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Solubilization of Active Somatostatin Receptors from Rat Brain

HAI-TAO HE, STEPHANIE RENS-DOMIANO, JEAN-MICHEL MARTIN, SUSAN F. LAW, STEVEN BORISLOW, MARILYN WOOLKALIS, DAVID MANNING, and TERRY REISINE

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

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SUMMARY

Rat brain somatostatin (SRIF) receptors were solubilized in an active form with the detergent 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Solubilized SRIF receptors were detected with the stable SRIF analog ^{125}I-MK 678. CHAPS solubilized approximately 30% of membrane-bound SRIF receptors. ^{125}I-MK 678 binding to the solubilized SRIF receptors reached equilibrium by 90 min and dissociated from the receptor with a $t_{½}$ of 60 min. The binding of ^{125}I-MK 678 to the solubilized SRIF receptor was of high affinity and was selective. The characteristics of ^{125}I-MK 678 binding to the solubilized and membrane-bound SRIF receptors were similar. The solubilized brain SRIF receptor specifically bound to a wheat germ agglutinin-Sepharose column, suggesting that it is a glycoprotein. Analysis of the solubilized SRIF receptor by gel exclusion chromatography on an AcA 34 Ultrogel column revealed that its molecular mass is approximately 400 kDa. This mass is probably representative of the receptor complexed with other proteins or molecules. Further characterization of the fractionated 400-kDa species by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting indicated that G_i and G_o may be associated with the solubilized SRIF receptor. This is supported by the finding that guanosine-5′-*O*-(3-thio)triphosphate abolished ^{125}I-MK 678 binding to the solubilized SRIF receptor. Antibodies directed against a synthetic peptide corresponding to a region of the C-terminal of G_{ia}, which specifically immunoprecipitate G_{ia}, immunoprecipitated over 24% of the solubilized SRIF receptor, suggesting that the receptor, in part, is coupled to G_i. These studies describe for the first time the characterization of the solubilized SRIF receptor in an active form. The ability to solubilize the SRIF receptor should allow for further characterization of its physical properties.

SRIF is a neurotransmitter in the brain and a neurohormone in peripheral target organs such as the anterior pituitary, pancreas, and gut (1). SRIF plays a major role in the regulation of hormone secretion and neurotransmitter release. SRIF is the predominant physiological inhibitor of growth hormone release from the anterior pituitary. In addition, it blocks the secretion of insulin and glucagon from the pancreas (1). The peptide also facilitates dopamine, norepinephrine, and serotonin release in brain (2).

SRIF induces its biological effects by acting upon membrane-bound receptors. SRIF receptors are coupled to multiple cellular effector systems such as the adenylyl cyclase complex and ionic conductance channels (1, 3–5). Results from a number of studies suggest that SRIF receptors couple to these cellular effectors via pertussis toxin-sensitive G proteins (3–5).

Most attempts to characterize the physical properties of SRIF receptors have relied on the use of covalent cross-linking or photo-cross-linking techniques to covalently tag the receptor with radiolabeled SRIF analogs. The labeled receptors are then subjected to SDS-PAGE and autoradiography in order to assess their size. The results of these studies have shown that the size of SRIF receptors varies from 60 to 94 kDa (6–11). Sakamoto *et al.* (6), as well as others (7–10), reported that the size of pancreatic acinar SRIF receptors is approximately 90 kDa. In contrast, Thermos *et al.* (11) and Sakamoto *et al.* (8) reported that the size of brain SRIF receptors is 60–70 kDa. These findings suggest that physical differences exist between the brain and pancreatic acinar SRIF receptors. Results from these and other studies have also suggested that SRIF receptors are glycoproteins (10, 11). Variations in oligosaccharide composition could be responsible in part for the size differences in SRIF receptors in various tissues and may also contribute to the differences in pharmacological characteristics of SRIF receptor subtypes (12, 13).

Further characterization of the physical properties of SRIF receptors would be facilitated by the solubilization of the receptors in their active form. Previously, Knuhtsen *et al.* (7) reported that membrane-bound SRIF receptors from pancreatic acinar membranes could be labeled with a radioactive SRIF

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ABBREVIATIONS: SRIF, somatotroph release inhibitory factor; G, protein, GTP-binding regulatory proteins; CHAPS, 3-[(cholamidopropyl)]dimethylammonio]-1-propanesulfonate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TACT, *N*,*N*′,*N*″-triacetylchitotriose; WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

analog and, upon solubilization, the SRIF analog did not dissociate from the receptor. These authors, however, did not demonstrate specific reversible binding of radioactive SRIF analogs to the solubilized SRIF receptor. Following solubilization, this characteristic is necessary in order to show that the receptor remains active and is able to maintain pharmacological sensitivity for SRIF agonists. He *et al.* (14) have recently reported on the purification of a putative SRIF receptor from brain. These authors were also unable to demonstrate reversible binding of a radioactive SRIF analog (^{125}I-CGP 23996) to the solubilized or purified SRIF receptor. For this reason, it was not possible to determine whether the material that was purified retained biological activity. To date, no investigator has reported the solubilization of active SRIF receptors.

In the present study, we describe a procedure to solubilize SRIF receptors from rat brain in an active form, using the nondenaturing zwitterionic detergent CHAPS. We were able to demonstrate specific reversible labeling of the solubilized SRIF receptor, due to the development of a new SRIF analog, ^{125}I-MK 678. Some of the properties of the CHAPS-solubilized SRIF receptor are revealed and, in particular, it is shown that solubilized SRIF receptors remain associated with G proteins.

