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 GTPyS 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, photofinity labeling of soluble receptors identified a major protein of Mr = 100,000 and two minor proteins of Mr = 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.

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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: Gs, 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'-triacetylchitotriose; BSA, bovine serum albumin; GTPyS, guanosine 5'-O-(3-thiotriphosphate); SMS, SMS 201-995.

1 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-drolyzable GTP analog GTPγS regulated the binding of [125]Tyr3JSMS to soluble receptors. It is well documented that somatostatin receptors are coupled to the adenylate cyclase system via the pertussis toxin-sensitive G-protein Gs 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 [125]Tyr3JSMS 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 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 (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-acetylgalcosamine (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.

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Experimental Procedures

Materials

Active Soluble Pancreatic Somatostatin Receptors

Preparation of pancreatic acinar cell plasma membranes

Dispersed pancreatic acini were obtained from male Wistar rats after pancreatic de-endothelialization of the pancreas with 0.25% trypsin solution in 0.25% acetic acid, as previously described (7). After thorough washing by filtration, acini were transferred to 0.2 M sucrose and homogenized by sonication at 0°C utilizing a Branson Sonifier. After sonication at 0°C for 10 min, the homogenate was centrifuged at 17,000 x g for 15 min, and the supernatant was collected and dialyzed against 0.2 M sucrose at 4°C overnight.

Stabilization of acinar membranes

Stabilization was carried out by incubating membranes at a concentration of 5 mg of protein per ml in 50 mM Tris-Cl (pH 7.4), 5 mM MgCl2, 1 mM CaCl2, 0.1% egg yolk lecithin, 0.02% MgSO4, 1 mM EGTA (pH 7.4) (tris-mediated buffer) with 1 mM CaCl2 for 10 min at 4°C. Following dialysis to a final concentration of 1% CaCl2 with tris-mediated buffer, the detergent was removed by centrifugation at 5,000 x g for 10 min at 4°C in a Sorvall HT-8500 ultracentrifuge. In these conditions, 60-65% of the membranes were recovered in the supernatant. The supernatant was collected, loaded onto a Sephadex G-25 column and dialyzed against 0.2 M sucrose at 4°C (5 ml of 3.75M sucrose in Tris-Cl buffer pH 8 with 0.02 M CaCl2). Stabilized acinar suspensions eluting in the void volume of the column were collected immediately after use for further experiments.

Binding of [125I]-Tyr380-DP

Binding to solubilized receptors was performed with the radiolabeled somatostatin analog [125I]-Tyr380-DP which has been previously demonstrated to bind to specific somatostatin receptors with a Kd of 2 nM and an affinity of 105 (9). In each experiment, the membranes were solubilized at a protein concentration of 0.05 mg/ml in a medium composed of 50 mM Tris-Cl (pH 7.4), 5 mM MgCl2, 1 mM CaCl2, 0.5% Brij-35, 0.1% sodium cholate, 0.02% MgSO4, 1 mM EGTA (pH 7.4) (tris-mediated buffer) with 1 mM CaCl2 for 10 min at 4°C. Following dialysis to a final concentration of 1% CaCl2 with tris-mediated buffer, the detergent was removed by centrifugation at 50,000 x g for 20 min at 4°C in a Sorvall HT-8500 ultracentrifuge. In these conditions, 60-65% of the membranes were recovered in the supernatant. The supernatant was collected, loaded onto a Sephadex G-25 column and dialyzed against 0.2 M sucrose at 4°C (5 ml of 3.75M sucrose in Tris-Cl buffer pH 8 with 0.02 M CaCl2). Stabilized acinar suspensions eluting in the void volume of the column were collected immediately after use for further experiments.

Cell permeability characterization of stabilized somatostatin receptors

The stabilized membranes were loaded at 4°C on a Tris-Sepharose 6B column (3 x 1 cm) pre-equilibrated with tris-mediated buffer containing 0.05% CaCl2. The column was run at 5 ml/min and 15% and fractions were collected and assayed for [125I]-Tyr380-DP binding as described above.

