Solubilization and Characterization of Active Somatostatin Receptors from Rat Pancreas*

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Somatostatin receptors were solubilized from rat pancreatic membranes with the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS). The binding of an iodinated somatostatin analog [125I-Tyr3]SMS to the soluble fraction was time-dependent, saturable, and reversible. Scatchard analysis of equilibrium binding data indicated that the soluble extract contained a single class of somatostatin binding sites with a Kd of 0.3 nM and a Bmax of 210 fmol/mg. As observed with membrane-bound receptors, soluble binding receptors were sensitive to the GTP analog GTP-7 indicating that they are functionally linked to a G protein. A molecular weight of about 400,000 was determined for soluble receptors under native conditions by gel filtration. In denaturing gel electrophoresis, photofluorimetry labeling of soluble receptors identified a major protein of M, = 100,000 and two minor proteins of M, = 56,000 and 21,000. Isoelectric focusing of soluble receptors revealed that the somatostatin receptor is an acidic protein with pI 4.8. The soluble somatostatin receptor is a glycoprotein which can be specifically bound to the wheat germ agglutinin lectin and eluted by triacylchitotriose.

Somatostatin, a widely distributed peptide, has been found to exert various inhibitory effects on secretory processes in tissues such as pituitary, gastrointestinal tract, and pancreas (1-3). In addition, somatostatin could also act as an antiproliferative hormone (4, 5). The mechanisms by which somatostatin initiates the cellular response involves its interaction with specific high affinity receptors which have been characterized in various tissues (6-9). However, the biochemical events involved in somatostatin action after binding to its receptor are not clearly elucidated. Indeed, membrane somatostatin receptors are known to exert various inhibitory effects on secretory processes such as pituitary, gastrointestinal tract, and pancreas (10-12). In addition, somatostatin reduces intracellular calcium (13) and promotes protein dephosphorylation (14).

The biochemical components of the somatostatin receptor have been characterized by affinity labeling, and various cross-linking patterns have been observed suggesting tissue and/or species difference (15-17). In the pancreas, we have recently identified the somatostatin receptor as a monomeric glycoprotein with a M, = 90,000 (18). Binding of radiolabeled [Tyr3]SMS somatostatin analog to somatostatin receptor prior to its solubilization allowed preliminary molecular characterization of the solubilized [Tyr3]SMS-receptor complex (19).

In the present work, we reported the successful solubilization of active somatostatin binding sites from rat pancreas using the zwitterionic detergent CHAPS. Partial purification of the receptors by lectin adsorption chromatography has also been performed. The binding properties of this soluble receptor was characterized, and its functionality was supported by its interaction with G-proteins.

EXPERIMENTAL PROCEDURES AND RESULTS

This paper describes the first report of the solubilization and partial purification of the rat pancreatic somatostatin receptor in an active and stable state. Both the choice of the zwitterionic detergent CHAPS and the gel filtration of CHAPS extracts are crucial to obtain active pancreatic soluble receptors capable of retaining binding activity.

After solubilization under optimal conditions, soluble somatostatin receptors display many characteristics of the native receptors such as saturable, reversible, and sodium-dependent binding of radiolabeled somatostatin analog (7). Soluble receptors exhibit a single class of high affinity binding sites with an apparent dissociation constant 5-fold higher than that measured for membrane-bound receptors (19), but in the same range as that reported for other membrane somatostatin receptors (6). The number of binding sites that are solubilized corresponds to less than 10% of those detected in pancreatic membranes with the same tracer (19). This yield is weak but quite comparable to that obtained in other solubilization systems using the same detergent (24).

Striking differences in pharmacological properties between soluble and membrane-bound receptors have been observed. Indeed, membrane somatostatin receptors are known to ex-

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The abbreviations used are: GTP, inhibitory GTP-binding protein; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; ANB-NOS, N-5-azido-2-nitrobenzoyloxyxysuccinimide; WGA, wheat germ agglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TACT, N,N',N'-triacetychitotriose; BSA, bovine serum albumin; GTPyS, guanosine 5'-O-(3-thiotriphosphate); SMS, SMS 201-995.