Experimental Procedures

Materials. SRIF, D-Trp^8 SRIF, somatostatin 28, and somatostatin 28[1–14] were obtained from Bachem (CA). MK 678 [cyclo(*N*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe] was a gift from Dr. R. Saperstein, Merck (Rahway, NJ). CHAPS and bicinchoninic acid protein assay reagents were obtained from Pierce (Rockford, IL). Silver-staining reagents were from Bio-Rad. AcA-34 Ultrogel was from IBF Biochemicals and WGA-Sepharose and Protein A-Sepharose were from Sigma (St. Louis, MO). The iodination of MK 678 was as previously described (13).

Solubilization of the SRIF receptor. For the solubilization of the SRIF receptor, rat brains minus the cerebellum were homogenized in 10 volumes of 50 mM Tris·HCL (pH 7.8) containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg of leupeptin, 2 μg of pepstatin, 200 μg of bacitracin, and 0.5 μg of aprotinin per ml (Buffer 1) as previously described (14). The homogenate was centrifuged at 600 × *g* for 5 min at 4° and the supernatant was saved. The pellet was resuspended in Buffer 1 and recentrifuged at 600 × *g* for 5 min. The two supernatants were combined and centrifuged at 45,000 × *g* for 30 min at 4°. The pellet was resuspended in Buffer 1 and centrifuged again at 45,000 × *g* for 30 min at 4°. The resulting membrane pellet either was used to detect membrane-bound SRIF receptor or was solubilized. For solubilization, the membrane pellet was resuspended in Buffer 1 containing 10 mM CHAPS at 20% (v/v) glycerol, stirred on ice for 60 min, and centrifuged for 60 min at 100,000 × *g*. The supernatant was removed and used immediately to detect solubilized SRIF receptors or frozen at −80°. The solubilized SRIF receptor remained active for at least 1 week when stored at −80°. For the solubilization of AtT-20 cell membranes, similar procedures as described above were used, except that 30–50 × 10^6 AtT-20 cells were used.

^{125}I-MK 678 binding assay. Solubilized SRIF receptors were reversibly labeled with the stable SRIF agonist ^{125}I-MK 678 (13). For the binding assay, aliquots of solubilized proteins (final CHAPS concentration of 2 mM) were incubated with 20 pM ^{125}I-MK 678 (specific activity of 2200 Ci/mmol), in a final volume of 0.88 ml of Buffer 1, for 90 min at 25°. Incubations were stopped by the addition of 3 ml of ice-cold 50 mM Tris·HCl (pH 7.8), followed by vacuum filtration over Whatman (GF/F) glass fiber filters that had been presoaked in 0.5% polyethylenimine at 4°. Filters were washed with 12 ml of ice-cold Tris·HCl (pH 7.8) buffer and the radioactivity retained on the filters was measured in a γ-counter. Specific ^{125}I-MK 678 binding was defined as total ^{125}I-MK 678 binding to tissue or solubilized proteins minus the amount bound in the presence of 1 μM D-Trp^8-SRIF. IC_{50} values were obtained from curve-fitting involving nonlinear regression analysis. The analysis was performed by the mathematical modeling program NEWFITSITES, available on the National Institutes of Health-sponsored PROPHET system. The dissociation constant (K_D) and maximal binding (B_{max}) of ^{125}I-MK 678 binding to solubilized SRIF receptors was determined by Scatchard analysis (15) of the competitive displacement experiments. Protein content was determined using the bicinchoninic acid protein assay procedure described by Smith *et al.* (16), with bovine serum albumin as a protein standard.

WGA affinity chromatography. Solubilized rat brain proteins were concentrated 10-fold using Amicon Centricon 30 microconcentrators, after which the CHAPS concentration of the solution was increased to 20 mM. Four milliliters of the solubilized concentrated rat brain proteins were applied to a WGA-Sepharose affinity column (0.7-ml bed volume) that had been prewashed with 10 ml of distilled water and preequilibrated with 10 ml of Buffer 1 containing 10% glycerol and 5 mM CHAPS. The column was recycled for 1.5 hr at a flow rate of 0.17 ml/min. The sample was then allowed to pass through the column. The column was washed with Buffer 1 containing 10% glycerol and 5 mM CHAPS and 1.6-ml fractions were collected. The bound proteins were then eluted with 4 mM TACT in Buffer 1 containing 10% glycerol and 5 mM CHAPS. Fractions of 0.8 ml were collected and each fraction was analyzed for specific ^{125}I-MK 678 binding.

Gel exclusion chromatography. The size of the rat brain SRIF receptor was estimated by gel exclusion chromatography. Four rat brains, minus cerebellum, were prepared as described above, except that they were solubilized in 6 ml of Buffer 1 containing 20% glycerol and 15 mM CHAPS. A 5-ml aliquot of the solubilized material was applied to an AcA 34 Ultrogel (1.6 × 73 cm) column that was preequilibrated with 50 mM Tris (pH 7.8), 1 mM EGTA, 5 mM $MgCl_2$, 10% glycerol, 5 mM CHAPS. The column was eluted at a flow rate of 4.8 ml/hr at 4°. Fractions of 1.2 ml were collected, diluted 2-fold with Buffer 1, and used in the ^{125}I-MK 678 binding assay.

Western blot analysis of the α subunits of G proteins. The presence of the α subunits of G proteins associated with the solubilized SRIF receptor was analyzed by immunoblotting, using procedures previously described (17–19). The antiserum employed (No. 1398) is directed against the peptide sequence CGAGESGKSTIVKQMK and has been shown to recognize, following Western blotting, the α subunits of G_i, G_o, G_t, and, with less affinity, G_s (17–19). The antiserum does not cross-react with the β/γ complex. The generation and specificity of antiserum 1398 is described elsewhere (17–19). G_i and G_o were purified from bovine brain as previously described (18).

