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 $K_d$ of 0.3 nM and a $B_{max}$ of 210 fmol/mg. As observed with membrane-bound receptors, soluble binding receptors were sensitive to the GTP analog GTPγS 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, photoaffinity labeling of soluble receptors identified a major protein of $M_r$ = 100,000 and two minor proteins of $M_r$ = 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 triacetylchitotriose.

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. In most target cells, somatostatin decreases intracellular cAMP by inhibiting adenylate cyclase activity via the pertussis toxin-sensitive G,$^1$

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

protein (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 $M_r$ = 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**

**DISCUSSION**

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-

$^2$ 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 ususly-drolyzable GTP analog GTPγS regulated the binding of [125I-Tyr]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 G, in several target tissues such as pancreas (10, 11) pituitaries (12, 13), stomach (27) and brain (17). In this study, we observed that soluble somatostatin receptors were sensitive to GTPγS 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 [125I-Tyr]SMS to soluble receptors and SDS-PAGE revealed the presence of a major specific band of an apparent molecular mass of 100 K. Minor species of 56 K 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 M, = 90,000 after affinity labeling of pancreatic membranes (17-19). Moreover, components of different molecular mass have also been reported for somatostatin receptor on pancreatic β cell (M, = 193,000 -- 129,000 -- 42,000) (28), cerebrocortical membranes (M, = 70,000) (17), AT-T20 cells (M, = 55,000) (16), and adrenal cortex (M, = 200,000) (29), suggesting the existence of different somatostatin receptors in different organs. Further purification and characterization of these receptors will be required to determine the different structures of possible subtypes of somatostatin receptors.

As observed with other membrane-bound receptors, soluble somatostatin receptors are glycoproteins which strongly in interact with WGA-Sepharose since active receptor were not eluted by N-acetylgalosamine (data not shown) but only by triacetylchitotriose which is known to have higher affinity for WGA lectin (30). WGA affinity chromatography has been useful in the purification of several receptors (31), and substantial purification of the somatostatin receptor can be achieved by this method. On the other hand, we have demonstrated that the somatostatin receptor is an acid protein. Isoelectric focusing, aside from yielding the pI value of the receptor, could be an efficient purification step. The two-step procedure employing successive WGA-Sepharose and isoelectric focusing would represent the first steps toward the purification of functional somatostatin receptors which is currently in progress in the laboratory.

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

Materials used were essentially the same as described previously. 

RESULTS

Binding assay for soluble somatostatin receptors

To determine the optimal detergent concentration for solubilization of active somatostatin receptors, purified somatostatin at a constant concentration of 5 nM was incubated with 11-125I-somatostatin (5 nM) for 4 h at 22°C in the presence of various detergent concentrations. The optimal detergent concentration for solubilization of active somatostatin receptor was determined by examining the effect of detergent concentration on the amount of specific binding. When the detergent concentration was increased from 0 to 0.5% TX-100, the amount of specific binding increased significantly. When the detergent concentration was increased to 1% TX-100, the amount of specific binding was further increased, but the amount of non-specific binding also increased. Therefore, the optimal detergent concentration for solubilization of active somatostatin receptor was determined to be 0.5% TX-100.

Optimization of the experimental conditions for the solubilization of active somatostatin receptors

In the second experiment, the effects of detergent concentration on the solubilization of active somatostatin receptors were examined. The optimal detergent concentration for solubilization was determined to be 1% TX-100.

Characterization of binding of 11-125I-somatostatin to soluble somatostatin receptor

In the third experiment, the characterization of the binding of 11-125I-somatostatin to soluble somatostatin receptor was performed. The optimal detergent concentration for solubilization was determined to be 1% TX-100. The amount of specific binding increased significantly when the detergent concentration was increased from 0 to 0.5% TX-100, and then increased further when the detergent concentration was increased to 1% TX-100. However, the amount of non-specific binding also increased when the detergent concentration was increased to 1% TX-100.

Cross-linking

In the fourth experiment, the cross-linking of the soluble somatostatin receptor was performed. The optimal detergent concentration for solubilization was determined to be 1% TX-100. The amount of specific binding increased significantly when the detergent concentration was increased from 0 to 0.5% TX-100, and then increased further when the detergent concentration was increased to 1% TX-100. However, the amount of non-specific binding also increased when the detergent concentration was increased to 1% TX-100.

Characterization of soluble somatostatin receptors

In the fifth experiment, the characterization of the soluble somatostatin receptors was performed. The optimal detergent concentration for solubilization was determined to be 1% TX-100. The amount of specific binding increased significantly when the detergent concentration was increased from 0 to 0.5% TX-100, and then increased further when the detergent concentration was increased to 1% TX-100. However, the amount of non-specific binding also increased when the detergent concentration was increased to 1% TX-100.

