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Structural Analysis and Functional Role of the Carbohydrate Component of Somatostatin Receptors*

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SRIF receptors are membrane-bound glycoproteins. To structurally identify the carbohydrate components of SRIF receptors, solubilized rat brain SRIF receptors were subjected to lectin affinity chromatography. Solubilized SRIF receptors specifically bound to wheat germ agglutinin-lectin affinity columns but not to succinylated wheat germ agglutinin. This finding, as well as the ability of the solubilized receptor to interact with a *Sambucus nigra* L. lectin affinity column suggested that sialic acid residues are associated with SRIF receptors. The inability of the receptor to bind to concanavalin A, *Dolichus biflorus* agglutinin, *Ulex europeaus* I, and Jacalin lectin affinity columns suggests that high mannose, *N*-acetylgalactosamine, fucose, and *O*-linked carbohydrates are not associated with receptor. To investigate the functional role of the carbohydrate groups in brain SRIF receptors, specific sugars were selectively cleaved from SRIF receptors and the subsequent effect on the specific high affinity binding of the agonist [^{125}I]MK 678 to SRIF receptors was determined. Treatment of the receptor with endoglycosidase D did not affect the specific binding of [^{125}I]MK 678 to the solubilized SRIF receptors, consistent with the finding from lectin affinity chromatography that high mannose-type carbohydrate structures were not associated with SRIF receptors. Treatment of solubilized SRIF receptors with peptide-*N*-glycosidase F and endoglycosidases H and F reduced [^{125}I]MK 678 binding to SRIF receptors indicating that either hybrid, or a combination of hybrid and complex *N*-linked carbohydrate structures, have a role in maintaining the receptor in a high affinity state for agonists. Treatment of solubilized SRIF receptors with neuraminidase from *Vibrio cholera* abolished high affinity agonist binding to the receptors, whereas treatment of the receptor with neuraminidase from Newcastle disease virus did not affect [^{125}I]MK 678 binding to the receptor. These findings suggest that sialic acid residues in an α2,6-configuration have a role in maintaining the SRIF receptor in a high affinity conformation for agonists. This is further indicated by studies on SRIF receptors in the pituitary tumor cell line, AtT-20. Treatment of AtT-20 cells in culture with neuraminidase (*V. cholera*) greatly reduces high affinity [^{125}I]MK 678 binding sites, but did not alter the maximal ability of SRIF to inhibit forskolin-stimulated cAMP accumulation in intact AtT-20 cells. This finding suggests that the desialylated SRIF receptor is functionally active and remains coupled to GTP-binding proteins, but exhibits a reduced affinity for agonists. Treatment of AtT-20 cell membranes with neuraminidase from *V. cholera* was also able to greatly reduce the affinity of SRIF receptors for [^{125}I]MK 678. However, treatment of AtT-20 cell membranes with neuraminidase in the presence of MK 678 diminished the ability of the neuraminidase to affect [^{125}I]MK 678 binding to SRIF receptors. The protection afforded the agonist occupied SRIF receptors to the effects of neuraminidase treatment suggests that the sialic acid residues involved in maintaining the receptor in a high affinity state for agonist are closely associated with the ligand-binding site. The results of these studies demonstrate for the first time that sialic acid residues, as well as certain other carbohydrate components, maintain the SRIF receptor in a high affinity state for agonists and therefore may have an important physiological role in the functional activity of SRIF receptors.

SRIF[1] is a cyclic tetradecapeptide found throughout the central and peripheral nervous systems, as well as in the pancreas and gastrointestinal system (1). In the central nervous system, SRIF acts as a neurotransmitter to regulate neuronal activity (2) and to facilitate release of other neurotransmitters such as dopamine, norepinephrine, and serotonin (3). Additionally, SRIF has a role in centrally mediated behaviors such as movement and cognition (1, 4). In the periphery, SRIF is the predominant physiological inhibitor of growth hormone release from cells in the anterior pituitary (5) and it blocks the secretion of both insulin and glucagon from the pancreas (6, 7).

SRIF induces its biological effects by stimulating cell surface receptors. Characterization of the physical properties of brain, pituitary, and pancreatic SRIF receptors by chemical or photocross-linking techniques have shown the size of the receptors to vary from 60 to 94 kDa (8–13). Thermos *et al.* (13) have additionally reported that the high affinity SRIF analogue [^{125}I]CGP 23996 could specifically label two receptors in the rat corpus striatum that were of the same molecular weight, but different physical charge. Purified SRIF receptors from brain (14) and a gastric tumor cell line (15) were also shown to be of different sizes, 60 and 90 kDa, respectively.

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[1] The abbreviations used are: SRIF, somatotroph release inhibitory factor; WGA, wheat germ agglutinin; ConA, concanavalin A; CHAPS, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate; Endo F, endoglycosidase F; Endo H, enoglycosidase H; Endo D, endoglycosidase D; PNGase F, peptide-*N*-glycosidase F; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

These results would suggest that physical heterogeneities exist between SRIF receptors.

Post-translational modifications have been shown to contribute to the heterogeneity of neurotransmitter and hormone receptors (16–20). In a similar manner, post-translational modifications of SRIF receptors may contribute to the observed physical heterogeneity described above. In this regard, a number of investigators have reported that SRIF receptors from brain, pituitary, and pancreas are glycoproteins. Therefore, it is conceivable that differences in carbohydrate processing may contribute to the physical heterogeneity of SRIF receptors. Previous studies have shown that SRIF receptors, affinity labeled with radioactive SRIF analogues, from brain, pituitary, and pancreatic membranes are all able to bind specifically to WGA lectin affinity columns (9, 13, 21–25), which the authors have suggested implies the presence of GlcNAc in the carbohydrate structure. Additionally, it has also been reported that SRIF receptors from pituitary and cerebrocortical membranes can specifically bind to *Ricinus communis* II lectin affinity columns, but not to ConA or lectil lectin columns (10, 21). However, to date, there has been no complete nor detailed analysis of the carbohydrate nature of SRIF receptors. Analysis of the carbohydrate composition of SRIF receptors is therefore necessary to establish whether physical heterogeneities of SRIF receptor subtypes are due to differences in carbohydrate processing.

Differences in the physical properties of SRIF receptors may also contribute to functional heterogeneity of the receptors. Brain SRIF receptors have been reported to couple to multiple cellular effector systems, including adenylylcyclase (8) and ionic conductance channels (27–30). Results from a number of studies suggest that brain SRIF receptors couple to these effector systems via pertussis toxin-sensitive GTP-binding proteins (26–30). Functionally distinct SRIF receptors have been shown to be expressed in pancreatic islet cells (6, 7, 31) and neocortical neurons (29, 30). In the pancreas, SRIF and its prohormone SRIF-28 have different rank order of potencies to inhibit glucagon *versus* insulin release (6, 7, 32), and, in neocortical neurons, they have opposing effects on a delayed rectifier K^+ current (29, 30). Variations in carbohydrate processing could be an underlying factor in the different pharmacological properties of SRIF receptor subtypes. In this regard, the carbohydrate component of SRIF receptors may have roles in determining ligand binding affinities and/or promoting the coupling of SRIF receptors to particular populations of GTP-binding proteins and specific cellular effector systems.