Immunoprecipitation of the soluble SRIF receptor complexed to G proteins with antibodies directed against G_{ia}. In order to immunoprecipitate soluble SRIF receptors coupled to G_{ia}, we used antiserum 8730 (19). This antiserum is directed against the peptide sequence KNNLKDCGLF. This sequence is shared by G_{ia-1}, G_{ia-2}, and, except for two consecutive substitutions, G_{ia-3}. This antiserum selectively immunoprecipitates G_{ia} (19) and is analogous to antiserum AS/7 described by Goldsmith *et al.* (20). For the immunoprecipitation studies, solubilized rat brain SRIF receptors were size fractionated by gel exclusion chromatography and peak SRIF receptor binding activity was pooled and concentrated using an Amicon Centriprep 10. Solubilized SRIF receptors (800 μl) were incubated overnight at 4° with antiserum 8730 or nonimmune control serum. Protein A-Sepharose CL-4B beads (50 μl) were added and the solution was incubated for 1 hr at 4°. The samples were reprecipitated by the addition of a 1:100 final dilution of antiserum 8730 or nonimmune serum and 30 μl of Protein A-Sepharose CL-4B beads. After a 3-hr incubation at 4°, the solution was microfuged and the supernatant was assayed for specific ^{125}I-MK 678 binding activity.

Results

To solubilize SRIF receptors, brain membranes were treated with the detergent CHAPS. The solubilized receptors were

detected using the stable SRIF agonist ^{125}I-MK 678. In previous studies, ^{125}I-MK 678 has been shown to selectively bind to rat brain and anterior pituitary membrane-bound SRIF receptors with high affinity and in a saturable manner (13). Rat brain membranes were treated with varying concentrations of CHAPS to determine the conditions that would allow the highest proportion of SRIF receptors to be solubilized. SRIF receptors appeared to be maximally solubilized by 10–15 mM CHAPS. At these concentrations, CHAPS solubilized approximately 20–30% of specific ^{125}I-MK 678 binding activity. Ten millimolar CHAPS was used in subsequent studies to solubilize SRIF receptors.

To investigate whether the material solubilized from brain membranes contained active SRIF receptors, ^{125}I-MK 678 binding to the solubilized material was characterized. Specific ^{125}I-MK 678 binding to the solubilized SRIF receptor accounted for approximately 70% of total ^{125}I-MK 678 binding. Specific ^{125}I-MK 678 binding to the solubilized SRIF receptor reached equilibrium by 90 min at 25° (Fig. 1). The half life of dissociation of ^{125}I-MK 678 binding from the solubilized SRIF receptor was approximately 60 min.

MK 678 potently inhibited ^{125}I-MK 678 binding to both the solubilized and membrane-bound SRIF receptors, with IC_{50} values of 1.2 and 1.3 nM, respectively (Fig. 2, *left*). Analysis of the MK 678 displacement of ^{125}I-MK 678 binding to the SRIF receptor by the method of Scatchard (15) revealed the presence of two classes of ^{125}I-MK 678 binding sites in both the solubilized material and membrane preparations (Fig. 2, *right*). In the solubilized material, the high affinity ^{125}I-MK 678 binding sites had a K_D of 1.1 nM and a B_{max} of 452 fmol/mg of protein and the low affinity ^{125}I-MK 678 binding sites had a K_D of 35 nM and a B_{max} of 1875 fmol/mg of protein. ^{125}I-MK 678 binding to the membrane-bound SRIF receptor had similar characteristics, with K_D values of 0.9 and 24 nM and B_{max} values of 403 and 1131 fmol/mg of protein, respectively.

^{125}I-MK 678 binding to the solubilized SRIF receptor was potently and effectively inhibited by SRIF, D-Trp^8-SRIF, and somatostatin-28 (Fig. 3). The IC_{50} values for these peptides to inhibit ^{125}I-MK 678 binding to solubilized and membrane-bound SRIF receptors were similar (see Fig. 3 legend). The inactive peptide SRIF-28[1–14], as well as biologically active peptides unrelated to SRIF (Leu-enkephalin, Met-enkephalin, vasopressin, cholecystokinin, angiotensin II, and corticotropin releasing factor), did not affect ^{125}I-MK 678 binding to the solubilized SRIF receptor (not shown).

The results of previous covalent cross-linking and photocross-linking studies have suggested that SRIF receptors are glycoproteins (10, 11). To test whether the solubilized brain SRIF receptor has an oligosaccharide moiety, it was applied to a WGA-Sepharose affinity column. Approximately 50% of the solubilized SRIF receptor bound to the WGA column and 67% of the bound receptor could be eluted from the column with the sugar TACT, suggesting that at least a population of the solubilized receptors are glycoproteins (Fig. 4).

To assess the size of the solubilized SRIF receptor, the receptor was subjected to gel exclusion chromatography. A small proportion of SRIF receptors detected by specific ^{125}I-MK 678 binding migrated near the void volume, along with most of the solubilized proteins (Fig. 5). However, the majority of specific ^{125}I-MK 678 binding activity was present in a peak migrating at a molecular mass of approximately 400 kDa (Fig. 5). This corresponds to a Stokes radius of 60 Å. The peak binding activity represented approximately a 5-fold purification of the SRIF receptor. To determine whether the characteristics of ^{125}I-MK 678 binding to the material migrating at 400 kDa were similar to the binding of this radioligand to the unfractionated solubilized SRIF receptor, fractions 32 to 43 (see Fig. 5) were pooled and the binding of ^{125}I-MK 678 to the fractionated material was analyzed. D-Trp^8-SRIF potently and selectively inhibited ^{125}I-MK 678 binding to the 400-kDa material.