2 Portions of this paper (including “Experimental Procedures,” “Results,” Figs. 1-10, and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
hibit different relative affinities for the biologically active somatostatin molecules, somatostatin and the analog somatostatin 28, depending on the tissues. In pancreatic acini (7, 15) and pituitary GH,C membranes (6), somatostatin receptors display higher affinity for somatostatin than for somatostatin 28. In contrast, in pituitary membranes (8), pancreatic β cells, and cerebral cortex membranes (9), somatostatin 28 is the most potent in displacing somatostatin binding. In the present study, we observed that soluble somatostatin receptors showed a greater selectivity for somatostatin 28 over somatostatin in contrast to that observed in pancreatic membrane-bound receptors (7, 15). Thus, the procedure of solubilization appears to induce conformational change in receptor molecules or dissociation of the receptors with other membrane components which might affect the selectivity of the receptors for agonists. Analogous changes in ligand affinities between membrane and soluble receptors have been previously reported (25, 26).

Another major observation of interest is that the newly hydrolyzable GTP analog GTP-S regulated the binding of [125l]-Tyr3-SMS to soluble receptors. It is well documented that somatostatin receptors are coupled to the adenylate cyclase system via the pertussis toxin-sensitive G-protein Gi in several target tissues such as pancreas (10, 11) pituitaries (12, 13), stomach (17) and brain (18). In this study, we observed that soluble somatostatin receptors were sensitive to GTP-S and Na+ ions, which are known to interact in a synergistic fashion to decrease the affinity of G-protein-coupled receptors (10, 23). These results suggest that receptor-associated G-proteins can be solubilized with somatostatin receptors. The high molecular weight of 400,000 observed by gel filtration chromatography of soluble somatostatin receptors probably represents aggregates of somatostatin receptor complexes due to the low detergent concentration required for preserving binding activity in solution. Covalent cross-linking of [125l]-Tyr3-SMS to soluble receptors and SDS-PAGE revealed the presence of a major specific band of an apparently molecular mass of 100 kDa. Minor species of 56 kDa and 21 kDa are also present; however, the intensity of these bands varied between experiments. Whether or not these two minor bands represent proteolytic cleavage products, receptor subunits, or a combination of these is not known. We and others previously reported a broad band centered at Mr = 90,000 after labeling of pancreatic membranes (17-19). Moreover, components of different molecular mass have also been reported for somatostatin receptor on pancreatic β cells, and cerebral cortex membranes (15). Thus, the procedure of solubilization appears to induce conformational change in receptor molecules or dissociation of the receptors with other membrane components which might affect the selectivity of the receptors for agonists. Analogous changes in ligand affinities between membrane and soluble receptors have been previously reported (25, 26).

In summary, we have established for the first time the conditions of rapid somatostatin receptor extraction from pancreatic membranes in a state capable of specifically binding somatostatin and closely associated with a GTP-binding protein. This is also the first report of characterization and partial purification of the pancreatic soluble somatostatin receptor.

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REFERENCES
Active Soluble Pancreatic Somatostatin Receptors

EXPERIMENTAL PROCEDURES

Materials

- The REPIJนอก-1131 and 1131 crystalline somatostatin (TPSS) (from Pan-AM, Inc.) was kindly donated by J. A. USER and from P. Rothenberg (Dept. Ser.). Somatostatin was prepared from animal sources by chemical synthesis.
- CRPM and CRPM (from Chromochemicals) were prepared by S. R. J. F. (Japan Drug Co.).
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Preparation of pancreatic acinar cell plasma membranes

Dispersed pancreatic acinar cells were obtained from male Wistar rats after enzymatic de-activation of the pancreas with 0.2 mg of collagenase (type 1,0 mg/ml) for 2 h at 37°C. After thorough washing by sedimentation, acinar cells were transferred to 0.2 M sucrose and homogenized at 5°C utilizing a Dounce homogenizer. After sedimentation of 15 min at 15,000 × g at 4°C, the purified membranes were collected from the interface and stored at -80°C until use.

Stabilization of pancreatic membranes

Stabilization was carried out by incubating membranes at a concentration of 0.2 g of protein per ml in 0.1 M Tris-HCl (pH 7.4) containing 0.5 M NaCl, 1% sucrose, 0.1% triton-X-100 (v/v) (v/v), 0.1% sodium azide, 0.3% sodium pyrophosphate, and 0.1 M NaCl. The mixture was then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was collected and mixed with 1 M sucrose containing 0.1 M NaCl and centrifuged at 100,000 × g for 10 min. The purified membranes were collected from the interface and stored at -80°C until use.