Cross-linking

The stabilized receptors (300 µg of protein) were incubated at 25°C for 15 min with 1 µM [125I]-Tyr380-DP and 50 µCi [125I]-Tyr380-DP in the presence of 1% Brij-35 in the presence of 0.05% CaCl2. The membranes were solubilized at a protein concentration of 0.05 mg/ml in a medium composed of 50 mM Tris-Cl (pH 7.4), 5 mM MgCl2, 1 mM CaCl2, 0.5% Brij-35, 0.1% sodium cholate, 0.02% MgSO4, 1 mM EGTA (pH 7.4) (tris-mediated buffer) containing 1% CaCl2. The labeled membranes were filtered through a nitrocellulose filter (pore size 0.45 µm) and the remaining activity was determined by liquid scintillation counting.

Gel filtration chromatography of stabilized somatostatin receptors

As shown in fig 6 the binding of [125I]-Tyr380-DP to soluble receptors is a slowly reversible process, whereby complex formation and disassociation occurs. The remaining activity was determined by liquid scintillation counting.

Furthermore, 125I concentration over time has been shown to affect the affinity of somatostatin receptors. In order to determine the potential influence of the solubilized somatostatin receptors, we incubated the [125I]-Tyr380-DP bound to solubilized receptors with 200 mM ouabain for 30 min at 4°C and the remaining activity was determined by liquid scintillation counting.

In summary, we have demonstrated that the solubilization of somatostatin receptors is a slow process, which is characterized by a high degree of specificity and selectivity.

Characterization of binding of [125I]-Tyr380-DP to CaCl2-stabilized rat pancreatic membranes

For all assays the conditions were at 35°C at pH 7.4. [125I]-Tyr380-DP (1 µM) was incubated with solubilized somatostatin receptors. Equilibrium was reached after 50 min of incubation and数控 rates were determined. The incubation buffer contained 50 mM Tris-Cl, 50 mM NaCl, 1% Brij-35, 0.01% sodium cholate, 0.02% MgSO4, 1 mM EGTA (pH 7.4) (tris-mediated buffer) containing 1% CaCl2. The remaining activity was determined by liquid scintillation counting.

Gel filtration chromatography of stabilized somatostatin receptors

In order to determine the potential influence of the solubilized somatostatin receptors, complex, solubilized receptors were incubated with 1 µM [125I]-Tyr380-DP in the presence of 0.05% CaCl2 for 15 min at 25°C and the remaining activity was determined by liquid scintillation counting.

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Active Soluble Pancreatic Somatostatin Receptors

The somatostatin receptor has been shown to be a pneumococcal by its interaction with arenicen and lactose (18, 27). Therefore, WGA affinity chromatography was used as the in situ step for somatostatin receptor purification. Soluble receptors were adsorbed onto a lactose affinity column which contained the lectin WGA covalently linked to Sepharose beads. Appr. 3 % of the receptors from the soluble receptor preparation bound to the WGA column. When 6 M urea/methylisobutylketone was added to the chromatography buffer, approximately 56 % of the receptors were eluted from the column (unpublished observations). The receptor activity of 4% [125I]Tyr-Ser binding to the receptor allowed to WGA column.

Table 1: Solubilization of somatostatin receptors as a function of CHAPS concentration. Membranes were solubilized with different concentrations of CHAPS for 1 hour. After centrifugation and gel filtration, the solubilized material was assayed for [125I]Tyr-Ser binding as described in "Experimental Procedures". The values are expressed as percentage of maximal specific binding observed with 1 % CHAPS and are the mean ± S.E. of three experiments.

| CHAPS CONCENTRATION | SPECIFIC BINDING |
|----------------------|------------------|
| 0.1%                 | 74 ± 8           |
| 0.5%                 | 74 ± 7           |
| 1%                   | 100              |
| 2%                   | 40 ± 0.7         |
| 3%                   | 54 ± 0.5         |

Figure 1: Specific binding of [125I]Tyr-Ser binding to soluble somatostatin receptors as a function of CHAPS concentrations. Pancreatic membranes (2 mg/ml) were solubilized with 1 % CHAPS for 1 hour. After centrifugation, the solubilized material was assayed for [125I]Tyr-Ser binding as described in "Experimental Procedures". The results are expressed as percentage of the specific binding observed at 1 % CHAPS.

