Molecular characterization of the solubilized receptor of somatostatin from rat pancreatic acinar membranes

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The somatostatin receptors on rat pancreatic acinar membranes were demonstrated by use of a radioiodinated (¹²⁵I-) analogue of somatostatin (SMS 204-090 or [Tyr³]SMS). The tracer was found to bind to the receptor with a K_d of 58 pM. The number of sites detected by this tracer (4.7 pmol/mg of protein) was 5–10 times higher than the number of sites previously found with other tracers. Since the level of non-specific binding was also very low as compared with findings with other tracers, ¹²⁵I-204-090 might be of interest in future attempts to characterize the somatostatin receptors in the pancreas. The prelabelled membranes were solubilized with 1% CHAPS, and the solubilized complexes were found to adsorb to wheat-germ-agglutinin-coupled agarose, from which they could be eluted with 4 mM-triacetylchitotriose. The complexes within this eluate were shown by gel filtration on Trisacryl GF-2000 to have an M_r of about 400 000. The dissociation of the complexes was augmented both within the membranes as well as in the solubilized state by incubation with the GTP analogue guanosine 5'-[γ-thio]triphosphate, indicating that the complexes are probably functionally linked to a guanine-nucleotide-binding regulatory protein. After SDS/slab-gel electrophoresis and autoradiography of cross-linked complexes after treatment with the heterobifunctional reagent *N*-5-azido-2-nitrobenzoyloxysuccinimide, a broad band occurred at ~ M_r 90 000 both in the membranes and in the eluates of complexes after lectin-adsorption chromatography. We conclude that the augmentation of the number of detectable sites for binding of somatostatin, as well as the very low level of non-specific binding obtained by the use of ¹²⁵I-[Tyr³]SMS as tracer, has made it possible for us to demonstrate the solubilization of the somatostatin receptor in conjunction with its ligand and a GTP-binding regulatory protein, and we have succeeded in cross-linking ¹²⁵I-[Tyr³]SMS to a binding subunit of M_r 90 000 in the membranes and in demonstrating the presence of the same labelled binding subunit within complexes solubilized and chromatographed on a lectin column before cross-linking.

INTRODUCTION

Originally purified from the hypothalamus and characterized as an inhibitor of growth-hormone secretion (Brazeau *et al.*, 1973), the tetradecapeptide somatostatin (SS-14) has recently attracted much attention owing to its inhibitory effects upon growth of human and guinea-pig pancreatic-cell lines (Redding & Schally, 1984; Liebow *et al.*, 1986). These effects are probably mediated through interaction with specific receptors for SS-14. Normal rat pancreatic membranes possess such receptors and these have been described previously both with regard to their binding characteristics (Esteve *et al.*, 1984), as well as to their M_r after covalent cross-linking of radioiodinated ligands (Sakamoto *et al.*, 1984; Srikant & Patel, 1986; Susini *et al.*, 1987). We decided to extend these observations by studying the binding of a radioiodinated stable analogue of SS-14, SMS 204-090 or [Tyr³]SMS, previously shown valuable in characterization of binding of SS-14 to receptors in rat brain (Reubi, 1985).

Here we report the characteristics of the binding of this tracer to rat pancreatic membranes, the molecular properties of the binding protein after solubilization as evidenced by lectin-adsorption chromatography, gel

filtration, covalent cross-linking followed by SDS/polyacrylamide-gel electrophoresis and autoradiography, and the association between the receptor and a GTP-binding regulatory protein, both within the membranes as well as in the solubilized state.

EXPERIMENTAL

Materials

The peptides SMS 201-995 and its tyrosine analogue [Tyr³]SMS or 204-090 were most kindly donated by Sandoz Ltd., Basel, Switzerland. Somatostatin-14 was a gift from Professor E. Wunsch, Max-Planck-Institut, München, Germany. Wheat-germ agglutinin, CHAPS and collagenase (from *Clostridium histolyticum*) were obtained from Serva Fine Chemicals, Tebu, France. Na¹²⁵I was purchased from Amersham France, Les Ullis, France. Soybean trypsin inhibitor (Type I-s) and triacetylchitotriose were from Sigma, St. Louis, MO, U.S.A. Reacti-Gel 6X (carbamyl-1,1'-diimidazole-activated agarose) and ANB-NOS were from Pierce Chemical Co., Rockford, IL, U.S.A. Sephadex G-25-SF gel was obtained from Pharmacia Fine Chemicals A.B.,

