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Agonist Binding to Rat Brain Somatostatin Receptors Alters the Interaction of the Receptors with Guanine Nucleotide-Binding Regulatory Proteins

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SUMMARY

To investigate the interaction of guanine nucleotide-binding regulatory proteins (G proteins) with the agonist-bound brain somatostatin (SRIF) receptor, rat brain SRIF receptor/G protein complexes were solubilized and immunoprecipitated with peptide-directed antisera selective for the different subtypes of G protein α subunits ($G_{i\alpha}$). In the absence of agonist, solubilized SRIF receptor/G proteins complexes could be immunoprecipitated by antiserum 8730, which is directed against the carboxyl-terminal region of $G_{i\alpha}$ and recognizes all $G_{i\alpha}$ subtypes, and by antiserum 3646, which selectively interacts with internal regions of $G_{i\alpha1}$. In contrast, antiserum 1521, which is directed against an internal region of $G_{i\alpha2}$, and antiserum 9072, which is directed against the carboxyl-terminal region of $G_{o\alpha}$, did not immunoprecipitate the SRIF receptor. After the binding of agonist to solubilized SRIF receptors, antisera 9072 and 1521, as well as antisera 8730 and 3646, were able to immunoprecipitate the agonist-bound SRIF receptor/G protein complexes, indicating that agonist interaction with SRIF receptors maintained receptor association with $G_{i\alpha1}$ and promoted receptor association with $G_{o\alpha}$ and, to a lesser extent, $G_{i\alpha2}$. Antiserum 1518, which is directed against $G_{i\alpha3}$, uncoupled SRIF receptors from $G_{i\alpha}$ and did not immunoprecipitate the agonist-bound or agonist-free brain SRIF receptor. These findings indicate that differences exist in the interaction of the agonist-free and agonist-bound SRIF receptors with G proteins. The binding of agonists to SRIF receptors promotes the association of the receptor with $G_{o\alpha}$ and, to a lesser extent, $G_{i\alpha2}$, indicating that these G proteins, along with $G_{i\alpha1}$ and $G_{i\alpha3}$, may be involved in coupling SRIF receptors to cellular effector systems.

Many neurotransmitter receptors are coupled to cellular effector systems via G proteins (1–3). It has been hypothesized that the binding of agonists to a membrane-associated receptor can induce conformational changes in the receptor to activate G proteins (1–3). This activation causes an increase in the GDP/GTP exchange rate of the α subunit of the G protein heterotrimer and the dissociation of the α subunit from the β/γ complex. Such dissociation frees the α subunit and the β/γ complex to interact with cellular effector systems, such as the catalytic subunit of adenylyl cyclase, other enzymes including phospholipase C, or ion channels (1–3). Although experimental evidence exists that, after receptor activation, the GDP/GTP exchange rate increases and the β/γ complex dissociates from the α subunit, relatively little is known about the physical interaction of receptors with specific G proteins after agonist binding.

Receptors for the neurotransmitter SRIF are coupled to pertussis toxin-sensitive G proteins (4, 5), and recent studies have shown that selectivity exists in the G proteins associated with the receptor (6). After solubilization of rat brain SRIF receptors under conditions in which G proteins remain coupled to the receptor, we reported that antiserum directed against the carboxyl-terminal region of $G_{i\alpha}$ could immunoprecipitate SRIF receptor/$G_{i\alpha}$ complexes (6). Using peptide-directed antisera selective for different $G_{i\alpha}$ subtypes as well as $G_{o\alpha}$, it was observed that brain SRIF receptors are tightly associated with $G_{i\alpha1}$, since SRIF receptor/$G_{i\alpha1}$ complexes could be immunoprecipitated, weakly associated with $G_{i\alpha3}$ since antisera selective for $G_{i\alpha3}$ uncoupled the receptor from $G_{i\alpha3}$ but not coupled to $G_{i\alpha2}$ or $G_{o\alpha}$, since antisera against these subunits neither immunoprecipitated SRIF receptor/G protein complexes nor uncoupled the receptors from G proteins, even though $G_{i\alpha2}$ and $G_{o\alpha}$ immunoreactivity is present in the solubilized brain samples and the antisera directed against these G protein α subunits immunoprecipitate G_{α}- as well as $G_{i\alpha1}$-directed antisera (6).