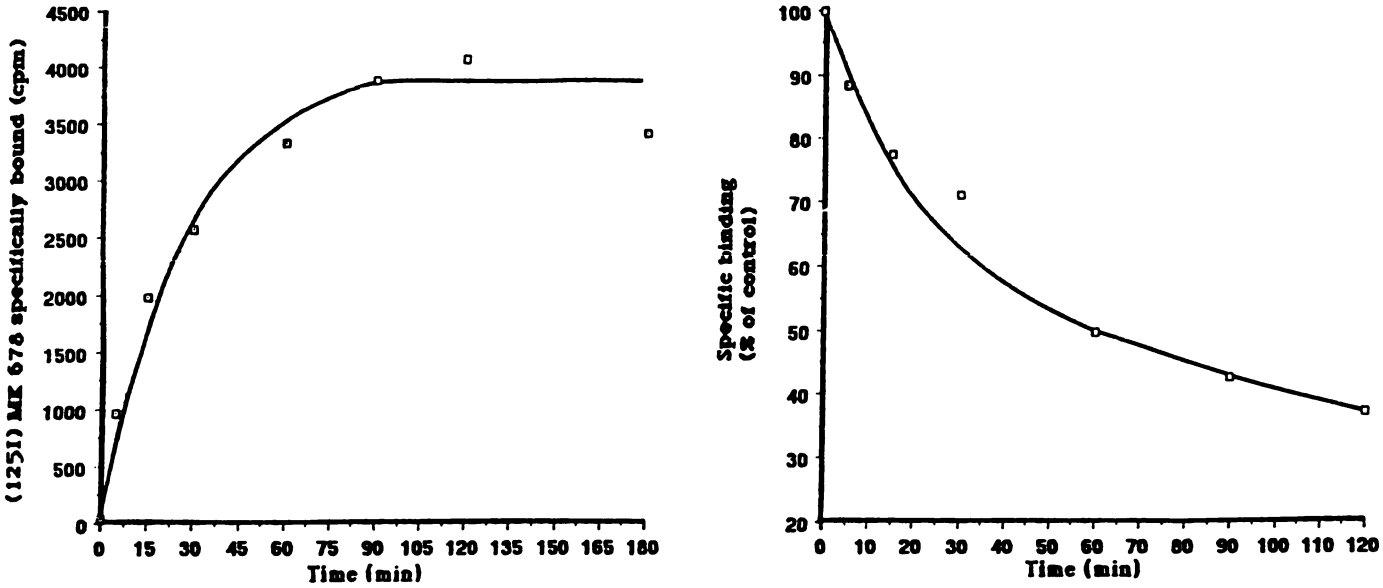

Fig. 1. Equilibrium binding of ^{125}I-MK 678 to the soluble SRIF receptor. The time course for association (*left*) and dissociation (*right*) of specific ^{125}I-MK 678 (20 pM) binding to the solubilized SRIF receptor is presented. This experiment is a representative study, which was repeated two other times with similar results. For the experiment, 50 μg of protein were included in each reaction tube. For the dissociation studies, the binding reaction was allowed to reach equilibrium after 90 min and then 1 μM D-Trp^8-SRIF was added to promote dissociation of ^{125}I-MK 678 from the receptor. At various times after the addition of unlabeled displacer, the reaction was terminated by vacuum filtration. The dissociation constant determined from the average kinetic analyses of ^{125}I-MK 678 binding to the solubilized SRIF receptor in three different experiments is 0.8 ± 0.2 nM.