To investigate the carbohydrate nature of brain SRIF receptors and the role of oligosaccharides in the functional activity of SRIF receptors, we have developed a method to solubilize SRIF receptors in an active form (25). The solubilized receptors from brain retain their high affinity and specificity for SRIF agonists and remain coupled to GTP-binding proteins. Because the solubilized receptor can be solubilized in a high affinity state, it is possible to investigate the influence of carbohydrates in SRIF receptors on agonist interaction with SRIF receptors. This provides a distinct advantage over past attempts to characterize the oligosaccharide composition of SRIF receptors after covalent cross-linking of the receptor with a radioactive ligand. These past studies were unable to investigate the role of carbohydrates in high affinity agonist binding to SRIF receptors, nor in the functional activity of SRIF receptors. In the present study, we have investigated the functional role of the carbohydrate moiety of solubilized high affinity SRIF receptors. These studies show, for the first time, that SRIF receptors contain hybrid, or a combination of hybrid and complex, oligosaccharide structures and that particular carbohydrate groups, specifically sialic acid residues, play a critical role in the binding of agonists to SRIF receptors. The SRIF receptors are the first neurotransmitter receptors for which carbohydrate groups play a fundamental role in the functional activity of the receptors.

EXPERIMENTAL PROCEDURES

Materials

Agarose-bound lectins were obtained from Vector Laboratories (Burlingame, CA). Purified *Vibrio cholera* neuraminidase was purchased from Behring Diagnostics, α-Fucosidase from Oxford Glycosystems (Rosedale, NY), and all other glycosidases from Boehringer Mannheim Biochemicals. CHAPS and bicinchoninic acid protein assay reagents were purchased from Pierce Chemical Co. and D-Trp^8-SRIF from Bachem (Torrence, CA). Newcastle disease virus was a gift from Dr. Robert Eckroade, University of Pennsylvania Veterinary School.

Solubilization of the SRIF Receptor

The solubilization of rat brain SRIF receptors was performed essentially as described by He *et al.* (25). Briefly, rat brains, minus cerebellum, were homogenized in 10 volumes of 50 mM Tris, pH 7.8, containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/ml of leupeptin, 2 μg/ml of pepstatin, 200 μg/ml of bacitracin, and 50 mM phenylmethylsulfonyl fluoride (Buffer 1). The homogenate was centrifuged at 600 × *g* for 5 min and the supernatant saved. The pellet was resuspended in 5 volumes of Buffer 1 and recentrifuged at 600 × *g* for 5 min. The two supernatants were combined and centrifuged at 45,000 × *g* for 30 min. The resulting pellet was then resuspended in Buffer 1 and centrifuged again at 45,000 × *g* for 30 min. The resulting membrane pellet was solubilized by resuspension in Buffer 1 containing 10 mM CHAPS and 20% glycerol, stirred on ice for 45 min, and centrifuged at 150,000 × *g* for 60 min. The supernatant was removed, diluted with Buffer 1, and used for radioligand binding studies of solubilized SRIF receptors. Protein content was determined by the bicinchoninic acid protein assay method (33) using bovine serum albumin as the protein standard.

[^{125}I]MK 678 Binding Assay to Solubilized SRIF Receptors

Solubilized SRIF receptors were reversibly labeled with the stable high affinity agonist, [^{125}I]MK 678, as described previously by He *et al.* (25). Specific [^{125}I]MK 678 binding was defined as total [^{125}I]MK 678 binding to solubilized SRIF receptors minus the amount bound in the presence of 1 μM D-Trp^8-SRIF. Specific [^{125}I]MK 678 binding accounted for 60–70% of total binding to solubilized SRIF receptors.

Lectin Affinity Chromatography

Agarose-bound lectin columns (1 ml bed volume) were equilibrated with 50 mM Tris, pH 7.8, containing 5 mM CHAPS and 10% glycerol. When appropriate, 1 mM $CaCl_2$ and $MgCl_2$ were added to the equilibration buffer and the proteins to be chromatographed solubilized in the absence of EGTA. All column procedures were conducted at 4 °C, except ConA chromatography, which was performed at room temperature. A 1-ml sample of solubilized brain membranes, in 5 mM CHAPS, was applied to the agarose-bound lectin and allowed to equilibrate overnight. The columns were then washed with 20 ml of equilibration buffer and 2-ml fractions were collected. The columns were then specifically eluted with 15 ml of equilibration buffer containing the appropriate sugar and 1-ml fractions were collected. All fractions were then assayed for specific [^{125}I]MK 678 binding. The sugars used to elute the glycoprotein from the lectin affinity columns were not observed to have any effect on the specific binding of [^{125}I]MK 678 to solubilized SRIF receptors. All of the lectin columns were tested with ovalbumin, α_1-acid glycoprotein, desialylated α_1-acid glycoprotein, or ribonuclease b to determine that the chromatographic conditions allowed for the selective and quantitative binding of the appropriate types of glycoproteins. CHAPS was not observed to interfere with the lectin affinity chromatography, except for ConA, which was circumvented by conducting those studies at room temperature instead of 4 °C.

Glycosidase Treatment

For glycosidase treatment, solubilized brain membranes were diluted to 2 mM CHAPS and the resulting solution concentrated 5-fold using Centricon 30 or Centriprep 30 microconcentrators (Amicon). All enzymatic treatments were for 16 h at 25 °C under the conditions described below. After the deglycosylation treatments, the solubilized membranes were assayed for specific [^{125}I]MK 678 binding. The overnight incubations in the absence of any treatment generally resulted in less than a 30% decrease in the original specific SRIF receptor binding and the enzymes themselves did not interfere with the specific binding of [^{125}I]MK 678 to solubilized SRIF receptors. Additionally, all enzymes were observed to completely deglycosylate the control glycoproteins α_1-acid glycoprotein, ribonuclease b, or ovalbumin under the treatment conditions described below (Fig. 1) and no contaminating proteolytic activity was observed. For these control studies, the glycoproteins were incubated under conditions identical to those used to deglycosylate solubilized SRIF receptors. After treatment, the glycoproteins were subjected to polyacrylamide gel electrophoresis, visualized by Coomassie Blue staining, and the change in migration of the control glycoproteins compared to previously reported changes in molecular weight after deglycosylation. As shown in Fig. 1, each glycosidase treatment resulted in sharp distinct shifts in migration of the control glycoproteins, consistent with the complete removal of the appropriate oligosaccharides, rather than incomplete cleavages of the sugars.

Endoglycosidase Digests—PNGase F (*Flavobacterium meningosepticum*) was used at a concentration of 30–60 units/ml and as shown in Fig. 1 was able to completely deglycosylate ribonuclease b. Endo D (*Diplococcus pneumonia*) was used at a concentration of 0.1 unit/ml and was able to completely deglycosylate ovalbumin. For Endo F (*F. meningosepticum*) (4.5 units/ml) and Endo H (*Streptomyces plicatus*) (0.3 unit/ml) treatments, brain membranes were solubilized as above, except that potassium phosphate buffer, pH 7.0, was used throughout. Both Endo F and Endo H were able to completely deglycosylate ribonuclease b (Fig. 1).