Although $G_{o\alpha}$ association with SRIF receptors from rat brain

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ABBREVIATIONS: G protein, guanine nucleotide-binding regulatory protein; G_i, inhibitory regulator of adenylyl cyclase; SRIF, somatostatin-14; G_o, guanine nucleotide-binding protein isolated from brain; G_{α}, α subunit of guanine nucleotide-binding proteins; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

was not detected previously (6), recent electrophysiological studies (7) have reported that $G_{o\alpha}$ functionally couples SRIF receptors to Ca^{2+} channels in GH_3 cells, suggesting that $G_{o\alpha}$ interacts with SRIF receptors and is involved in mediating this important physiological action of SRIF. Differences in the results of biochemical and physiological studies have also been reported for α_2 receptor/G protein interactions, because $G_{o\alpha}$ has been shown to mediate α_2 receptor inhibition of Ca^{2+} conductance (8), whereas reconstitution studies with purified α_2 receptors and recombinant $G_{o\alpha}$ showed no stable association (9). The discrepancies observed in the biochemical and electrophysiological studies on SRIF receptors may be due to differences in the methodology used to study SRIF receptor/$G_{o\alpha}$ interactions. In particular, the electrophysiological studies (7) investigated the agonist-occupied SRIF receptor, whereas the biochemical studies (6) examined $G_{o\alpha}$ coupling to the agonist-free SRIF receptor. Therefore, it is important to test whether the biochemical approach used previously (6) can detect agonist-induced changes in the association of G protein subunits with the SRIF receptor.

To investigate whether the binding of SRIF to brain receptors affects the interaction of the receptor with different α subunits, we have solubilized rat brain SRIF receptor/G protein complexes and treated them with agonist. Then, using our immunoprecipitation approach, we tested whether the agonist-occupied receptors form stable complexes with different $G_{i\alpha}$ and $G_{o\alpha}$ subunits. We report that agonist binding to solubilized rat brain SRIF receptors promotes the association of the receptor with $G_{i\alpha}$, and, to a lesser extent, $G_{i\alpha2}$, while maintaining its coupling to $G_{i\alpha1}$. These findings suggest that agonist binding to SRIF receptors modifies the conformation of the receptor, such that it is able to interact with G proteins with which it does not associate in the absence of agonist.

Experimental Procedures

Materials. For these studies, frozen rat brains were obtained from Pel Freeze. AcA22 was obtained from IBF Biochemicals, and Centriprep 30 was purchased from Amicon. MK 678 was obtained from Dr. D. Veber at Merck (Rahway, NJ) and was iodinated as previously described (10). Trp^8-SRIF was purchased from Bachem (Torrence, CA).

Preparation of the solubilized SRIF receptor from rat brain. The SRIF receptor was solubilized from three rat brains minus cerebellums, as previously described (6). The sample was centrifuged at 100,000 × *g* for 60 min at 4°. After centrifugation, the supernatant was removed and loaded onto an Ultrogel AcA22 gel exclusion column. The running buffer for the column consisted of 50 mM Tris (pH 7.8), 1 mM EGTA, 5 mM $MgCl_2$, 10% glycerol, and 5 mM CHAPS (6). The column dimensions were 36 × 1.6 cm, with a flow rate of 6.0 ml/hr at 4°. The eluted fractions were analyzed for specific ^{125}I-MK 678 binding to solubilized SRIF receptors. The peak of specific ^{125}I-MK 678-binding activity, which eluted at a position corresponding to approximately 400 kDa, was pooled and concentrated with Centriprep 30. This sample was subsequently used in the SRIF receptor immunoprecipitation studies. This sample contains $G_{i\alpha1}$, $G_{i\alpha2}$, $G_{i\alpha3}$, and $G_{o\alpha}$ immunoreactivity (6).