Binding of [125I]-TPSS

Rabbits to be used were prepared with the inactivated pancreatic extract (125I)-TPSS which has been previously demonstrated to be specific for somatostatin receptors. Rabbits were immunized with 0.1 M Tris-HCl (pH 7.4) containing 0.5 M NaCl, 1% sucrose, 0.1% triton-X-100 (v/v), 0.1% sodium pyrophosphate, and 0.1 M NaCl. The mixture was then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was collected and mixed with 1 M sucrose containing 0.1 M NaCl and centrifuged at 100,000 × g for 10 min. The purified membranes were collected from the interface and stored at -80°C until use.

Preparation of membranes for isothiocyanate labeling

The membranes were suspended in 50 mM Tris-HCl (pH 7.4) containing 0.5 M NaCl, 1% sucrose, 0.1% triton-X-100 (v/v), 0.1% sodium pyrophosphate, and 0.1 M NaCl. The mixture was then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was collected and mixed with 1 M sucrose containing 0.1 M NaCl and centrifuged at 100,000 × g for 10 min. The purified membranes were collected from the interface and stored at -80°C until use.

Cross-linking

The membranes were suspended in 50 mM Tris-HCl (pH 7.4) containing 0.5 M NaCl, 1% sucrose, 0.1% triton-X-100 (v/v), 0.1% sodium pyrophosphate, and 0.1 M NaCl. The mixture was then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was collected and mixed with 1 M sucrose containing 0.1 M NaCl and centrifuged at 100,000 × g for 10 min. The purified membranes were collected from the interface and stored at -80°C until use.

Isothiocyanate labeling of membranes

Membrane pellets were suspended in 0.5 M guanidine (pH 7.4) and 0.5 M Cys (pH 7.4) for 1 h at room temperature. The membranes were then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was collected and mixed with 1 M sucrose containing 0.1 M NaCl and centrifuged at 100,000 × g for 10 min. The purified membranes were collected from the interface and stored at -80°C until use.

Electrophoretic analysis of membranes

Electrophoretic analysis was carried out using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Following electrophoresis, the gels were stained with Coomassie blue R-250 and destained in 10% acetic acid.

Protein determination

Protein concentration was determined by the Bradford method with serum albumin as the standard (21).
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The somatostatin receptor has been shown to be a disulfide bond by its interaction with intact or less with intact (18, 19). Therefore, WGA affinity chromatography and in situ cross-linked to phosphatase. Disulfide bonds were subjected to a lectin affinity column which contained the lectin WGA cross-linked to phosphatase beads. Approximately 1% of the receptor from the soluble receptor preparation bound to the WGA column. When 20 mCi of radioiodinated ligand was added to the chromatography buffer, approximately 2% of the receptor was added to the column (Fig. 2). Sandwich receptor preparation of the ligand activity of [111]Inty-DSM binding to

Table 1: Solidification of somatostatin receptors as a function of CM-Sepharose concentration. Membranes were solidified with different concentrations of CM-Sepharose for 6 h. After solidification and gel filtration, the solidified material was assayed for [111]Inty-DSM binding as described in "Experimental procedures." The results are expressed as percentage of maximal specific binding observed with 0.01 M Tris and are the mean ± SD of three experiments.

<table>
<thead>
<tr>
<th>CM-Sepharose Concentration</th>
<th>Specific Binding</th>
</tr>
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<tbody>
<tr>
<td>0.1 M</td>
<td>100 ± 5</td>
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<tr>
<td>0.5 M</td>
<td>40 ± 7</td>
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<tr>
<td>1.0 M</td>
<td>15 ± 0.5</td>
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</tbody>
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Figure 1: Specific binding of [111]Inty-DSM to pancreatic soluble somatostatin receptors as a function of CM-Sepharose concentration. Pancreatic membranes (1 mg/ml) were incubated with 0.1 M Tris at 4°C. After centrifugation, the solidified receptor material was assayed for [111]Inty-DSM binding as described in "Experimental procedures." The results are expressed as percentage of maximal specific binding measured in the absence of CM-Sepharose at 0.01 M Tris buffer. Each point is the mean of triplicate determinations from three different experiments.