Exoglycosidase Treatment—β-*N*-Acetyl-D-glucosaminidase (*D. pneumonia*) was used at a concentration of 0.17 unit/ml and α-fucosidase from bovine epididymis at a concentration of 0.2 unit/ml.

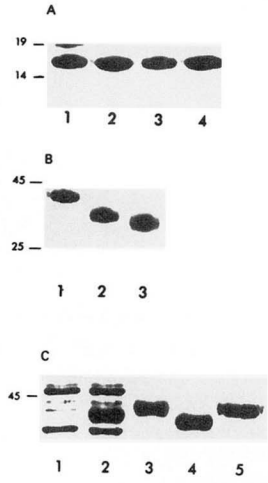

FIG. 1. **Deglycosylation of control proteins by endo- and exoglycosidases.** Control glycoproteins (0.1 mg/ml) were subjected to treatment with endo and exoglycosidases as described under "Experimental Procedures." The proteins were treated overnight at 25 °C, the reaction stopped by the addition of sodium dodecyl sulfate sample buffer. The proteins were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie Blue. *Panel A*, treatment of 0.1 mg of ribonuclease b with buffer (*lane 1*), Endo F (*lane 2*), Endo H (*lane 3*), and PNGase F (*lane 4*). The upper of the two bands in *lane 1* represents the glycosylated form of ribonuclease b and the lower band, the deglycosylated form. *Panel B*, treatment of desialylated α_1-acid glycoprotein with buffer (*lane 1*), β-galactosidase (*lane 2*), and β-galactosidase and β-*N*-acetylglucosaminidase (*lane 3*). *Panel C*, Newcastle disease virus preparation alone (*lane 1*) or treatment of α_1-acid glycoprotein with Newcastle disease virus (*lane 2*), buffer (*lane 3*), or α_1-acid glycoprotein with neuraminidase from *V. cholera* (*lane 4*) or buffer (*lane 5*). Size markers (in kDa) are indicated to the left of each gel.

For treatments with β-galactosidase (*D. pneumonia*) (0.1 unit/ml), solubilized membranes were prepared in the absence of EGTA and enzymatic treatments were also conducted in the absence of EGTA. β-Galactosidase and β-*N*-acetyl-D-glucosaminidase were able to completely remove Gal and GlcNac from desialylated- and desialylated-degalactosylated α_1-acid glycoprotein, respectively (Fig. 1).

Neuraminidase Treatment—Neuraminidase from *V. cholera* was used at a concentration of 0.17 unit/ml. Newcastle disease virus was prepared as described by Paulson *et al.* (34) and had a neuraminidase content of 0.20 unit/ml and a specific activity of 0.37 unit/mg of protein as determined by the thiobarbituric assay method of Warren (35). One unit of Newcastle disease virus neuraminidase activity was equal to 1 μmol of product/min. Newcastle disease virus neuraminidase was used at a concentration of 0.08 unit/ml. Under the assay conditions employed, neuraminidase from *V. cholera* was able to completely desialylate α_1-acid glycoprotein as shown in Fig. 1. α_1-Acid glycoprotein contains approximately 50% α2,3-linked sialic acids (34) and, accordingly, Newcastle disease virus neuraminidase was only able to remove about 50% of the sialic acid residues from α_1-acid glycoprotein as demonstrated in Fig. 1. These results are in agreement with previously published results on the types of sialic acid linkages present on α_1-acid glycoprotein and its ability to be desialylated by these two types of neuraminidases (34).

Neuraminidase Treatment of AtT-20 Cells—AtT-20 cells were grown and subcultured as previously described (14). Cells were plated in six-well culture plates at an initial density of 1×10^6 cells/well and used in the experiments 3–4 days after subculturing when the cells were 70–80% confluent. For treatment of AtT-20 cells in culture with neuraminidase from *V. cholera*, the media was replaced with 1 ml of serum-free Dulbecco's modified Eagle's medium containing either neuraminidase buffer or buffer containing 0.17 unit/ml of neuraminidase from *V. cholera* and incubated for 24 h. After incubation, the cells were removed from the plates with phosphate-buffered saline, washed 3 times with phosphate-buffered saline to remove all traces of neuraminidase, and the cells homogenized in Buffer 1. The homogenate was centrifuged at 40,000 $\times$ *g* for 20 min and the crude membrane pellet resuspended in Buffer 1. [^{125}I]MK 678 binding to the control and treated crude membranes was accomplished by vacuum filtration as previously described (25). K_d and B_{max} values were calculated from homologous displacement curves analyzed by the curve fitting program LIGAND (36).

For the effect of neuraminidase treatment on SRIF inhibition of forskolin-stimulated cAMP production, AtT-20 cells were plated and treated as above except that 24-well plates were used and the cells plated at an approximate density of 0.5×10^6 cells/well. After treatment, the cells were preincubated with 10 mM isobutylmethylxanthine for 30 min, then treated with either 50 mM forskolin alone or forskolin and 0.1 μM SRIF in the presence of 10 mM isobutylmethylxanthine for 30 min. The reaction was stopped by the removal of the culture media and the addition of 0.5 ml of 0.1 M HCl. cAMP accumulation in the control and treated cells was measured using a commercially available radioimmunoassay from Du Pont New England Nuclear following the manufacturer's directions.

For the agonist protection studies, AtT-20 cell membranes were prepared as described above. The crude membranes were preincubated for 1.5 h at 25 °C in the presence of buffer or 100 nM MK 678 to attain equilibrium with the agonist. After the preincubation treatment, 0.1 unit/ml of neuraminidase from *V. cholera* was added and the mixture allowed to incubate for an additional 5 h. The samples were then rinsed free of the MK 678 and neuraminidase by washing the membranes twice with an Hanks' balanced salt solution/sodium acetate, pH 5, buffer; a treatment which was able to completely remove any traces of agonist with no deleterious effects on the SRIF receptors (62). The washed, control and treated membranes were then measured for their ability to bind [^{125}I]MK 678 as described previously (25).

RESULTS

In previous studies (25), we have shown that rat brain SRIF receptors can be solubilized using the detergent CHAPS. The solubilized receptors retain their high affinity and specificity for SRIF agonists, including the highly stable SRIF analogue [^{125}I]MK 678. [^{125}I]MK 678 was shown to bind reversibly and with high affinity to the solubilized SRIF receptors with the binding reaction attaining equilibrium by 90 min at 25 °C. The solubilized SRIF receptors maintained their coupling to

GTP-binding proteins. This was indicated by the finding that stable GTP analogues abolished high affinity [^{125}I]MK 678 binding to solubilized SRIF receptors. Overall, the properties of [^{125}I]MK 678 binding to membrane-bound and solubilized SRIF receptors are very similar (25).