^{125}I-MK 678 binding assay. The presence of solubilized SRIF receptors was detected with the stable SRIF analog ^{125}I-MK 678, which was iodinated to a specific activity of 2200 Ci/mmol (10). Solubilized SRIF receptors were incubated with 20 pM ^{125}I-MK 678, in a total volume of 0.4 ml of buffer A (6). Nonspecific binding of ^{125}I-MK 678 was determined as the amount of binding remaining in the presence of 1 μM D-Trp^8-SRIF (6, 7) and accounted for <20% of total ^{125}I-MK 678 binding. The binding reaction was carried out at 25° for 90 min. Under these conditions, the binding reaction reaches equilibrium. The binding reaction was terminated by the addition of 9 ml (three consecutive additions of 3 ml) of 50 mM Tris·HCl, pH 7.8, at 4°. The samples were vacuum filtered over Whatman (GF/F) glass fiber filters, which had been presoaked in 0.5% polyethylenimine at 4°. The filters were dried and radioactivity was measured in a γ counter (80% efficiency). A similar procedure was used to detect immunoprecipitated SRIF receptors.

G protein-directed antisera. The G protein-directed antisera used were all obtained after injection of synthetic peptides, corresponding to specific regions of the G_α, into rabbits. Antiserum 8730 is directed against a carboxyl-terminal region of $G_{i\alpha}$ and selectively detects and immunoprecipitates $G_{i\alpha1}$, $G_{i\alpha2}$, and, to a lesser extent, $G_{i\alpha3}$ (11). Antisera 3646 (anti-$G_{i\alpha1}$) and 1521 (anti-$G_{i\alpha2}$) are made to the same internal region of $G_{i\alpha}$, which is divergent in sequence for $G_{i\alpha1}$ and $G_{i\alpha2}$. The antisera are selective on Western blots in detecting recombinant $G_{i\alpha}$ subtypes (11, 12). Antiserum 1518 specifically interacts with $G_{i\alpha3}$, as shown by its selectivity for recombinant $G_{i\alpha3}$ (12). Antiserum 9072 (anti-$G_{o\alpha}$) is directed against the same region (peptide ANNLRGCGLY) of G_α, which differs in sequence between $G_{o\alpha}$ and $G_{i\alpha}$, as is 8730 (peptide KNNLKDCGLF). It selectively detects recombinant forms of $G_{o\alpha}$ by Western blotting.[1] Antiserum 2353 is directed against an internal region of $G_{o\alpha}$ and selectively interacts with $G_{o\alpha}$ (6, 11, 12). Antisera 8730, 3646, 1521, 1518, 9072, and 2353 were used at a dilution of 1/20, which yielded maximal immunoprecipitation of the G protein subunit under study as well as SRIF receptor/G protein complex.

Immunoprecipitation of SRIF receptor/G protein complexes. The fractionated brain SRIF receptor/G protein complex (obtained from gel exclusion chromatography, as described above) was incubated with an aliquot of G protein-specific antiserum for 4–6 hr and, as with all subsequent steps, the samples were placed on a rotator at 4°. One hundred microliters of 50% (w/v) Protein A-Sepharose beads (CL-4B; Sigma), washed three times and diluted in buffer A, were added to the sample and incubated overnight. Another aliquot of antiserum was then added, bringing the total antiserum dilution to 1/20, which was the optimal concentration of antiserum needed to immunoprecipitate both G protein and SRIF receptor coupled to G protein. At higher concentrations of the antisera, no further immunoprecipitation of SRIF receptor/G protein complexes were observed. The samples were incubated for an additional 3 hr and then centrifuged at 10,000 rpm for 2 min, in an Eppendorf microcentrifuge. The immunoprecipitate was resuspended in buffer A and centrifuged as described above. The supernatant was then discarded, the immunoprecipitate was resuspended in buffer A, and the presence of SRIF receptors was detected using the ^{125}I-MK 678 binding assay, as described above. Data were analyzed by paired *t* test, using the Number Cruncher Statistical Systems, version 501 (Kaysville, UT).

