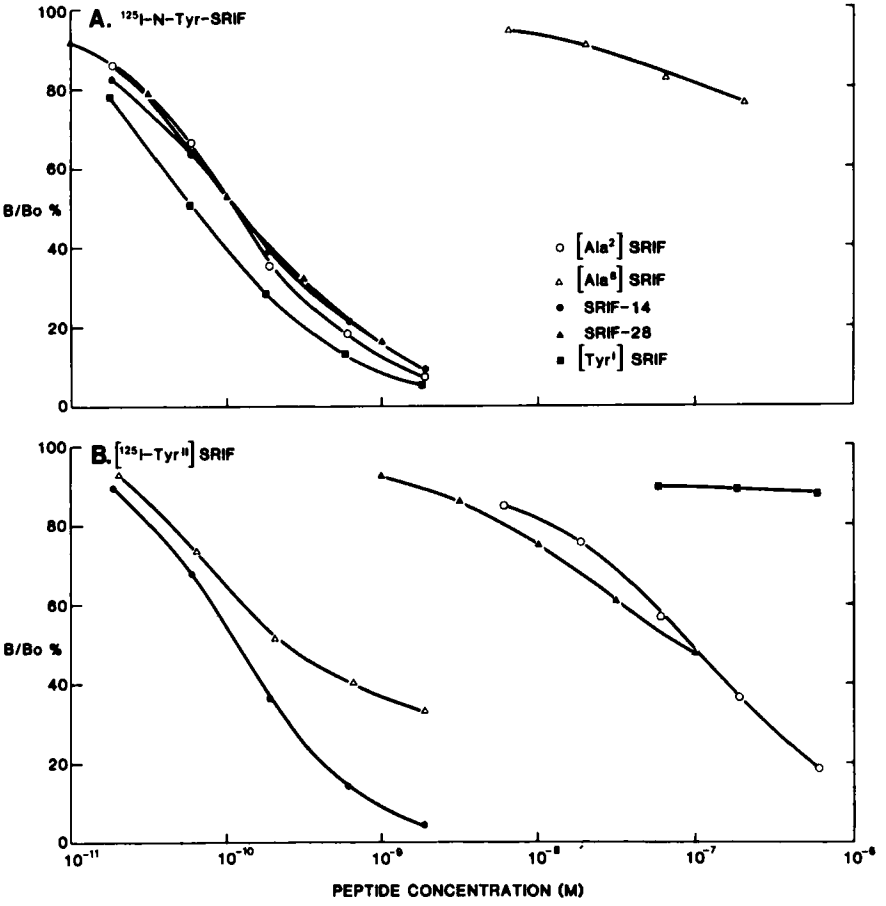

FIGURE 2. Binding of (A) ^{125}I-N-Tyr-SRIF and (B)[^{125}I-Tyr^{11}]-SRIF to antiserum SB as a function of the concentration of unlabelled SRIF analogs. B/Bo, ratio of cpm of radioligand specifically bound to antiserum in the presence of unlabelled peptide to cpm specifically bound in the absence of unlabelled peptide. Each point is the mean of duplicate determinations.

TABLE 2

ID_{50} of SRIF Analogs (M x 10^{10}).

Analog	Sheep A			Sheep B		
	[^{125}I-Tyr^{1}] SRIF	^{125}I-N-Tyr-SRIF	[^{125}I-Tyr^{11}] SRIF	[^{125}I-Tyr^{1}] SRIF	^{125}I-N-Tyr-SRIF	[^{125}I-Tyr^{11}] SRIF
SRIF-14	3.1	2.6	45	1.2	0.9	1.2
SRIF-28	3.5	2.7	470	1.2	0.9	850
N-Tyr-SRIF	3.1	2.1	210	0.9	0.7	1540
[Tyr^{1}]SRIF	1.9	1.8	4000	1.2	0.6	>5750
[Ala^{2}]SRIF	4.0	2.9	1500	1.7	1.2	900
[Ala^{4}]SRIF	3.3	2.5	180	1900	1400	4.5
[Ala^{6}]SRIF	1700	1500	2600	7470	>2000	3.4
[Ala^{7}]SRIF	>2020	>2020	>4000	1330	800	6.6
[Ala^{8}]SRIF	210	760	>4000	9300	>2000	2.3
[Ala^{9}]SRIF	860	740	1350	7.9	7.0	3.0
[Ala^{10}]SRIF	130	170	90	16	13	3.2
[Ala^{11}]SRIF	>2020	>2020	1050	4.5	2.3	115
[Tyr^{11}]SRIF	605	400	170	3.3	1.7	1.0
[Ala^{12}]SRIF	12.5	11	170	4.4	5.0	4.3
[Ala^{13}]SRIF	11	10.8	240	5.0	4.4	3.0
H_2-SRIF	12	7.8	96	5.7	3.7	6.1