Figure 2: Extraction of somatostatin receptors as a function of time of solubilization. Pancreatic membranes (5 mg/ml) were solubilized at 4 °C with 1% CHAPS for different times. After centrifugation and gel filtration, the solubilized material was assayed for [125I]Tyr-Ser binding as described in "Experimental Procedures". The results are expressed as percentage of the specific binding observed at 1 % CHAPS for 1 hour at 4 °C (100%).

Figure 3: Specific binding of [125I]Tyr-Ser binding to soluble somatostatin receptors as a function of temperature. Pancreatic membranes (5 mg/ml) were solubilized at 4 °C with 1% CHAPS for 1 hour. After centrifugation and gel filtration, the solubilized material was assayed for [125I]Tyr-Ser binding as described in "Experimental Procedures". The results are expressed as percentage of the specific binding observed at 25 °C.

Figure 4: Time course of dissociation of [125I]Tyr-Ser binding to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated for 60 min at 25 °C with 0.2 μg [125I]Tyr-Ser binding, and the dissociation of bound radioactivity was measured. The dissociation of bound radioactivity was measured at 25 °C by addition of 0.2 M NaCl and 0.1 M Tris (pH 7.4) and was continued for an additional hour. Each point is determined in triplicate and represents the mean ± S.E. of three different experiments.

Figure 5: SDS-PAGE analysis of [125I]Tyr-Ser binding to soluble somatostatin receptors. Solubilized receptors (50 μg of protein) were incubated at 25 °C for 60 min with increasing concentrations of [125I]Tyr-Ser binding. The results are expressed as percentage of the specific binding measured in the absence of 0.2 M NaCl. Each point is determined in triplicate and represents the mean ± S.E. of three different experiments.

Figure 6: Specific binding of [125I]Tyr-Ser binding to soluble somatostatin receptors as a function of concentration. Solubilized receptors (50 μg of protein) were incubated at 25 °C for 60 min with 0.2 μg [125I]Tyr-Ser binding and various concentrations of somatostatin (100-1 μM) or somatostatin (2 M). The results are expressed as percentage of the specific binding measured in the absence of somatostatin. Each point is determined in triplicate and represents the mean ± S.E. of three different experiments.

Figure 7: Gel filtration of soluble somatostatin receptors. 4 μg of solubilized receptors were loaded on a Sepharose 2B-200 column (1 x 1.4 cm) equilibrated with 50 mM Tris-Cl pH 7.4 (buffer C). The column was washed with buffer C and 1 ml fractions were collected. The specific binding of [125I]Tyr-Ser binding to aliquots of each fraction was measured. The data were expressed as percentage of the specific binding observed in the absence of specific binding.

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Figure 8: Autoradiogram of SDS polyacrylamide gel electrophoresis of [125I-Tyr]Arg-200,116,94,67,116,94,67 immunoprecipitated to soluble somatostatin receptors. Solubilized receptors (150 μg of protein) were incubated with 1 nM [125I-Tyr]Arg-200,116,94,67 in the presence or absence of 10 μM somatostatin. Free hormone was removed by a second antibody precipitation. Preparative gel was scanned until no protein could be detected elution from the column, and the gel was stained with 3% Coomassie brilliant blue to demonstrate the presence of solubilized receptors. The indicated elution was assayed for binding activity, which is shown as [125I-Tyr]Arg binding (hatched and protein).

Figure 9: Electrophoresis of soluble somatostatin receptors. 4.3 mg of protein were diluted to a final volume of 1 ml, with 0.1 M sodium phosphate buffer, pH 7.2, 2.5% sodium deoxycholate, and 0.05% sodium dodecyl sulfate. The gel was stained with 3% Coomassie brilliant blue and scanned until no protein could be detected elution from the column. The gel was stained with 3% Coomassie brilliant blue to demonstrate the presence of solubilized receptors. The indicated elution was assayed for binding activity, which is shown as [125I-Tyr]Arg binding (hatched and protein).
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