Agonist treatment of solubilized SRIF receptors. To determine the effect that agonist binding to SRIF receptors has on the coupling of the receptors to different G protein subunits, subfractionated, solubilized, rat brain SRIF receptors were treated with or without 10 μM D-Trp^8-SRIF for 1 hr at room temperature, as previously described (6). The reaction mixture was constantly shaken on a rotator. A 1/20 dilution of the different antisera was added to the sample, which was rotated at 4° for 4–6 hr. One hundred microliters of 50% (w/v) Protein A-Sepharose were added, and the mixture was incubated overnight under the same conditions as described above. The sample was centrifuged at 10,000 rpm for 2 min, in an Eppendorf microcentrifuge, and the supernatant was removed. The immunoprecipitate was resuspended in buffer A and incubated on a rotator at room temperature for 15 min. The sample was then centrifuged again at 10,000 rpm for 2 min, and the supernatant was removed. The immunoprecipitate was resuspended and incubated in the same manner as described above. This cycle of

[1] D. Manning, personal communication.

washing was repeated one more time, so that the immunoprecipitates containing SRIF receptors pretreated with agonist were washed a total of three times, to completely remove any D-Trp^8-SRIF from the immunoprecipitate, and then the immunoprecipitate was assayed for the presence of SRIF receptors by using the ^{125}I-MK 678 binding assay. Similar washing procedures were used for immunoprecipitates containing SRIF receptors that had not been pretreated with agonists, to ensure that any differences in ^{125}I-MK 678 binding to the receptors in the immunoprecipitates were not due to the washing procedure.

Results

To investigate the interaction of SRIF receptors with G proteins, rat brain SRIF receptors were solubilized and partially purified as described previously (6). The receptor migrates with a mass of 400 kDa and contains all the pertussis toxin-sensitive G proteins (6). The solubilized receptors were then incubated with antiserum 8730, which is directed against the carboxy-terminal region of $G_{i\alpha}$ and recognizes all forms of $G_{i\alpha}$ (11). In agreement with previous findings (6), antiserum 8730 immunoprecipitated SRIF receptors, as indicated by the appearance of specific ^{125}I-MK 678 binding sites in the immunoprecipitate (Fig. 1A). However, the anti-$G_{o\alpha}$ antiserum 9072 did not significantly immunoprecipitate rat brain SRIF receptors (Fig. 1A).