ID_{50}, concentration (M x 10^{10}) of analog that displaced 50% of specifically-bound radioligand from antiserum. Antiserum dilutions were used that resulted in 50-60% specific binding of the radioligand in the absence of unlabelled peptide. The ID_{50} was determined from dose response curves for each peptide as illustrated in Fig. 2.

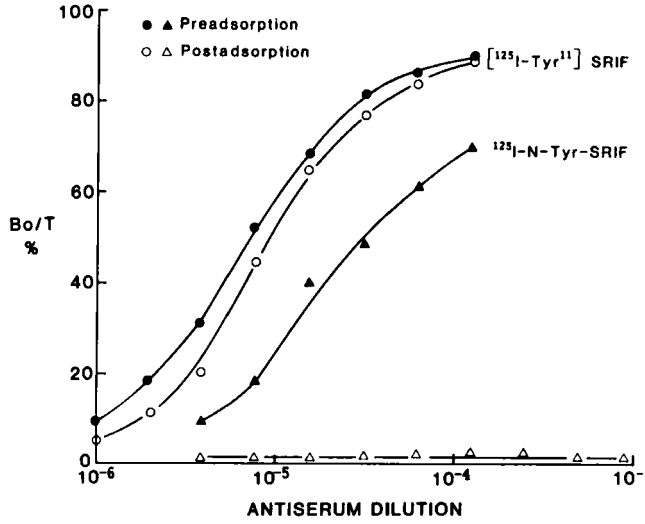

FIGURE 3. Binding of antiserum SB (immunoglobulin fraction) to [^{125}I-Tyr^{11}]-SRIF (○,●) and ^{125}I-N-Tyr-SRIF (△,▲) before and after adsorption of the antiserum to immobilized [Tyr^{1}]-SRIF. Solid symbols (●,▲), preadsorption; open symbols (○,△), postadsorption. Each point is the mean of duplicate determinations.

capacity to bind ^{125}I-N-Tyr-SRIF, indicating that the centrally reactive population of antibodies had been removed from solution (Fig. 3). The N-terminus directed binding activity of the SB immunoglobulin was only slightly reduced post-adsorption.

The nonspecific bindings pooled for experiments using all five antisera with the three radioligands were: [^{125}I-Tyr^{1}]-SRIF, 4.94 ± 0.56% (mean ± SD); ^{125}I-N-Tyr-SRIF, 3.73 ± 0.57%; and [^{125}I-Tyr^{11}]-SRIF, 2.50 ± 0.35%.

DISCUSSION

Use of different radioligands may modify the binding specificity of certain SRIF antisera, such as SA and SB, under

RIA conditions. In contrast, other antisera, such as RA and RB, recognize the same region of SRIF regardless of whether they are used with N-terminal or [Tyr^{11}] radioligands. Antiserum SB had a high titer (1:100,000) of antibodies that bound the N-terminal region of SRIF. These antibodies failed to bind to [^{125}I-Tyr^{1}]-SRIF and ^{125}I-N-Tyr-SRIF, which are considerably modified at the N-terminus. Consequently, the lower titer (approx. 1:18,000) antibodies directed against the region Lys^{4}-Trp^{8} manifested themselves by binding the N-terminus radiolabelled analogs. In the studies using [^{125}I-Tyr^{11}]-SRIF the low titer antibodies directed against the Lys^{4}-Trp^{8} region were not manifested at the high dilution of antiserum used (1:100,000). Higher titers for antiserum binding of [^{125}I-Tyr^{11}]-SRIF than [^{125}I-Tyr^{1}]-SRIF have been noted previously, including one study that used antiserum SB (9,10,14). Alteration of the specificity of a particular SRIF antiserum by use of different radioligands has not been reported.