Lectin Affinity Chromatography of Solubilized SRIF Receptors—Lectin affinity chromatography has been shown to be very useful in the qualitative structural characterization of glycoproteins. Therefore, to characterize the carbohydrate moieties present in rat brain SRIF receptors, we subjected solubilized SRIF receptors to lectin affinity chromatography. The results of these studies are summarized in Table I. Under the chromatographic conditions described under "Experimental Procedures" all of the lectin affinity columns utilized in these studies were able to bind control glycoproteins selectively and quantitatively. Previously it was shown that solubilized rat brain SRIF receptors were able to specifically interact with a WGA-lectin affinity column and could be eluted with the potent, haptenic sugar *N-N′-N″*-triacetylchitotriose (25). Additionally, as shown in Fig. 2 (*top*), approximately 75% of the recovered solubilized brain SRIF receptors can be specifically eluted with GlcNAc. This specific interaction of SRIF receptors with WGA would suggest that the solubilized SRIF receptor contains poly-(GlcNAc) residues (37, 38), and/or terminal sialic acid residues (39, 40). To determine specifically which carbohydrate residues on the SRIF receptor were interacting with WGA, solubilized SRIF receptors were chromatographed on a succinylated WGA-lectin affinity column. After succinylation, WGA retains the capability to interact with poly-(GlcNAc) residues, but not sialic acid (41). In our control studies, α_1-acid glycoprotein, which interacts with WGA via sialic acid residues, bound completely to the WGA-lectin affinity column but not to succinylated WGA column, while approximately 20% of the loaded ribonuclease b (prepurified over ConA), which does not contain sialic acids, but does contain GlcNac, bound to both columns (data not shown). These results are in agreement with the previously reported binding of glycoproteins to

TABLE I

Specific binding of solubilized rat brain SRIF receptors to lectin affinity columns

Solubilized rat brain membranes were prepared as described under "Experimental Procedures." The solubilized brain proteins were diluted to 5 mM CHAPS and a 1-ml sample applied to an agarose-bound lectin affinity column (1 ml) which had been equilibrated with 50 mM Tris, pH 7.8, containing 5 mM CHAPS, 10% glycerol, and where appropriate 1 mM $CaCl_2$ and 1 mM $MgCl_2$. After an overnight equilibration the columns were washed with buffer and 2-ml fractions were collected. Each column was then specifically eluted with 15 ml of equilibration buffer containing the appropriate haptenic sugar and 1-ml fractions were collected. All fractions were then assayed for specific [^{125}I]MK 678 binding. The equivalent of an average of 60,000 cpm of specific, D-Trp^8-SRIF displaceable, [^{125}I]MK 678 binding was applied to each column. The data represents the percentage of the recovered specific [^{125}I]MK 678 binding that was specifically eluted by the haptenic sugar and each value is the mean of at least two experiments.

Column	Specifically eluted
	%
WGA	75.4
S. nigra L.	20.6
R. communis I	3.9
R. communis II	3.0
ConA	0.0
Succinylated WGA	0.0
D. biflorus	0.0
Jacalin	0.0
U. europeaus I	0.0

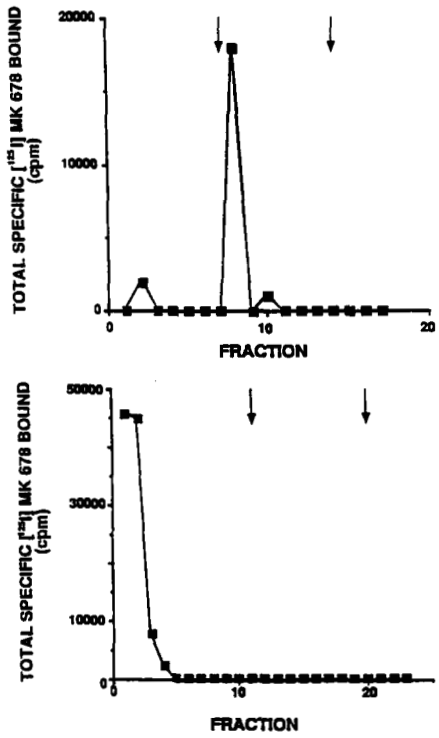

FIG. 2. **Elution profile of solubilized rat brain SRIF receptors from WGA and succinylated WGA-lectin affinity columns.** Solubilized rat brain membranes were prepared as described under "Experimental Procedures." The solubilized brain membranes were diluted to 5 mM CHAPS and a 1-ml sample applied to either an agarose-bound WGA (top) or succinylated WGA (bottom) lectin affinity column (1 ml) which had been equilibrated with 50 mM Tris, pH 7.8, containing 5 mM CHAPS and 10% glycerol. Approximately the equivalent of 80,000 and 200,000 cpm of specific [^{125}I]MK 678 binding to solubilized SRIF receptors was loaded onto the WGA and succinylated WGA columns, respectively. After an overnight equilibration the column was washed with equilibration buffer and 2-ml fractions collected. The column was then specifically eluted with 15 ml of equilibration buffer containing 0.5 M GlcNAc followed by 7 ml of 4 mM *N-N′-N″*-triacetylchitotriose. One-ml fractions were then collected. All fractions were assayed for specific [^{125}I]MK 678 binding and representative elution profiles are depicted. The *arrows* indicate the points at which 0.5 M GlcNAc and then 4 mM *N-N′-N″*-triacetylchitotriose were each added to the column.

WGA and succinylated WGA-lectin affinity columns (41). As shown in Fig. 2 (*bottom*), solubilized SRIF receptors applied to the succinylated WGA column were present only in the wash buffer and were not specifically eluted from the column by either GlcNAc or *N-N′-N″*-triacetylchitotriose, suggesting that the solubilized SRIF receptor did not bind specifically to succinylated WGA. This finding would indicate that solubilized SRIF receptors are binding primarily to WGA via its affinity for sialic acid.

Since the solubilized SRIF receptors appeared to be interacting with WGA via its interaction with sialic acids, we subjected solubilized SRIF receptors to chromatography over a *Sambucus nigra* L. lectin affinity column (Fig. 3). This lectin is able to specifically bind terminal sialic acids present in α2,6-conformations (42). In our control experiments, *S. nigra* L. lectin was able to specifically bind approximately 40% of the α_1-acid glycoprotein loaded onto the column, an amount similar to the quantity of α2,6-linked sialic acid present (34). Solubilized SRIF receptors were specifically eluted from the lectin column by lactose, suggesting the presence of α2,6-linked sialic acids in the SRIF receptors. Additionally, solubilized SRIF receptors were able to bind