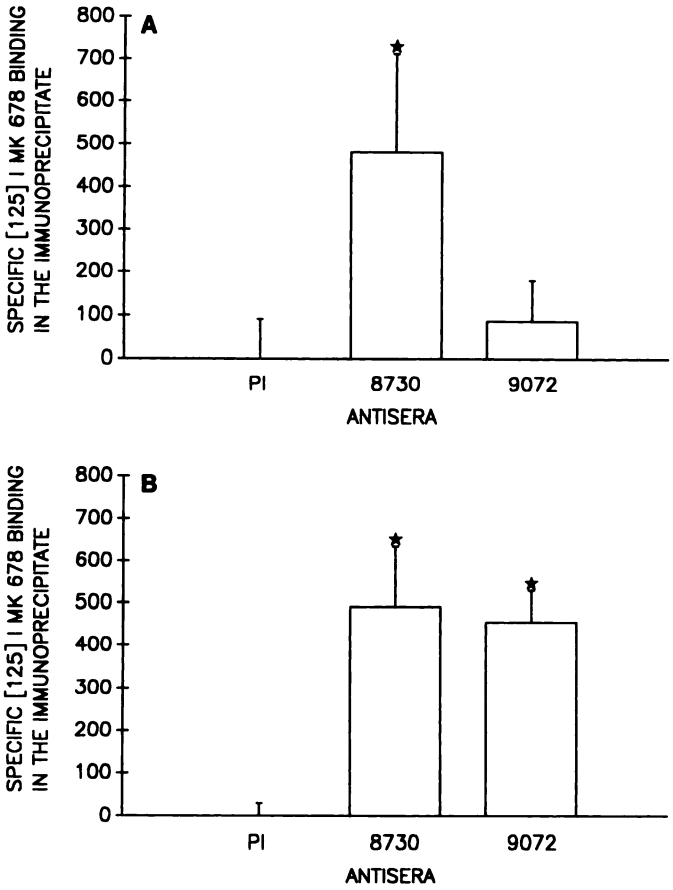

Fig. 1. Binding of agonists to brain SRIF receptors promotes association of the receptor with $G_{o\alpha}$. Solubilized brain SRIF receptors were treated with (B) or without (A) 10 μM D-Trp^8-SRIF for 1 hr and then immunoprecipitated with either the anti-$G_{i\alpha}$ antiserum 8730 or the anti-$G_{o\alpha}$ antiserum 9072 (1/20 final dilution for each). The immunoprecipitates were washed and SRIF receptor/G protein complexes were detected using the ^{125}I-MK 678 binding assay. Values (in cpm) are the means ± standard errors of 10 different experiments. *, Values significantly different ($p < 0.05$) from preimmune (*PI*) control values.

Antiserum 9072 is directed against the same region of G_α as is antiserum 8730, but it is selective for $G_{o\alpha}$. Its selectivity is shown by its inability to cross-react with $G_{i\alpha}$ in 293 cell membranes, which is similar to the results obtained with another $G_{o\alpha}$ antiserum, 2353 (Fig. 2). The results obtained with antiserum 9072 are in agreement with our previous findings that antiserum 2353 did not immunoprecipitate rat brain SRIF receptors (6). After the addition of 10 μM Trp^8-SRIF to the solubilized rat brain SRIF receptors, antiserum 9072 was able to immunoprecipitate a similar amount of G protein-coupled SRIF receptor as was antiserum 8730 (Fig. 1B). The effect of antiserum 9072 was maximal at the concentration of antiserum used and was additive with that of antiserum 8730 (Fig. 3), indicating that the antisera immunoprecipitated different populations of SRIF receptor/G_α complexes. These findings suggest that agonist binding to the solubilized SRIF receptor does not alter $G_{i\alpha}$ associated with the receptor so that anti-$G_{o\alpha}$ antiserum 9072 recognizes it; instead, they show that agonist binding to solubilized SRIF receptors promotes the association of the receptor with $G_{o\alpha}$. Because the association does not exist with the agonist-free SRIF receptor, the results indicate that agonist binding to the receptor increases the affinity of the receptor for $G_{o\alpha}$ present in the solubilized sample.

In previous studies, we showed that differences existed in the ability of antisera directed against the subtypes of $G_{i\alpha}$ to immunoprecipitate brain SRIF receptors (6). Antiserum 3646, which is directed against $G_{i\alpha1}$, immunoprecipitated a similar amount of SRIF receptor/G_α complex as did antiserum 8730. In contrast, antiserum 1521, which is selective for $G_{i\alpha2}$, did not immunoprecipitate SRIF receptors, even though antisera 3646 and 1521 are equally effective in immunoprecipitating $G_{i\alpha}$ from the solubilized brain sample (6). Similar results were obtained in the present study (Table 1). However, agonist binding to the solubilized SRIF receptor facilitated the ability of antiserum 1521 to immunoprecipitate SRIF receptor/$G_{i\alpha}$ complexes (Table 1). It should be noted that, whereas antiserum 1521 could

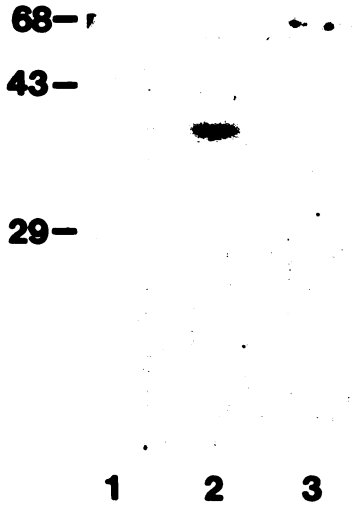

Fig. 2. Anti-$G_{o\alpha}$ antiserum 9072 does not cross-react with $G_{i\alpha}$. To test whether the carboxyl terminus-directed anti-$G_{o\alpha}$ antiserum 9072 cross-reacts with $G_{i\alpha}$, membranes from 293 cells, which lack $G_{o\alpha}$ but express $G_{i\alpha}$, were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, as described (6), and were reacted with antiserum 2353 (anti-$G_{o\alpha}$) (*lane 1*), 8730 (anti-$G_{i\alpha}$) (*lane 2*), or 9072 (anti-$G_{o\alpha}$) (*lane 3*). All antisera were used at a dilution of 1/100. Size markers (kDa) are to the *left* of the immunoblots.