[Tyr^{1}]-, N-Tyr-, and [Tyr^{11}]-SRIF all show substantial biological activity - 110%, 100% and 65% relative to SRIF (15) - indicating that there exists little, if any, conformational disruption of the biologically active central region of SRIF in these analogs. Iodination of these analogs could theoretically alter their conformations and reduce their ability to bind certain antisera. For instance, the binding titers of centrally-directed antisera RA and RB to [^{125}I-Tyr^{11}]-SRIF were less than to the N terminal radiolabelled ligands (Table 1).

antibodies with desired specificity from a heterogenous antiserum by immunoaffinity adsorption (19). In the present study, the N-terminal and central specific populations of antibodies in the SB antiserum were separable by immunoaffinity adsorption of the central specific antibodies to [Tyr^1]-SRIF immobilized on Sepharose. iv. Selective use of radiolabelled ligands. In addition to the present report, use of different radiolabels has modified the specificity of antisera raised to angiotensin (20), human gastrin 2-17 (21) and physalaemin (22).

In the instance where two or three of the available SRIF radioligands result in identical desirable antiserum specificities, one may consider other factors in deciding which radiolabel would be preferred for RIA: i. The titer of antiserum that can be used; ii. The sensitivity of the RIA; iii. The stability of the radioligand; iv. The nonspecific binding of the radioligand. ^{125}I-N-Tyr-SRIF may be generally preferable to [^{125}I-Tyr^1]-SRIF because of reported greater stability (5,6) and slightly lower nonspecific binding. The sensitivities to tracer displacement using the three radioligands in this study were generally comparable, with the exception of antiserum SA, although this question was not studied specifically. Others have reported equal (5) or greater (6) sensitivity with use of ^{125}I-N-Tyr-SRIF compared to [^{125}I-Tyr^1]-SRIF in RIA. In the present study [^{125}I-Tyr^{11}]-SRIF was highly stable in storage and was associated with the lowest nonspecific binding.

Because the recognition sites for RA and RB partially include Phe^{11}, substitution with tyrosine and iodination at this residue may be expected to have more effect on antiserum binding than substitution and iodination at the N-terminus.

Single amino acid substitutions may exert their effect on antibody binding by replacing essential amino acid side chain groups or by altering the peptide conformation. The aromatic amino acids Phe^{6}, Phe^{7}, Trp^{8} and Phe^{11}, which are believed responsible for maintaining the conformation of SRIF (16-18), were also the most important residues for binding of centrally directed antisera. Replacement of Trp^{8} with Ala^{8}, which substantially reduced immunoreactivity with entrally directed antisera, is known to markedly alter the conformation from that of SRIF (16).

Although very often a desired specificity of an antiserum to a peptide is achieved by chance, certain procedures may be employed in an attempt to rationally direct the specificity of an antiserum. These include: i. Immunization with the fragment of the larger peptide to which one desires antiserum specificity (19). ii. Selective conjugation of the peptide to a larger molecule so that the portion of the immunogen peptide to which one desires specificity is spatially removed from the site of conjugation and unaltered in conformation compared to the site of conjugation. This approach has generated N-terminus (8) and central specific (5) antisera to SRIF. iii. Separation of

The present observations have practical significance for the measurement of SRIF-like immunoreactive peptides in body fluids and tissues. SRIF-like immunoreactivity is comprised of SRIF-14, SRIF-28 and at least one larger form (23,24). Centrally-specific SRIF RIAs detect both SRIF-14 and the larger molecular weight forms whereas N-terminal specific RIAs are selective for SRIF-14 (23). N-terminal specificity of an antiserum may be masked if one exclusively uses [^{125}I-Tyr^{1}]-SRIF or ^{125}I-N-Tyr-SRIF, whereas use of [^{125}I-Tyr^{11}]-SRIF may reveal N-terminal specificity in some antisera. The potential to perform both N-terminal and central specific SRIF RIAs greatly enhances the utility of such an antiserum.

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