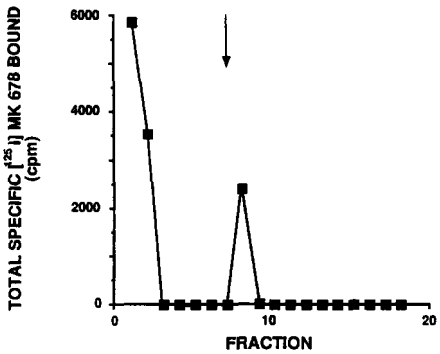

FIG. 3. **Elution profile of solubilized rat brain SRIF receptors from a *S. nigra* L. lectin affinity column.** Solubilized brain membranes were prepared as described under "Experimental Procedures." The solubilized rat brain membranes were diluted to 5 mM CHAPS and a 1-ml sample applied to an agarose-bound *S. nigra* L. lectin affinity column (1 ml) which had been equilibrated with 50 mM Tris, pH 7.8, containing 5 mM CHAPS and 10% glycerol. Approximately the equivalent of 25,000 cpm of specific [^{125}I]MK 678 binding to solubilized SRIF receptors was applied to the column. After an overnight equilibration the column was washed with equilibration buffer and 2-ml fractions were collected. The column was then specifically eluted with 15 ml of equilibration buffer containing 0.5 M lactose and 1-ml fractions collected. All fractions were then assayed for specific [^{125}I]MK 678 binding and a representative elution profile is depicted. The *arrow* indicates the point at which 0.5 M lactose was added to the column.

specifically to *R. communis* I and II lectins (Table I), albeit to a small extent, suggesting that (Gal → GlcNAc)$_n$ polysaccharides found in hybrid and complex carbohydrate structures are also present (43, 44). This result is consistent with a previous report where only a small fraction of photocross-linked SRIF receptors from cerebrocortical membranes were specifically eluted from a *R. communis* II column (10). As shown in Table I, there appeared to be no specific binding of solubilized SRIF receptors to ConA, *Dolichos biflorus* agglutinin, *Ulex europeaus* I, nor Jacalin lectins, although control proteins bound selectively and quantitatively to these lectins, thus suggesting the lack of high mannose-type carbohydrates, *N*-acetylgalactosamine, fucose, and *O*-linked carbohydrates, respectively (45).

Endoglycosidase Digestion of Solubilized SRIF Receptors—The functional role of carbohydrate groups on receptors and proteins in general is not well understood. Glycosylation has been observed to have a role in protein trafficking, as well as modulating biological activity (46). However, for most neurotransmitter systems, glycosylation has not been shown to have a role in the functional activity of the neurotransmitter receptors. In particular, complete removal of the carbohydrate groups of the β-adrenergic (16, 17) and muscarinic cholinergic (47, 48) receptors had no effect on agonist binding to these receptors. To investigate the role of glycosylation in the biological functioning of SRIF receptors, solubilized SRIF receptors were subjected to selective hydrolysis with glycolytic enzymes and the effect on specific agonist binding to SRIF receptors examined. PNGase F has been shown to hydrolyze most types of *N*-linked carbohydrate groups from glycoproteins at a point between the di-GlcNAc core and the Asn to which the carbohydrate is linked (49, 50). Treatment of solubilized SRIF receptors with PNGase F resulted in a decrease of 24.6 ± 8.2% in specific [^{125}I]MK 678 binding to solubilized SRIF receptor (Table II). As shown in Fig. 1, under the incubation conditions employed for these studies the control protein ribonuclease b was completely deglycosylated, with no evidence of proteolysis, by treatment with PNGase F. These findings suggest that removal of some *N*-linked carbohydrates from SRIF receptors reduces the affinity of the receptor for agonist. This is the first evidence that oligosaccharides have a role in the functional activity of SRIF receptors.

TABLE II

Effect of endoglycosidase treatment on [^{125}I]MK 678 binding to solubilized SRIF receptors

Brain membranes were solubilized as described under "Experimental Procedures." After solubilization, the proteins were diluted to 2 mM CHAPS and concentrated 5-fold before enzymatic treatments. Solubilized proteins were treated for 16 h at 25 °C with the appropriate enzyme. After treatment, specific [^{125}I]MK 678 binding to solubilized SRIF receptors was assayed and the percent decrease in specific binding was determined by comparison with a control sample incubated in the absence of exoglycosidase. Average control net specific [^{125}I]MK 678 binding was equal to 921 ± 94 cpm. Data are means ± S.E. of 3–9 separate assays conducted in triplicate.

Enzyme	Decrease of [^{125}I]MK 678 binding
	%
PNGase F	24.6 ± 8.2 (9)
Endoglycosidase F	53.9 ± 11.9 (8)
Endoglycosidase H	39.9 ± 13.1 (9)
Endoglycosidase D	0.0 (3)

While PNGase treatment is able to establish the presence of *N*-linked carbohydrates, this treatment is unable to provide information as to the specific types of carbohydrate structures present. Therefore, to determine more specifically the type(s) of *N*-linked carbohydrate group(s) present on the brain SRIF receptors that are involved in agonist binding, the solubilized brain SRIF receptors were treated with specific endoglycosidases (Table II). These enzymes all cleave *N*-linked carbohydrates between the two GlcNAc residues of the core sequence of the oligosaccharide with varying substrate specificities. First, Endo D, which can cleave only unsubstituted high mannose-type carbohydrates (51), had no effect on agonist binding. However, under the same incubation conditions Endo D was able to completely deglycosylate the control protein ovalbumin. The lack of effect of Endo D on agonist binding to SRIF receptors was consistent with the inability of the solubilized SRIF receptors to bind specifically to ConA-lectin affinity columns and suggest that high mannose carbohydrate groups are not associated with the SRIF receptor. Endo H, which, in general, cleaves high mannose- and hybrid-type carbohydrates (52), was able to decrease specific [^{125}I]MK 678 binding approximately 39.9 ± 13.1%. Endo F, which, in general, can remove high mannose, hybrid, and certain complex carbohydrate structures (52), was able to decrease specific [^{125}I]MK 678 binding to solubilized SRIF receptors by 53.9 ± 11.9%. Both Endo H and Endo F were able to completely deglycosylate the control protein ribonuclease b, demonstrating that the incubation conditions were suitable for deglycosylation of glycoproteins by these enzymes (Fig. 1). The seemingly differential effects on binding by PNGase F, Endo H, and Endo F could either be due to variability in the efficiencies of each of the enzymes to deglycosylate SRIF receptors under nondenaturing conditions, that is the enzymes have differing capabilities of accessing the cleavage site on native SRIF receptors, or these findings could be suggestive of heterogeneity in the carbohydrate component of SRIF receptors. Therefore, at least qualitatively, these results would suggest that solubilized SRIF receptors specifically contain either hybrid, or a combination of hybrid and complex carbohydrate structures that are involved in high affinity agonist binding to SRIF receptors. This is the first such evidence that carbohydrate groups are involved in the high affinity binding of agonists to the SRIF receptor.