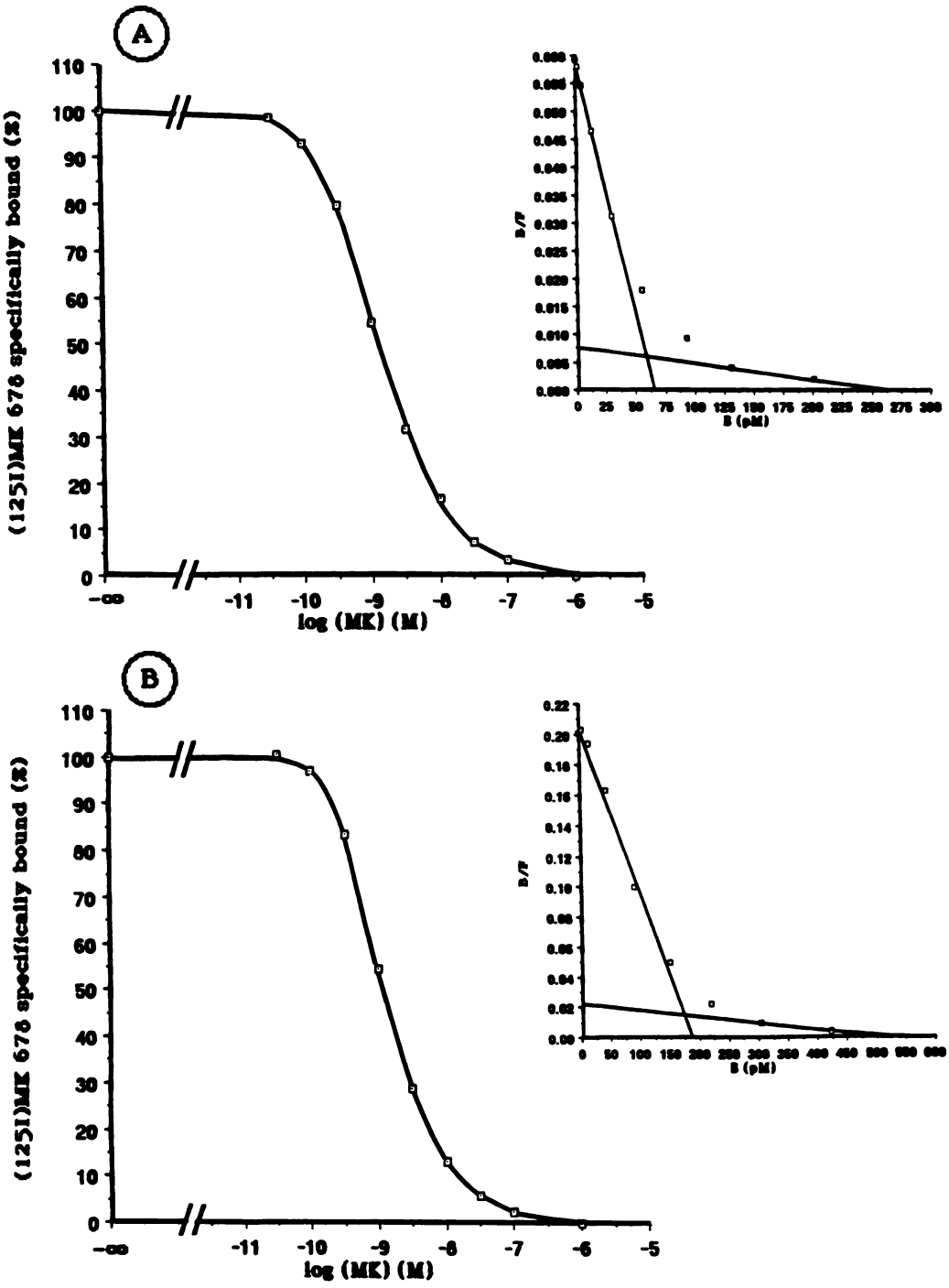

Fig. 2. Scatchard analysis of ^{125}I-MK 678 binding to the solubilized and membrane-bound SRIF receptors. ^{125}I-MK 678 (20 pM) binding to the solubilized (A) and membrane bound (B) SRIF receptors was inhibited by varying concentrations of MK 678 (*MK*). The IC_{50} values for MK 678 inhibition of ^{125}I-MK 678 binding to the solubilized and membrane-bound SRIF receptors were 1.2 and 1.3 nM, respectively. The data from the inhibition curves were analyzed by the method of Scatchard, using the National Institutes of Health-based PROPHET program, and revealed that ^{125}I-MK 678 bound to two sites in both the soluble and membrane preparations. The linearization of the inhibition curves is presented in the two insets. The K_d and B_{max} values for each site are described in Results. *B*, bound; *F*, free.

The IC_{50} value for D-Trp^8-SRIF to inhibit ^{125}I-MK 678 binding to the 400-kDa SRIF receptor was 0.9 nM, which is similar to its potency to inhibit ^{125}I-MK 678 binding to the unfractionated and membrane-bound SRIF receptors (see Fig. 3). The inactive peptide somatostatin-28[1–14] did not affect ^{125}I-MK 678 binding to the 400-kDa SRIF receptor.

The 400-kDa material may represent, in part, SRIF receptors complexed with other proteins. G proteins may be one component of the SRIF receptor complex, because GTPγS abolished the binding of ^{125}I-MK 678 to the solubilized SRIF receptor (Fig. 6) as well as specific binding of ^{125}I-MK 678 to the 400-kDa material (not shown). GTPγS did not affect nonspecific ^{125}I-MK 678 binding. GDP (100 μM) and ATP (100 μM) did not affect specific ^{125}I-MK 678 binding to the solubilized SRIF receptor.

In an attempt to further identify the G proteins coupled to the SRIF receptor, the fractionated (fractions 32 to 43; see Fig. 5) SRIF receptor complex was subjected to SDS-PAGE (Fig. 7, *lane A*) and the proteins were identified by silver stain. The proteins were then transferred to nitrocellulose membranes. The nitrocellulose membrane was reacted with antiserum 1398, which is selective for a common determinant of the α subunit of G proteins (17–19). The Western blot revealed that antiserum 1398 interacted with proteins of 39 and 40 kDa from the pooled fractions containing SRIF receptor binding activity (Fig. 7, *lane C*). These proteins may be G_i and G_o, because they co-

