Solubilization and Characterization of Active Somatostatin Receptors from Rat Pancreas*

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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-

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 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 [Tyr9]SMS somatostatin analog to somatostatin receptor prior to its solubilization allowed preliminary molecular characterization of the solubilized [Tyr9]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).

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 cells, 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 nonhydrosoluble GTP analog GTPγS regulated the binding of [125I]-Tyr3SMS 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 (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]-Tyr3SMS to soluble receptors and SDS-PAGE revealed the presence of a major specific band of an apparent 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 cleavages 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 cells (Mr = 193,000-129,000-42,000) (28), cerebrocortical membranes (Mr = 70,000) (17), AT-T20 cells (Mr = 55,000) (16), and adrenal cortex (Mr = 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 receptors were not eluted by N-acetylgalactosamine (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 acidic 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.

Acknowledgments—We thank Anne-Marie Remaury and Christian Mora for excellent technical assistance.

REFERENCES

Active Soluble Pancreatic Somatostatin Receptors

**EXPERIMENTAL PROCEDURES**

**Materials**

**RESULTS**

Binding assay for soluble somatostatin receptors

We previously reported that non-pancreatic somatostatin receptors which were not completely precluded with [125I]-Tyrotyl could be extracted in a stable state from the membrane using non-depolarizing pertussis toxin (PTX) and [125I] (6-12), which is described below. Therefore, we used CRPA for solubilizing pancreas somatostatin receptors in an active state. To establish a binding assay for soluble receptors, we solubilized purified pancreas receptors with 10% CHAPS for 5 h at 4°C. Following solubilization, we determined the binding of [125I]-Tyrotyl to the supernatant of microsomal membranes. This binding assay, which is described in detail elsewhere, is identical to the solubilization assay (3) (Figure 1). After the binding assay, the membranes were recovered by centrifugation. The supernatant containing the 125I-labeled peptide was then collected and stored at 4°C for further use.

Characterization of binding of [125I]-Tyrotyl to CRPA-solubilized rat pancreatic membranes

In Figure 1, the binding of [125I]-Tyrotyl to CRPA-solubilized pancreatic membranes was examined. The results show that the binding of [125I]-Tyrotyl to CRPA-solubilized membranes is specific and saturable, with a dissociation constant of 2.5 nM and a maximum binding capacity of 12.5 pmol/mg protein.

**Optimization of the experimental conditions for the extraction of active somatostatin receptors**

To determine the optimal detergent concentration for solubilization of active somatostatin receptors, purified membranes at a concentration of 5 mg/ml were incubated with various concentrations of CHAPS (0, 1, 2, 4, and 8% w/v CHAPS) for 5 h at 4°C. The results show that 1% CHAPS is the optimal concentration, as it results in the highest binding activity and recovery of active receptors. However, at concentrations above 1%, the binding activity decreases due to detergent-induced precipitation of the membranes. Therefore, 1% CHAPS was used for all subsequent experiments.

**Discussion**

The results of this study demonstrate that the solubilization and recovery of active somatostatin receptors from the membrane can be achieved using CHAPS. The optimal concentration of CHAPS for solubilization and recovery is 1%, which is in agreement with previous studies (6-12). This finding confirms the applicability of CHAPS as a detergent for the solubilization of active somatostatin receptors.

**Conclusions**

In conclusion, the results of this study provide evidence for the presence of active somatostatin receptors in the rat pancreas. The solubilization and recovery of these receptors using CHAPS allows for the development of a specific binding assay, which can be used for further studies.
Active Soluble Pancreatic Somatostatin Receptors

Table 1: Stabilization of somatostatin receptors as a function of CMPS concentration. Membranes were solubilized with different concentrations of CMPS for 90 min. After centrifugation, the precipitated material was assayed for [125I]-Tyr3([9]-Somatostatin 1-28)-Mab binding as described in "Experimental procedures". The results are expressed as percentage of maximal specific binding observed with 1% CMPS and are the mean ± SEM of three experiments.

<table>
<thead>
<tr>
<th>CMPS CONCENTRATION (1% CMPS)</th>
<th>SPECIFIC BINDING (1% CMPS)</th>
</tr>
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<tbody>
<tr>
<td>0.1</td>
<td>76 ± 6.7</td>
</tr>
<tr>
<td>1.0</td>
<td>30 ± 2.2</td>
</tr>
<tr>
<td>2.0</td>
<td>48 ± 3.7</td>
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<tr>
<td>3.0</td>
<td>56 ± 5.9</td>
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Figure 1: Time course of dismutation of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25°C with 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The dismutation of bound radioactivity was initiated by addition of 100 μM somatostatin 1-28 to a final concentration of 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The incubation was continued for an additional 30 min. Each point is the mean ± SEM of triplicate determinations.

Figure 2: Effect of CMPS on the equilibrium dissociation constant (Kd) of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25°C with various concentrations of [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The equilibrium dissociation constant (Kd) of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors was determined as described in "Experimental procedures". Data are expressed as percentage of the specific binding measured in the absence of non-specific competitor.

Figure 3: Effect of temperature on the equilibrium dissociation constant (Kd) of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 4°C with various concentrations of [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The equilibrium dissociation constant (Kd) of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors at 4°C was determined as described in "Experimental procedures". Data are expressed as percentage of the specific binding measured in the absence of non-specific competitor.

Figure 4: Time course of stabilization of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25°C with 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The dismutation of bound radioactivity was initiated by addition of 100 μM somatostatin 1-28 to a final concentration of 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The incubation was continued for an additional 30 min. Each point is the mean ± SEM of triplicate determinations.

Figure 5: Time course of dismutation of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25°C with 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The dismutation of bound radioactivity was initiated by addition of 100 μM somatostatin 1-28 to a final concentration of 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The incubation was continued for an additional 30 min. Each point is the mean ± SEM of triplicate determinations.

Figure 6: Time course of dismutation of [125I]-Tyr3([9] Somatostatin 1-28)-Mab bound to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25°C with 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The dismutation of bound radioactivity was initiated by addition of 100 μM somatostatin 1-28 to a final concentration of 0.2 nM [125I]-Tyr3([9] Somatostatin 1-28)-Mab. The incubation was continued for an additional 30 min. Each point is the mean ± SEM of triplicate determinations.
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Figure 8: Autoradiogram of SDS polyacrylamide gel electrophoresis of [35S]-Somatostatin cross-linked to soluble somatostatin receptors. Solubilized receptors (3 mg of protein were solubilized with 1 mM [35S]-Somatostatin in the presence or absence of 10 mM congo red) were cross-linked with a 1:1 ratio of [35S]-Somatostatin to cross-linking material. The cross-linked material was analyzed by SDS polyacrylamide gel electrophoresis and autoradiography.

Figure 9: Isoelectric focusing of soluble somatostatin receptors. 2 mg of protein were diluted to a final volume of 1 ml prior to addition of 0.1% ampholine as described in Experimental Procedures. The focusing was followed by measuring the pH (pH) and the specific binding of [35S]-Somatostatin to aliquots of each fraction (○).
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