Exoglycosidase Treatment of Solubilized SRIF Receptors—As described above, the use of endoglycolytic enzymes suggested that the carbohydrate groups on solubilized SRIF receptors were involved in high affinity binding of agonists to the receptor. In order to establish whether specific monosaccharides in the carbohydrate structures are important for agonist binding to solubilized SRIF receptors, the effect of a variety of exoglycosidases on [^{125}I]MK 678 binding to SRIF receptors was examined. These enzymes are able to cleave only those residues on the nonreducing terminus of an oligosaccharide branch. As shown in Table III, β-*N*-acetylglucosaminidase (*D. pneumonia*), reduced specific binding of [^{125}I]MK 678 by approximately 30%. At the low concentrations employed in this study, only β-GlcNAc residues present in β1,2-conformations at the nonreducing terminus of sugar chains, and not the bisecting GlcNAc residue present in a β1,4-conformation, will be cleaved by the β-*N*-acetylglucosaminidase from *D. pneumonia* (53). This would suggest that a portion of the nonreducing chains of the oligosaccharides associated with solubilized SRIF receptors terminate in β-GlcNAc. β-Galactosidase (*D. pneumonia*) was also found to decrease specific [^{125}I]MK 678 binding by approximately 25% (Table III). Both of these enzymes were able to completely remove the appropriate carbohydrates from desialylated α_1-acid glycoprotein under the incubation conditions employed in this study (Fig. 1). In contrast, α-fucosidase (bovine epididymis) appeared to have no effect on specific binding to solubilized SRIF receptors.

The high degree of binding of solubilized rat brain SRIF receptors to WGA and *S. nigra* L. lectin affinity columns, but not succinylated WGA-lectin affinity columns, suggested that sialic acids were a component of the carbohydrate groups present on the SRIF receptor. To determine if sialic acids influence the functional activity of SRIF receptors, solubilized rat brain SRIF receptors were treated with neuraminidase from *V. cholera*. This enzyme was able to completely disialylate α_1-acid glycoprotein under the incubation conditions used in this study (Fig. 1). As shown in Fig. 4, treatment of solubilized SRIF receptors with neuraminidase from *V. cholera* resulted in the complete loss of specific high affinity [^{125}I]MK 678 binding to SRIF receptors. The effect of neuraminidase treatment was time dependent with the complete loss of high affinity [^{125}I]MK 678 binding by 24 h. The complete loss of high affinity binding to the SRIF receptor after neuraminidase treatment suggests that sialic acids play a critical role in the directing of agonist binding to SRIF receptors.

V. cholera neuraminidase is able to cleave terminal sialic acids that are present on nonreducing termini in α2,3-, α2,6-, as well as α2,8-conformations (34). Another exoglycosidase, Newcastle disease virus neuraminidase, has been demonstrated to specifically cleave only those terminal sialic acids present in α2,3- and α2,8-conformations (34). α_1-Acid glycoprotein contains equal amounts of α2,3- and α2,6-linked sialic acids (34). As shown in Fig. 1, under the incubation conditions used in this study, neuraminidase from *V. cholera* was able to remove all of the sialic acids, while neuraminidase from Newcastle disease virus was only able to remove approximately 50% of the sialic acids on the control protein, indicating that both neuraminidases were cleaving the appropriate types of sialic acids found on α_1-acid glycoprotein. Treatment of solubilized SRIF receptors with Newcastle disease virus neuraminidase did not result in any changes in specific [^{125}I]MK 678 binding (Fig. 4). The dramatic loss of high affinity [^{125}I]MK 678 binding to solubilized SRIF receptors following treatment with neuraminidase from *V. cholera*, suggests an important role for terminal sialic acid residues, present in α2,6-linkages, in the functional activity of SRIF receptors.

Sialic acids are negatively charged molecules at physiological pH (54). Interestingly, structure-activity relationship studies have demonstrated that the portion of SRIF critical for binding to its receptor contains a positively charged lysine residue (55). Since sialic acid residues are associated with the extracellular surface of cells, they may be associated with the ligand-binding site of the receptor and may serve to attract SRIF agonists to the receptor through electrostatic interactions. To investigate this hypothesis, studies were conducted on the effect on neuraminidase treatment of intact AtT-20 cells. AtT-20 cells are a pituitary-derived tumor cell line which express a high density of SRIF receptors with the same physical, pharmacological, and functional characteristics as brain SRIF receptors (14, 64). Overnight treatment of intact AtT-20 cells in culture with neuraminidase from *V. cholera* reduced specific [^{125}I]MK 678 binding to SRIF receptors in membranes prepared from these cells by about 50%. The decrease in binding was due to a loss of high affinity [^{125}I]MK 678 binding sites as indicated by the Scatchard analysis of homologous displacement of [^{125}I]MK 678 binding to membranes prepared from control and neuraminidase-treated AtT-20 cells (Fig. 5). The loss of high affinity binding sites could either be due to a conversion of high affinity sites to lower affinity sites which cannot be detected with [^{125}I]MK 678 or a physical loss or inactivation of a population of SRIF

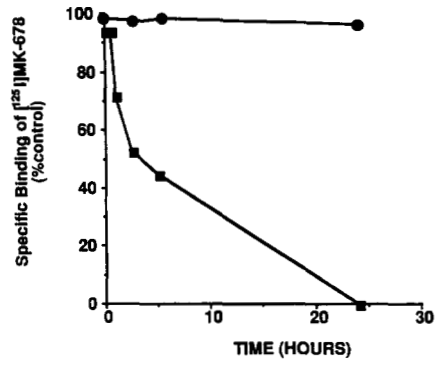

FIG. 4. **Time course of treatment of solubilized rat brain SRIF receptors with neuraminidase from *V. cholera* and Newcastle disease virus.** Solubilized rat brain membranes were prepared as described under "Experimental Procedures," diluted to contain 2 mM CHAPS, and concentrated 5-fold. The solubilized membranes were incubated for increasing amounts of time with 0.167 unit/ml of *V. cholera* (■) or 0.08 unit/ml of Newcastle disease virus neuraminidase (●). Data are representative of two separate experiments conducted in triplicate.

TABLE III

Effect of exoglycosidase treatment on [^{125}I]MK 678 binding to solubilized SRIF receptors

Brain membranes were solubilized as described under "Experimental Procedures." After solubilization, the proteins were diluted to 2 mM CHAPS and concentrated 5-fold before enzymatic treatments. Solubilized membrane proteins were treated for 16 h at 25 °C with the appropriate enzyme. After treatment, specific [^{125}I]MK 678 binding to solubilized SRIF receptors was assayed and the percent decrease in specific binding was determined by comparison with a control sample incubated in the absence of endoglycosidase. Net specific [^{125}I]MK 678 binding in control samples was equal to 924 ± 138 cpm. Data are means ± S.E. of 3–7 separate assays conducted in triplicate.