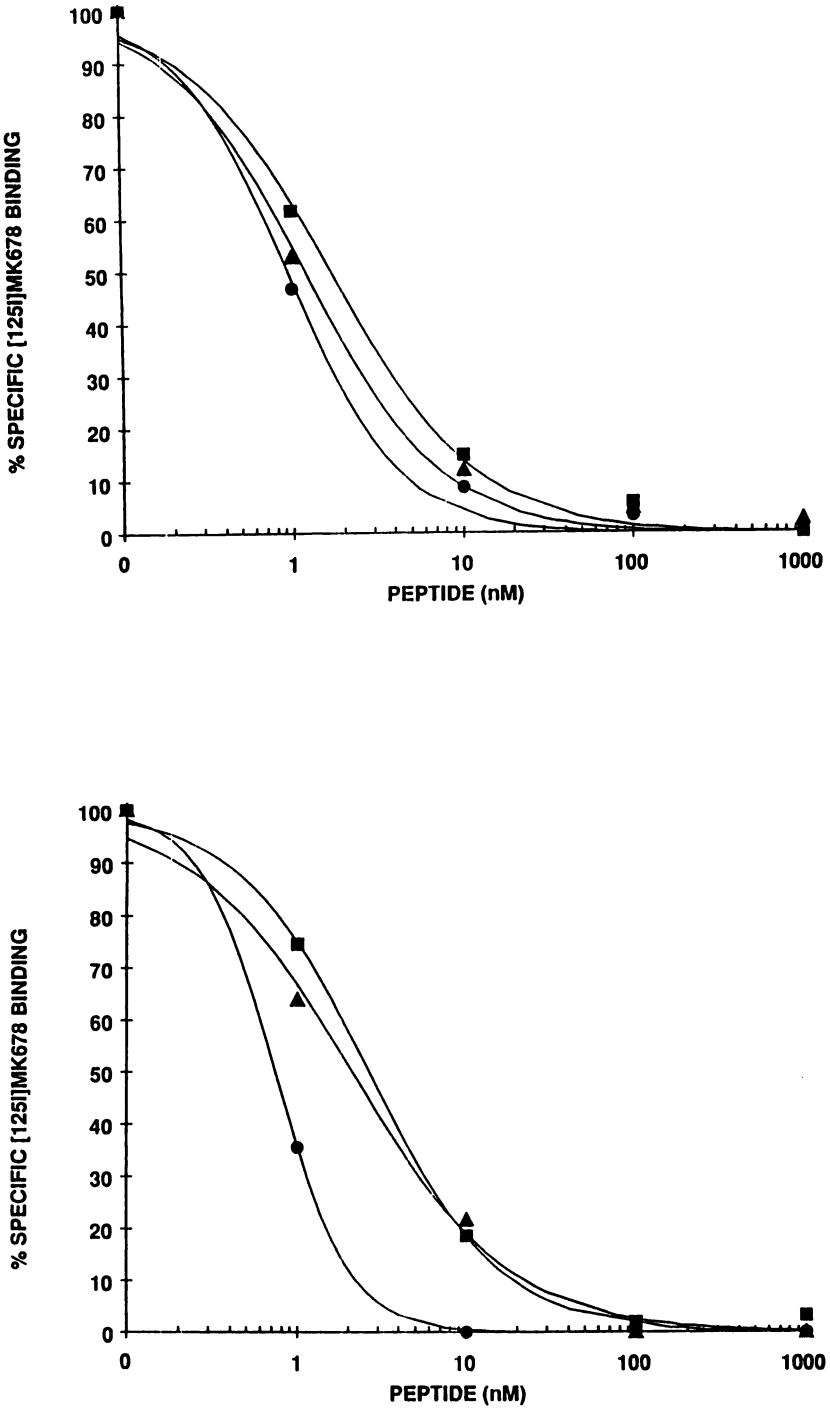

Fig. 3. Specificity of ^{125}I-MK 678 binding to the solubilized and membrane-bound SRIF receptors. ^{125}I-MK 678 (20 pM binding to the solubilized (*top*) and membrane-bound (*lower*) SRIF receptor was inhibited by various concentrations of D-Trp^8-SRIF (●), SRIF (▲), and somatostatin-28 (■). The results of a representative experiment repeated 2 times are presented. The average IC_{50} values in these experiments, derived from the displacement curves, are: solubilized, D-Trp^8-SRIF = 1.0 ± 0.2 nM, SRIF = 1.2 ± 0.3 nM, and somatostatin-28 = 1.7 ± 0.3 nM, membrane-bound, D-Trp^8-SRIF = 0.8 ± 0.2 nM, SRIF = 2.1 ± 0.4 nM, and somatostain-28 = 2.7 ± 0.3 nM. Somatostatin-28[1–14], Met-enkephalin, Leu-enkephalin, cholecystokinin, vasopressin, angiotensin II, and corticotropin releasing factor, at a concentration of 1 μM, did not affect ^{125}I-MK 678 binding to either the solubilized or membrane-bound SRIF receptor.

migrate with purified G_i and G_o that were run in adjacent lanes in the SDS gel (Fig. 7, *lane B*), transferred to the nitrocellulose membranes, and detected with antiserum 1398 by immunoblotting (Fig. 7, *lane D*).

To further identify the G protein coupled to the solubilized SRIF receptor, attempts were made to immunoprecipitate the SRIF receptor/G protein complex with antiserum 8730. Antiserum 8730 was generated by immunizing a rabbit against a synthetic peptide that corresponds to a fragment of the C-terminal region of $G_{i\alpha}$ (19). Antibody 8730 has been previously shown to selectively and specifically immunoprecipitate $G_{i\alpha}$ (19). For these studies, the size-fractionated solubilized SRIF receptor was reacted with either the antiserum or control nonimmune serum overnight and then precipitated with Protein A-Sepharose CL-4B beads. The SRIF receptor binding activity remaining in solution was then measured. Antiserum 8730 immunoprecipitated 24.4 ± 1.8% of the solubilized SRIF receptor. This is the average ± standard error reduction in specific SRIF receptor binding activity, compared with control nonimmune serum-treated samples, of four different experiments done in duplicate tubes. A representative example of one of those experiments is presented in Table 1. Antiserum 8730 reduced the amount of total and specific ^{125}I-MK 678 binding remaining in solution but did not affect nonspecific binding.