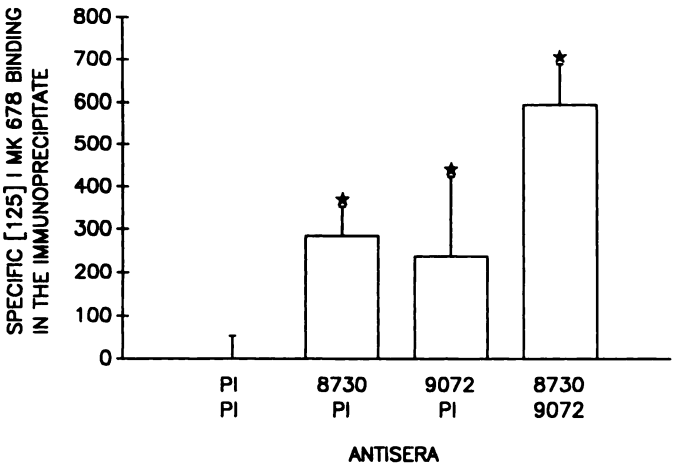

Fig. 3. Antisera 8730 and 9072 are additive in immunoprecipitating the agonist-bound brain SRIF receptor. To test whether antisera 8730 and 9072 immunoprecipitate different populations of agonist-bound brain SRIF receptors, solubilized receptors were treated with D-Trp^8-SRIF for 1 hr and were then immunoprecipitated with either antiserum 8730, antiserum 9072, or their combination. Preimmune (*PI*) control serum was added to some samples to equalize the total serum content (final dilution of 1/10) of each sample. The immunoprecipitates were washed, and then the presence of SRIF receptors was detected with the ^{125}I-MK 678 binding assay. Note that the level of specific ^{125}I-MK 678 binding in the 8730 and 9072 immunoprecipitates is lower in these studies than in those presented in Fig. 1. This is due to the greater total amount of serum incubated with the receptors in these studies, which test the additive ability of 8730 and 9072 to immunoprecipitate the receptor. Such large amounts of serum can nonselectively lower specific ^{125}I-MK 678 binding. Values (in cpm) are the means ± standard errors of four different experiments. *, Values significantly different ($p < 0.05$) from preimmune values.

TABLE 1

Agonist binding to solubilized brain SRIF receptors facilitates the association of the receptor with $G_{i\alpha 2}$, but does not affect association with $G_{i\alpha 3}$.

Solubilized SRIF receptors were treated either with (+D-Trp^8) or without (−D-Trp^8) D-Trp^8-SRIF and then were immunoprecipitated with either preimmune serum or the anti-$G_{i\alpha 1}$ (3646), anti-$G_{i\alpha 2}$ (1521), or anti-$G_{i\alpha 3}$ (1518) antisera. The presence of SRIF receptors was detected in the immunoprecipitate by using the ^{125}I-MK 678 binding assay. Values are the means ± standard errors of ^{125}I-MK 678 binding, of seven different experiments.

Antisera	^{125}I-MK 678 binding	
	+D-Trp^8	−D-Trp^8
	cpm	
Preimmune	0 ± 24	0 ± 50
3646	833 ± 251	545 ± 290
1521	306 ± 114	0 ± 48
1518	219 ± 103	138 ± 151

immunoprecipitate the agonist-bound SRIF receptor, antiserum 3646 immunoprecipitated much more SRIF receptor, suggesting that, even after agonist binding to the receptor, $G_{i\alpha 1}$ is the predominant $G_{i\alpha}$ subtype coupled to SRIF receptors. As an additional control for these studies, attempts were made to immunoprecipitate solubilized SRIF receptors with antiserum 1518, which is directed against $G_{i\alpha 3}$. In previous studies, this antiserum was found to uncouple brain SRIF receptors from G proteins but not to immunoprecipitate SRIF receptor/$G_{i\alpha 3}$ complexes (6). Similar results were observed in the present study, and agonist binding to the SRIF receptor did not facilitate the ability of antiserum 1518 to immunoprecipitate SRIF receptors (Table 1). The results of these studies indicate that agonist binding to the SRIF receptor promotes the association of the receptor with $G_{i\alpha 2}$, while maintaining its coupling to $G_{i\alpha 1}$ and not affecting its association with $G_{i\alpha 3}$.