Enzyme	Decrease of [^{125}I]MK 678 binding
	%
β-Galactosidase	24.9 ± 14.4 (3)
β-*N*-Acetylglucosaminidase	30.6 ± 13.7 (5)
α-Fucosidase	0.0 (3)

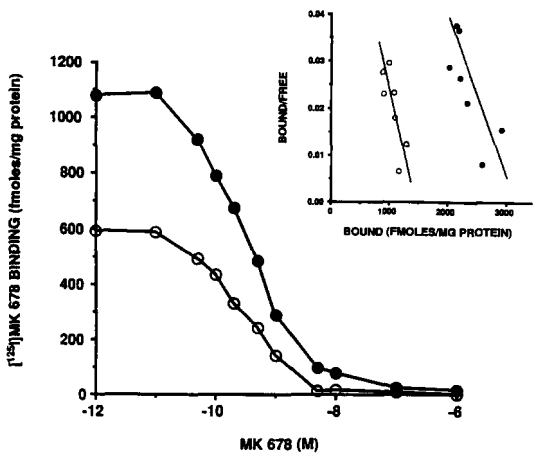

FIG. 5. **[^{125}I]MK 678 binding to neuraminidase-treated AtT-20 cells in culture.** AtT-20 cells in culture were treated for 24 h with 0.1 unit/ml of neuraminidase from *V. cholera* as detailed under "Experimental Procedures." Membranes were prepared from control (●) and treated AtT-20 (○) cells and [^{125}I]MK 678 (50 pM) binding was inhibited by varying concentrations of MK 678. The data from the inhibition curves was analyzed by the method of Scatchard using the LIGAND curve fitting program (*inset*). The linearization of the inhibition curves revealed a single [^{125}I]MK 678 binding site with K_d values of 0.15 ± 0.04 and 0.11 ± 0.03 nM and B_{max} values of 2848 ± 508 and 1609 ± 420 fmoles/mg of protein for control and treated cells, respectively. Data are representative examples of three studies conducted in duplicate.

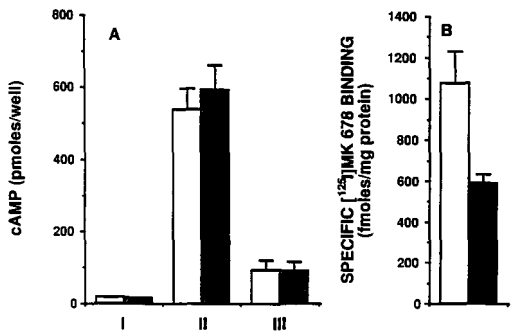

FIG. 6. **cAMP accumulation in AtT-20 cells after neuraminidase treatment.** AtT-20 cells in culture were treated with 0.1 unit/ml of neuraminidase from *V. cholera* for 24 h as described under "Experimental Procedures." Control (*open bars*) and treated (*hatched bars*) AtT-20 cells were then preincubated with 50 mM isobutylmethylxanthine for 30 min and treated with *I*, buffer, *II*, 50 mM forskolin and 10^{-7} M SRIF for 30 min. The cAMP accumulated was measured by radioimmunoassay (*A*). Specific [^{125}I]MK 678 binding to SRIF receptors in these same cultures was also measured (*B*). Data averages ± S.E. of four experiments conducted in duplicate.

receptors. To investigate whether desialylated SRIF receptors were still functionally active we examined the ability of SRIF to inhibit forskolin-stimulated cAMP production in control and neuraminidase-treated AtT-20 cells in culture. Based on the data in Fig. 6, the apparent loss of high affinity [^{125}I]MK 678 sites after neuraminidase treatment appears to be due to the conversion of SRIF receptors to a low affinity conformation for agonists since SRIF maximally inhibited forskolin-stimulated cAMP accumulation to the same extent in control and neuraminidase-treated AtT-20 cells. Since SRIF induced the same maximal response in both groups of cells, the neuraminidase treatment did not inactivate SRIF receptors. No differences in basal or forskolin-stimulated cAMP accumulation were observed between the control and neuraminidase-treated cells, nor were there any changes in morphology or protein content of the cells, suggesting that the effects on high affinity [^{125}I]MK 678 binding to SRIF receptors were not due to nonselective effects of the neuraminidase treatments. This partial loss of high affinity binding to SRIF receptors in plated intact AtT-20 cells is most likely reflective of the inaccessibility of some sialic acid residues on native SRIF receptors to the neuraminidase, since treatment of membranes prepared from AtT-20 cells with neuraminidase for 24 h resulted in a 90% loss of [^{125}I]MK 678 binding, similar to that which was observed with solubilized brain SRIF receptors (data not shown). Since SRIF inhibition of forskolin-stimulated cAMP formation requires the coupling of SRIF receptors to the catalytic subunit of adenylylcyclase via GTP-binding proteins, the ability of SRIF to inhibit forskolin-stimulated cAMP accumulation to the same extent in control and neuraminidase-treated cells, suggests that despite the loss of high affinity agonist binding to a substantial population of SRIF receptors, all of the receptors remain functionally active and coupled to GTP-binding proteins. The partial loss of high affinity [^{125}I]MK 678 binding sites, but the maintenance of full functional responsiveness could be due to the existence of spare receptors. However, there is no evidence that spare SRIF receptors exist on AtT-20 cells (56, 57). This being the case, neuraminidase treatment appears to have removed sialic acid residues from the extracellular surface of a large population of SRIF receptors in AtT-20 cells to reduce high affinity agonist binding without altering GTP-binding protein coupling to the receptor.

The finding that the cleavage of sialic acid residues from the extracellular surface of AtT-20 cells greatly reduces high affinity agonist binding to SRIF receptors, suggests that sialic acids are near, or at, the ligand binding site of SRIF receptors. To test this hypothesis, we determined whether neuraminidase treatment could still affect SRIF receptors when the receptor was occupied with agonist. As shown in Fig. 7, pretreatment of AtT-20 cell membranes with a saturating concentration of MK 678 (100 nM), prior to binding [^{125}I]MK 678 to SRIF receptors, did not affect the ability of [^{125}I]MK 678 to bind to SRIF receptors after extensive washing. These findings indicate that residual MK 678 was not bound to SRIF receptors. In AtT-20 cell membranes exposed to MK 678 (100 nM) and neuraminidase, neuraminidase treatment had little effect on SRIF receptors, whereas in AtT-20 cell

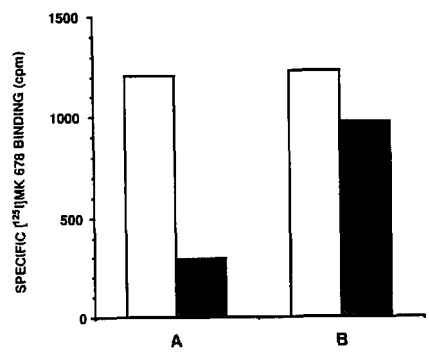

FIG. 7. **Treatment of SRIF receptors from AtT-20 cell membranes in the presence of agonist.** AtT-20 cell membranes were prepared as described under "Experimental Procedures" and the crude membranes preincubated for 1.5 h at 25 °C in the presence of *I*, buffer or *II*, 100 nM MK 678. After the preincubation, either buffer (*open bars*) or 0.1 unit/ml of neuraminidase from *V. cholera* (*hatched bars*) was added for an additional 5 h. The samples were then rinsed free of MK 678 and neuraminidase by washing the membranes twice with Hanks' balanced salt solution/sodium acetate, pH 5. The washed, control, and treated membranes were then measured for their ability to bind [^{125}I]MK 678. Depicted are the results of a representative example of three studies conducted in duplicate.

membranes incubated with neuraminidase alone, specific [^{125}I]MK 678 binding was reduced by 75%. The protection from the effects of the neuraminidase treatment afforded the SRIF receptors by MK 678 was specific since the inactive SRIF analogue, SRIF 28 (1–14), did not prevent neuraminidase from reducing high affinity agonist binding to SRIF receptors (data not shown).