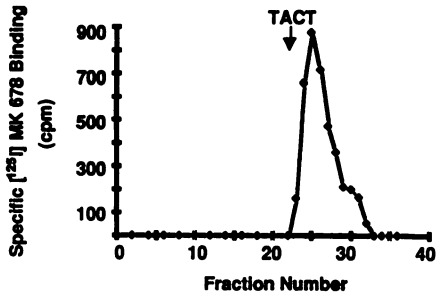

Fig. 4. Selective binding of solubilized SRIF receptors to WGA-Sepharose. Solubilized SRIF receptors were applied to a WGA-Sepharose affinity column. The proteins were allowed to equilibrate with the lectin column and then the column was extensively washed with buffer. Fractions were collected. Glycoproteins were eluted following the application of 4 mM TACT to the column and 0.8-ml fractions were collected. Aliquots of the fractions collected from the column before and after the addition of TACT were examined for specific ^{125}I-MK 678 binding. The data presented are representative of three separate studies.

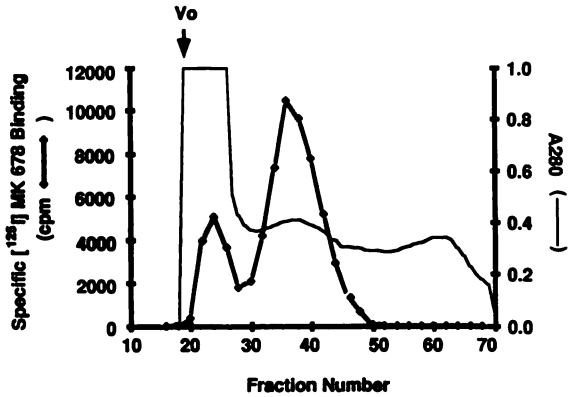

Fig. 5. Analysis of the size of the solubilized SRIF receptor by gel exclusion chromatography. Solubilized SRIF receptors were applied to a AcA 34 Ultrogel column, as described in Experimental Procedures. Fractions (1.2 ml) were collected and the protein content (A_{280}) and specific ^{125}I-MK 678 binding were measured in each fraction. Void volume (V_o) was determined using blue dextran and the column was calibrated with ferritin (M_r 440,000), catalase (M_r 232,000), aldolase (M_r 158,000), and ovalbumin (M_r 43,000).

The relatively small amount of immunoprecipitation of SRIF receptor binding activity by antiserum 8730 may be due to the limited amount of G_{i_a} that this antiserum is capable of precipitating. In previous studies in human platelets, antiserum 8730 was found to maximally precipitate only 38% of the G_{i_a} (19). However, the loss of ^{125}I-MK 678 binding following treatment with an antiserum that selectively interacts with G_{i_a} suggests that the solubilized SRIF receptor is coupled to G_{i_a}.

Discussion

The solubilization of SRIF receptors should allow for a more extensive identification of the molecular mechanisms by which SRIF induces its biological actions. The present study describes a procedure by which SRIF receptors can be solubilized in an active form. The receptors were solubilized with the nondenaturing, zwitterionic detergent CHAPS. The solubilized receptors were detected with the newly developed SRIF analog ^{125}I-MK 678. ^{125}I-MK 678 bound to the solubilized SRIF receptor selectively and with high affinity. The characteristics of the binding of ^{125}I-MK 678 to the solubilized and membrane-bound SRIF receptor were similar, suggesting that the agonist binding

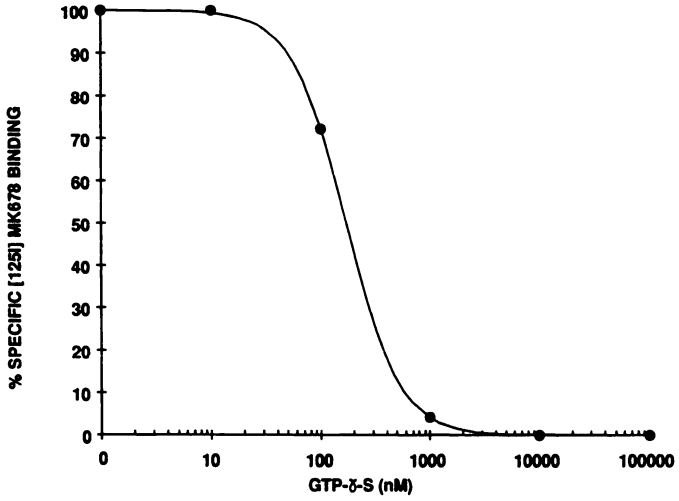

Fig. 6. GTPγS inhibition of specific ^{125}I-MK 678 binding of the solubilized SRIF receptor. The effect of varying concentrations of GTPγS on specific ^{125}I-MK 678 binding to the solubilized SRIF receptor was tested. An experiment representative of three separate studies is presented. One hundred micromolar GDP and ATP did not affect specific ^{125}I-MK 678 (20 pM) binding to the solubilized SRIF receptor.