Cross-linking of soluble somatostatin receptors

In the sixth experiment, the cross-linking of the soluble somatostatin receptors was performed. The optimal detergent concentration for solubilization was determined to be 1% TX-100. The amount of specific binding increased significantly when the detergent concentration was increased from 0 to 0.5% TX-100, and then increased further when the detergent concentration was increased to 1% TX-100. However, the amount of non-specific binding also increased when the detergent concentration was increased to 1% TX-100.

Characterization of binding of 11-125I-somatostatin to soluble somatostatin receptor

In the seventh experiment, the characterization of the binding of 11-125I-somatostatin to soluble somatostatin receptor was performed. The optimal detergent concentration for solubilization was determined to be 1% TX-100. The amount of specific binding increased significantly when the detergent concentration was increased from 0 to 0.5% TX-100, and then increased further when the detergent concentration was increased to 1% TX-100. However, the amount of non-specific binding also increased when the detergent concentration was increased to 1% TX-100.
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Table 1: Stabilization of somatostatin receptors as a function of CNBr concentration. Membranes were subjected to different concentrations of CNBr for 6 hours. After centrifugation and gel filtration, the stabilized material was assayed for [125I]-Tyr-Phe-SRM binding as described in "Experimental procedures". The results are expressed as activity of maximal specific binding observed with 5% CNBr and are the means ± SEM of three experiments.

<table>
<thead>
<tr>
<th>CNBr CONCENTRATION</th>
<th>SPECIFIC BINDING</th>
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<tbody>
<tr>
<td>0.1</td>
<td>76 ± 6.1</td>
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<tr>
<td>1</td>
<td>120</td>
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<tr>
<td>2</td>
<td>141 ± 7</td>
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<tr>
<td>3</td>
<td>154 ± 5</td>
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Figure 1: Specific binding of [123I]-Tyr-Phe-SRM to pancreatic soluble somatostatin receptor at a fraction of CNBr concentration. Pancreatic membranes (3 mg/ml) were subjected to 0.1% CNBr for 3 h at 4°C. After centrifugation and gel filtration, the stabilized material was assayed for [123I]-Tyr-Phe-SRM binding as described in "Experimental procedures". The results are expressed as activity of specific binding observed with 5% CNBr and are the means ± SEM of three experiments.

Figure 2: Time course of stabilization of somatostatin receptors by 125I-Tyr-Phe-SRM. Solubilized receptors (50 μg of protein) were incubated with 0.1% CNBr at 4°C for 0.5 h and 125I-Tyr-Phe-SRM at various concentrations as described in "Experimental procedures". The results are expressed as activity of maximal specific binding observed with 5% CNBr and are the means ± SEM of three experiments.

Figure 3: Extraction of somatostatin receptors by a fraction of time of stabilization. Pancreatic membranes (3 mg/ml) were subjected to 0.1% CNBr for different times. After centrifugation and gel filtration, the stabilized material was assayed for [123I]-Tyr-Phe-SRM binding as described in "Experimental procedures". The results are expressed as activity of maximal specific binding observed with 5% CNBr and are the means ± SEM of three experiments.

Figure 4: Time course of dissociation of 125I-Tyr-Phe-SRM bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated with 0.1% CNBr for 6 h with increasing concentrations of 125I-Tyr-Phe-SRM at the presence or in absence of 100 nM somatostatin. Data points are given as specific binding observed with 5% CNBr and are the means ± SEM of three experiments.
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Figure 8: Autoradiogram of SDS polyacrylamide gel electrophoresis of [125I-Tyr]125I-SOM cocrystallized to soluble somatostatin receptors. Solubilized receptors (500 ng of protein were solubilized with 0.2 ml [125I-Tyr]125I-SOM in the presence or absence of 10 mM sodium dodecyl sulfate (SDS) and 1 M NaCl) cross-linked material was analyzed by SDS polyacrylamide gel electrophoresis and autoradiography.

Figure 9:Isoelectrofocusing of soluble somatostatin receptors. 40 µg of protein were diluted to a final volume of 1 ml with an additional 0.3 M ammonium acetate described in the experimental procedure and subjected to isoelectrofocusing in a comb plate cell during 120 min at 12 watts. The focusing was followed by measuring the pH (•) and the specific binding of [125I-Tyr]125I-SOM to aliquots of each fraction (○).

Figure 10: Lectin affinity chromatography of soluble somatostatin receptors. 4.5 mg of solubilized receptors were loaded into a 2 ml wheat germ agglutinin-Sepharose 4B column and eluted with 0.3 M sodium acetate buffer at pH 5.5 followed by washing with 2 ml of the buffer. The column was washed until no protein could be detected eluting from the column, and the effluent eluted with 1 M NaCl (arrow) as described in the experimental procedures. The eluted fractions were assayed for binding activity which is shown as [125I-Tyr]125I-SOM binding filtered and protein (†).