Figure 2: Extraction of somatostatin receptors as a function of time of solidification. Pancreatic membranes (1 mg/ml) were incubated with 0.1 M Tris at 4°C. After centrifugation, the solidified material was washed with a buffer (0.25 M polybuffer) which was preincubated and diluted with CM-Sepharose (1 mg/ml) for 6 h. After centrifugation, the supernatant was assayed for [111]Inty-DSM binding as described in "Experimental procedures." The results are expressed as percentage of maximal specific binding observed after solidification of membranes for 15 min with 0.01 M Tris buffer.

Figure 3: Time course of association of [111]Inty-DSM binding to soluble somatostatin receptors. Solidified membranes (10 μg of protein) were incubated with 0.5 mM [111]Inty-DSM in the presence or absence of 10 μM somatostatin at two temperatures: 2°C and 25°C. The specific binding is expressed as percentage of maximal specific binding measured in the absence of somatostatin. Each point is the mean of triplicate determinations from three different experiments.

Figure 4: Time course of dissociation of [111]Inty-DSM bound to soluble somatostatin receptors. Solidified membranes (10 μg of protein) were incubated with 0.01 M Tris at 2°C with 0.2 M [111]Inty-DSM. The dissociation of bound radioactivity was initiated by addition of 0.01 M sodium citrate (pH 5.0) to some of the samples and the incubation continued for an additional hour. Each point is the mean of triplicate determinations from three different experiments.

Figure 5: Scatchard analysis of [111]Inty-DSM binding to soluble somatostatin receptors. Solidified receptors (10 μg of protein) were incubated at 2°C for 18 h with increasing concentrations of [111]Inty-DSM in the absence or presence of 10 μM somatostatin. Data and trend line were generated using GraphPad Prism and re-plotted to show the best fit of association equation.

Figure 6: Inhibition of binding of [111]Inty-DSM to soluble somatostatin receptors by somatostatin analogues. Solidified receptors (10 μg of protein) were incubated at 2°C for 18 h with 0.2 M [111]Inty-DSM and various concentrations of somatostatin (1, 10, 100, and 1000 nM) or somatostatin 28 (1, 10, 100, and 1000 nM). Data are expressed as percentage of the specific binding measured in the absence of analogues. Each point is the mean of triplicate determinations from three different experiments.

Figure 7: Gel filtration of soluble somatostatin receptors. 4 mg of solidified receptors were loaded on a Sepharose 2B-200 column (1.5 x 4.6 cm) equilibrated with 0.1 M Tris at 4°C. The eluted receptor was solubilized in 0.25 M polybuffer 74 (pH 7.5), concentrated to 3 mg/ml, and dialyzed against 0.01 M Tris at 4°C. The fraction volume was 2.5 ml and the flow rate 15 ml/min.
Figure 8: Autoradiogram of 2D polyacrylamide gel electrophoretogram of [125I-Tyr]OMG cross-linked to soluble somatostatin receptors. Solubilized receptors (750 μg of protein were cross-linked with 1 mM [125I-Tyr]OMG in the presence of absence of 10 μM somatostatin. The cross-linked material was analysed by 2D polyacrylamide gel electrophoresis and autoradiography.

Figure 9: Isoelectrofocusing of soluble somatostatin receptors. 4.3 mg of protein were dialyzed to a final volume of 1 ml at room temperature of 0.1 mM ethylenediamine as described in 'Experimental procedures' and subjected to isoelectrofocusing in a combi cell during 10 min at 12 watts. The focusing was followed by measuring the pH ( ) and the specific binding of [125I-Tyr]OMG to aliquots of each fraction ( ).

Figure 10: Lectin affinity chromatography of soluble somatostatin receptors. 4.5 mg of solubilized receptors were loaded onto a 3 ml wheat germ agglutinin Sepharose 4B column. Every 10 ml of the flow-through fraction was collected and the column was washed until no protein could be detected eluting from the column and the elution started with 3 ml 10 mM calcium as described in 'Experimental procedures'. The indicated fractions were assayed for binding activity which is shown as [125I-Tyr]OMG binding ( ) and protein ( ).
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