Discussion

Our previous findings indicated that, in the absence of agonist, brain SRIF receptors are coupled to $G_{i\alpha 1}$ and $G_{i\alpha 3}$ but not $G_{o\alpha}$ or $G_{i\alpha 2}$, even though $G_{o\alpha}$ and $G_{i\alpha 2}$ are present in solubilized brain samples and are freely accessible to the SRIF receptor (6). The results of our present study show that the binding of agonists to the SRIF receptor promotes the association of the receptor with $G_{o\alpha}$ and $G_{i\alpha 2}$, possibly by increasing the affinity of the receptor for these α subunits. Thus, after the binding of SRIF to its receptor, the receptor is able to couple with all the subtypes of $G_{i\alpha}$, as well as $G_{o\alpha}$.

Agonist binding to the SRIF receptor could induce conformational changes in the receptor so that it is able to associate with $G_{o\alpha}$ and $G_{i\alpha 2}$. This hypothesis would suggest that $G_{i\alpha 2}$ and $G_{o\alpha}$ have different contact sites with the SRIF receptor than do $G_{i\alpha 1}$ and $G_{i\alpha 3}$. This is possible, because $G_{i\alpha 1}$ and $G_{i\alpha 3}$ share the highest degree of amino acid sequence similarity of any pertussis toxin-sensitive G proteins (13). Alternatively, $G_{i\alpha 2}$ and $G_{o\alpha}$ may be coupled to the agonist-free SRIF receptor but the epitopes for antisera 1521 (anti-$G_{i\alpha 2}$) and 9072 (anti-$G_{o\alpha}$) are hidden and are exposed only after agonist binding to the SRIF receptor, due to conformational changes in $G_{i\alpha 2}$ and $G_{o\alpha}$. This seems unlikely, because the epitopes of antisera 3646 (anti-$G_{i\alpha 1}$) and 1521 (anti-$G_{i\alpha 2}$), as well as 8730 (anti-$G_{i\alpha}$) and 9072 (anti-$G_{o\alpha}$), are the same, and antisera 3646 and 8730 can immunoprecipitate agonist-free SRIF receptors. Furthermore, it is possible that agonist binding to solubilized SRIF receptors could propagate conformational changes in $G_{i\alpha 1}$ associated with the receptor, such that antisera 9072 and 1521 can recognize the SRIF receptor/$G_{i\alpha 1}$ complex. This seems unlikely, because antisera 9072 and 8730 were additive in their ability to immunoprecipitate agonist-bound SRIF receptors, indicating that the antisera immunoprecipitated distinct populations of SRIF receptor/G protein complexes. Furthermore, it is unlikely that antiserum 1521 immunoprecipitated SRIF receptor/$G_{i\alpha 1}$ complexes, because antisera 1521 and 3646 are equally effective in immunoprecipitating pertussis toxin-sensitive G proteins from the brain sample (6) but antiserum 3646 immunoprecipitates 4-fold more SRIF receptor than does antiserum 1521.

The agonist-bound SRIF receptor is capable of coupling equally well with $G_{i\alpha}$ and $G_{o\alpha}$. However, differences exist in the association of $G_{i\alpha}$ subtypes with the receptor, because $G_{i\alpha 1}$-directed antiserum was capable of immunoprecipitating much more SRIF receptor/$G_{i\alpha}$ complex than the antisera directed against the other $G_{i\alpha}$ subtypes. This is not due to differences in the ability of the antisera to immunoprecipitate G_α (6) but, instead, is likely to reflect a tighter association of $G_{i\alpha 1}$ with the SRIF receptor, compared with either $G_{i\alpha 2}$ or $G_{i\alpha 3}$. Recent studies (14) have shown that $G_{i\alpha 1}$ plays a critical role in the functioning of SRIF receptors, because inactivation of $G_{i\alpha 1}$ with the peptide-directed antiserum 3646 blocked SRIF inhibition of adenylate cyclase activity, whereas inactivation of $G_{i\alpha 2}$, $G_{i\alpha 3}$, and $G_{o\alpha}$ with peptide-directed antisera did not. This finding indicates that $G_{i\alpha 1}$ selectively couples SRIF receptors to adenylyl cyclase.