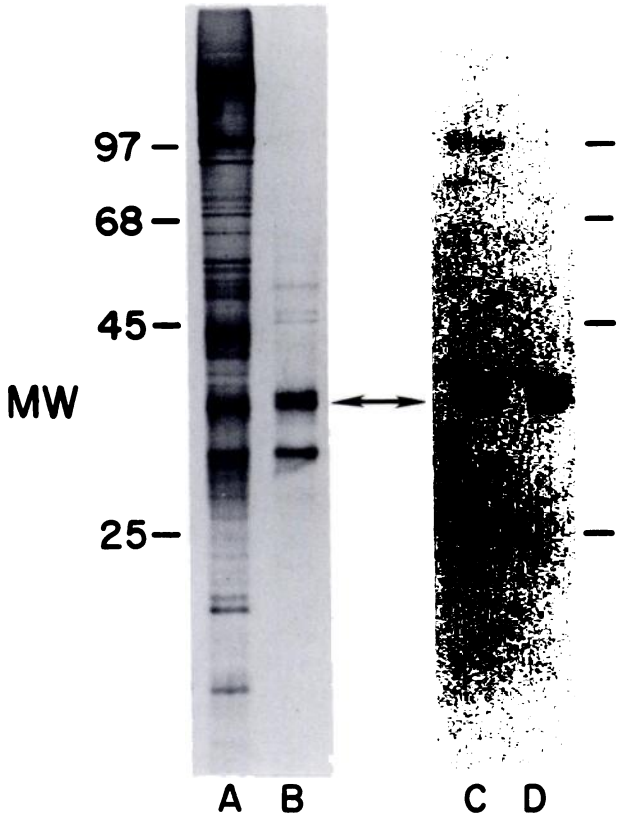

Fig. 7. Identification of G proteins co-migrating with the 400-kDa solubilized SRIF receptor. The fractionated (pooled fractions 32–43 depicted in Figure 5) 400-kDa solubilized SRIF receptor eluted from the gel exclusion column (described in Figure 5) was subjected to SDS-PAGE (*lane A*) and transferred to nitrocellulose membranes (*lane C*). Purified G_o/G_i was also subjected to SDS-PAGE (*lane B*) and transferred to nitrocellulose membranes (*lane D*). The proteins in the SDS gel were identified by silver stain and the proteins bound to the nitrocellulose membranes were screended with antiserum 1398. The *arrow* indicates where the purified α subunits of G_o and G_i migrated in the SDS gels and were detected on the nitrocellulose membranes by Western blotting.

TABLE 1

Immunoprecipitation of solubilized SRIF receptor binding activity with an antiserum directed against G_{ia}

This is a representative example of one experiment that was repeated three other times. Immunoprecipitation experiments were performed as described in Experimental Procedures. SRIF receptor binding activity remaining in solution after the immunoprecipitation is presented. Values represent ^{125}I-MK 678 binding to solubilized SRIF receptors. Nonspecific binding refers to the binding remaining in the presence of 1 μM D-Trp^8-SRIF.

^{125}I-MK 678 binding	Control	Antiserum-treated
	cpm	
Total	6335	5594
Nonspecific	3115	3142
Specific	3220	2452

site of the SRIF receptor was not altered following solubilization. To our knowledge, this is the first report on the solubilization of the SRIF receptor in an active form.

Membrane-bound SRIF receptors are known to couple with different cellular effector systems via G protein (3–5, 21, 22). The coupling of SRIF receptors and G proteins appears to be maintained following solubilization, because GTPγS completely abolished ^{125}I-MK 678 binding to the solubilized SRIF receptor. Other hormone receptors have also been shown to retain their coupling to G proteins following solubilization (23, 24). The maintenance of coupling of SRIF receptors to G proteins most likely allows for the detection of the solubilized receptors with ^{125}I-MK 678. Otherwise, the affinity of the SRIF receptor for agonists, such as ^{125}I-MK 678, would be too low to be measured. No SRIF receptor antagonist is available to detect the SRIF receptors uncoupled from G proteins.

The solubilized SRIF receptor appears to be complexed with other proteins or molecules. This is indicated by the large size (400 kDa) of SRIF receptor binding activity, as detected by gel exclusion chromatography. Previously, Knuhtsen *et al.* (7) reported that prelabeled SRIF receptors solubilized from pancreatic acinar membranes also migrated as a mass of 400 kDa. Analysis of the size of the SRIF receptor from brain under denaturing conditions indicates that the receptor migrates as a mass of 60 to 70 kDa (8, 11). The larger mass of the SRIF receptor binding activity detected in the present study and that of Knuhtsen *et al.* (7) most likely indicates that the solubilized receptor is noncovalently linked to other proteins, other cellular material, or detergents.

Results from a number of studies suggest that SRIF receptors are coupled to G proteins (3–5, 21, 22). The identity of the species of G protein coupled to the SRIF receptors has not been previously established. Several studies have suggested that brain SRIF receptors couple to both G_i and G_o, because SRIF can inhibit adenylyl cyclase activity as well as regulate multiple ionic conductance channels (3–5, 19, 22, 25–27). Our findings indicate that one of the G proteins to which brain SRIF receptors are coupled is G_i. This was first suggested by the finding that fractionation of the solubilized SRIF receptor by gel exclusion chromatography and analysis of the proteins by SDS-PAGE and immunoblotting with antiserum 1398, which is selective for the α subunit of G proteins, revealed that proteins of 39 and 40 kDa co-migrated with active solubilized SRIF receptors. This immunoreactive material migrated in SDS gels in a similar manner as G_i and G_o purified from bovine brain. It is conceivable that the G proteins detected by Western blots are associated with other neurotransmitter receptors or proteins co-migrating with the SRIF receptor and are not coupled to the SRIF receptor. However, we also found that antiserum 8730, which selectively interacts with G_{ia} and has been reported to specifically immunoprecipitate G_{ia} (19), immunoprecipitated SRIF receptor binding activity. Although these studies indicate that G_{ia} is associated with the brain SRIF receptor, they do not exclude the possibility that other G proteins are coupled to the receptor. In fact, antiserum 8730 only precipitated 24% of soluble SRIF receptor binding activity. This may be due in part to the limited amount (38%) of G_{ia} that antiserum 8730 can maximally precipitate (19) or it may indicate that other G proteins that antiserum 8730 does not react with, such as G_o, are coupled to the SRIF receptor. To our knowledge, this is the first direct demonstration that G_{ia} is coupled to the brain SRIF receptor.

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Send reprint requests to: Dr. Terry Reisine, Department of Pharmacology, University of Pennsylvania, School of Medicine, 36th Street and Hamilton Walk, Philadelphia, PA 19104.