DISCUSSION

We have recently reported that rat brain SRIF receptors can be solubilized in a high affinity state using the detergent CHAPS and can be detected by the SRIF analogue [^{125}I]MK 678 (25). These solubilized receptors were shown to retain their pharmacological specificities for SRIF analogues and have provided a model system by which we could investigate the carbohydrate nature of brain SRIF receptors. In this report, we have studied the composition of the carbohydrate found on solubilized rat brain SRIF receptors by lectin affinity chromatography, and determined their functional role by enzymatic deglycosylation. By using these techniques, we have shown that solubilized rat brain SRIF receptors are post-translationally modified to contain one or more polysaccharides *N*-linked to Asn residues. Asparagine-linked carbohydrates are classified into three types, high mannose, hybrid, and complex, based on the sugar substitutions present on the core oligosaccharides. The *N*-linked carbohydrates present in solubilized SRIF receptors that are involved in high affinity binding appear to be either of hybrid or a combination of hybrid and complex types. This is based on the effects of Endoglycosidases F and H treatment on high affinity agonist binding to solubilized SRIF receptors and is supported by the ability of some solubilized SRIF receptors to specifically bind to *R. communis* I and II lectin affinity columns. Furthermore, the lack of binding to ConA suggested the absence of any high mannose-type carbohydrate on the SRIF receptor, which is a finding consistent with other previous studies (10, 21). This conclusion was also supported by the lack of effect of Endo D treatment on agonist binding to the solubilized receptors and, additionally, in preliminary studies, treatment of solubilized SRIF receptors with α-mannosidase (almond) did not induce clear alterations in the high affinity binding of [^{125}I]MK 678 to SRIF receptors.

Aside from the determination of the types of carbohydrate groups present on the solubilized SRIF receptor, more detailed descriptions of the oligosaccharides associated with the SRIF receptors can be drawn from the results presented herein. First, the polysaccharide component involved in maintaining the solubilized SRIF receptor in a high affinity state for agonist binding does not appear to contain a bisecting GlcNAc residue. This would be evidenced by the lack of interaction of solubilized SRIF receptors with succinylated WGA, which has highest affinity for the β-GlcNac-(1→4)-β-GlcNac-(1→4)-GlcNac structure which is present in carbohydrate structures containing a bisecting GlcNAc residue. Second, the polysaccharide chains do not appear to be fucosylated, since the solubilized receptor did not specifically bind to a *U. europaeus* I lectin affinity column, nor was there any affect on agonist binding when the receptor was treated with α-fucosidase. Third, there appears to be terminal sialic acid residues present in α2,6-linkages on the carbohydrate structures of solubilized SRIF receptors. This was demonstrated by the specific bonding of solubilized SRIF receptors to the *S. nigra* L. lectin and the loss of high affinity agonist binding to SRIF receptors after treatment with neuraminidase from *V. cholera*, but not with Newcastle disease virus neuraminidase.

In our study we have shown that the carbohydrate component of rat brain SRIF receptors appears to play a role in promoting high affinity agonist binding. The results of the deglycosylation studies indicated that terminal residues, primarily sialic acid, found in either complex or hybrid structures, are required for the high affinity binding of the radioactive agonist [^{125}I]MK 678 to solubilized SRIF receptors. The role of the carbohydrate portion of the SRIF receptors in maintaining the high affinity state for agonists are in contrast to what has been described for agonist binding to other neurotransmitter receptors, such as the α_1- (58) and β-adrenergic (16, 17), A_1 adenosine (59), D2 dopamine (60), and M1 (47) and M2 (48) muscarinic receptors. Many of these studies have shown that the sugar component of neurotransmitter receptors do not have a role in agonist binding, but instead may function to direct the transportation and accumulation of the receptors at the cell surface. Interestingly, sialic acids have been suggested to play a role in the binding of vasoactive intestinal peptide to its receptor (61). The requirement for sialic acid residues and/or intact polysaccharide chains for high affinity agonist binding to SRIF and vasoactive intestinal receptors may represent an inherent property of the active sites of these peptidergic receptors that differs from the hydrophobic binding domains of nonpeptidergic receptors.

In general, sialic acid residues are oriented towards the extracellular surface of cells. Therefore, sialic acid residues associated with the ligand-binding site of SRIF receptors could influence agonist binding to the receptor through electrostatic interactions. This is suggested by the highly negative charge density of sialic acid residues at physiological pH (54) and the positively charged lysine observed to be crucial for SRIF's ability to bind to its receptors with high affinity (55). In fact, substitution of the lysine with non-charged amino acids has been reported to greatly reduce the binding affinity of the peptide to SRIF receptors (55), and in preliminary studies we have found that acetylation of the lysine residue in MK 678, to remove the positive charge, reduces the affinity of AtT-20 cell SRIF receptors for MK 678 by 50-fold.[2] Further evidence supporting a role for sialic acid residues in promoting high affinity binding to SRIF receptors is provided by the results of the studies on intact AtT-20 cells, in which the intact cells were treated with neuraminidase to remove sialic acid residues from the extracellular surface of the cells. There was a significant decrease in high affinity agonist binding to the membranes of treated cells, while in contrast, the ability of the receptors to mediate SRIF inhibition of cAMP accumulation was not hindered by treatment of the cells with neuraminidase. These findings suggest that neuraminidase treatment reduced the affinity of most SRIF receptors for agonists, but did not significantly affect SRIF receptor/GTP-binding protein coupling, since SRIF receptors must be coupled to G_i to mediate SRIF inhibition of cAMP accumulation and adenylylcyclase activity (63, 65). Therefore, it is possible that the removal of sialic acid residues from the extracellular surface of SRIF receptors reduces the affinity of the receptors for agonist, but these lower affinity sites are still functionally able to mediate the effects of high concentrations of SRIF on adenylylcyclase activity. Thus, both sialic acid residues associated with SRIF receptors, in addition to GTP-binding protein coupling with the receptors, contribute to the maintenance of SRIF receptors in a high affinity biologically active conformation.

The ability fo the stable SRIF agonist MK 678 to protect SRIF receptors from the effects of neuraminidase treatment, suggests that sialic acid residues may be closely associated with the ligand-binding site of the receptor. However, these

[2] K. Raynor and T. Reisine, unpublished observations.

results may also be explained by the conversion of the agonist-bound SRIF receptor into a conformation in which the sialic acid residues are no longer accessible to the neuraminidase. Clear identification of the localization of the sialic acid residues in the SRIF receptor, and their role in the functioning of the receptor, will require further detailed physical characterization.

In conclusion, we have shown that the carbohydrate component of SRIF receptors are involved in promoting high affinity agonist binding. We have also provided evidence that terminal sialic acid residues are specifically involved in the promotion of high affinity binding to SRIF receptors. The specific mechanism by which sialic acid residues influence agonist binding to SRIF receptors is currently being investigated. Furthermore, the role of the sugar groups in the determining physical and functional heterogeneity of SRIF receptors is also currently being studied.

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