The finding that agonist binding to brain SRIF receptors promotes its stable association with $G_{o\alpha}$ is the first biochemical evidence that $G_{o\alpha}$ couples with brain SRIF receptors and is also

consistent with the findings of recent electrophysiological studies, which have shown that $G_{o\alpha}$ selectively couples SRIF receptors to Ca^{2+} channels in GH_3 cells (7) and that SRIF receptors in brain neurons are coupled to voltage-dependent Ca^{2+} channels via pertussis toxin-sensitive G proteins (15). $G_{i\alpha 1}$ and $G_{o\alpha}$, therefore, selectively couple SRIF receptors to distinct cellular effector systems. These findings indicate that different G proteins may couple SRIF receptors to different cellular effector systems and contribute to diversifying the cellular actions of SRIF.

Recent studies by Murray-Whelan and Schlegel (16) have also investigated the interaction of brain SRIF receptors with G proteins. These investigators solubilized brain SRIF receptor/G protein complexes and tested different carboxy terminus-directed antisera for their ability to uncouple SRIF receptors from G proteins, as measured by a reduction in radiolabeled agonist (^{125}I-MK 678) binding to the receptors. They reported that antiserum directed against $G_{i\alpha 3}$ uncoupled SRIF receptors from $G_{i\alpha 3}$, which is similar to our previous findings (6). In contrast, they showed that antiserum directed against $G_{o\alpha}$ did not uncouple SRIF receptors from $G_{o\alpha}$, even when agonist was bound to the receptor. They concluded from their studies that $G_{o\alpha}$ does not associate with SRIF receptors (16). This conclusion differs from our results and the results of electrophysiological studies (7) showing SRIF receptor/$G_{o\alpha}$ coupling in the presence of agonist. A major difference between the approaches used by Murray-Whelan and Schegel (16) and by us is that we were able to immunoprecipitate SRIF receptor/G protein complexes with G protein-directed antisera, whereas they did not. As a result, we were able to show that agonist binding to the solubilized SRIF receptor induced a stable coupling with $G_{o\alpha}$, so that the receptor/G protein complex could be immunoprecipitated with antiserum 9072. In fact, the complex is so stable that $G_{o\alpha}$-directed antiserum was not able to uncouple $G_{o\alpha}$ from the receptor. Thus, by using the immunoprecipitation approach, we were able to demonstrate SRIF receptor/$G_{o\alpha}$ complexes.

Thus, the present study shows, for the first time, that the binding of agonists to solubilized SRIF receptors promotes the association of the receptor with $G_{o\alpha}$ and $G_{i\alpha 2}$, while not affecting the interaction of the receptor with $G_{i\alpha 1}$ or $G_{i\alpha 3}$. It also reveals differences in the interaction of brain SRIF receptors with the subtypes of $G_{i\alpha}$, because the predominant $G_{i\alpha}$ coimmunoprecipitated with the receptor is $G_{i\alpha 1}$, only a relatively small proportion of the receptor stably associates with $G_{i\alpha 2}$, and the receptor interacts only weakly with $G_{i\alpha 3}$, because $G_{i\alpha 3}$-directed antiserum did not immunoprecipitate the SRIF receptor/$G_{i\alpha}$ complex but uncoupled SRIF receptor/$G_{i\alpha}$ complexes (6). Identification of the nature of the conformational changes induced in SRIF receptors, after agonist binding, to modify G protein interactions and a further understanding of the functional consequences of the differences in stability of the SRIF receptor/G protein complexes will aid in the elucidation of the molecular basis of SRIF receptor activation.

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