Abbreviations used: ANB-NOS, *N*-5-azido-2-nitrobenzoyloxysuccinimide; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulphonic acid; GTP[S], guanosine 5-[γ-thio]triphosphate; WGA, wheat-germ agglutinin; SS-14, somatostatin; SMS 201-995, a synthetic stable analogue of somatostatin consisting of eight amino acids; SMS 204-090, corresponding to SMS 201-995, but the phenylalanine in position 3 has been replaced by tyrosine (the compound can thus also be referred to as '[Tyr³]SMS'; IC₅₀, concentration causing half-maximal (50%) inhibition.

Uppsala, Sweden. Trisacryl GF-2000 was from Réactifs IBF, Villeneuve la Garenne, France. GTP[S] was from Boehringer, Mannheim, Germany. Centrisart I tubes were from Sartorius G.m.b.H, Göttingen, Germany. Protein standards for calibration of gel-chromatographic columns and SDS/polyacrylamide electrophoretic gels were obtained from Bio-Rad, München, Germany. All other chemicals were of highest purity commercially available.

Preparation of rat pancreatic acinar membranes

Dispersed pancreatic acini were obtained from Wistar rats after enzymic degradation of the organ with 0.2 unit of collagenase/ml in an oxygenated Krebs-Ringer medium as described by Amsterdam *et al.* (1978). After thorough washing by sedimentation, acini were transferred to 0.3 M-sucrose. In 0.3 M-sucrose the acini were homogenized with a Dounce homogenizer by the method of Meldolesi *et al.* (1971). After sedimentation at 1500 *g* for 12 min the homogenized membranes were resuspended in 1.56 M-sucrose, covered with 0.3 M-sucrose and centrifuged at 27000 rev./min for 2 h in a Beckman L5-50 ultracentrifuge utilizing a type SW41 rotor. The purified membranes were collected at the interface between the sucrose solutions.

Iodination of SMS 204-090

The tracer was produced essentially as previously reported for other somatostatin-related peptides (Antonioti *et al.*, 1984). To 10 μ g of SMS 204-090 suspended in 50 μ l of a 0.25 M-sodium phosphate buffer, pH 7.5, was added 10 μ l of an Na¹²⁵I solution (1 mCi), followed by five additions of 5 μ l of Chloramine-T (0.5 μ g/ μ l) in water, an interval of 10 s being allowed between each addition. Finally, 120 μ l of a tyrosine solution (2 μ g/ μ l) was added. The mixture was loaded on to a μ Bondapak C₁₈ column (30 cm $\times$ 0.39 cm internal diameter) and, by utilizing a Waters Associates liquid chromatograph (consisting of a U6K injector, two 6000 A pumps and a Schoeffel model 770 multi-wavelength detector), the tracer was eluted at a flow of 1 ml/min in a pre-programmed non-linear gradient (gradient no. 7 as given by a Waters Associates model 660 solvent programmer) going from 20 to 80% (v/v) acetonitrile in 0.25 M-triethylammonium phosphate, pH 3.5, in 40 min. The tracer was eluted after 15 min. Its specific radioactivity was calculated from the peptide content (estimated from A₂₁₀) and recovered radioactivity in the fractions containing the tracer, and was found to be 900 Ci/mmol.

Binding of ¹²⁵I-SMS 204-090 to pancreatic membranes

Binding of the tracer was performed at 25°C by incubating membranes (4–45 μ g of protein/ml) in Tris (50 mM)/CaCl₂ (0.2 mM)/bacitracin (0.5 mM)/soybean trypsin inhibitor (0.02%)/benzamidine (1 mM)/bovine serum albumin (2 mg/ml), pH 8.0 (binding buffer) with appropriate quantities of tracer (200 pM for competition studies and 1.0 nM for studies of saturated receptors). Separation of bound and free ligand was obtained by centrifugation at 10000 *g* for 2 min and washing once with binding buffer. Non-specific binding was estimated as membrane-associated radioactivity in the presence of 1 μ M-SS-14, and specific binding was calculated as the difference between total and non-specific membrane-associated radioactivity. Dissociation constants, rates of dissociation and association and

values of IC₅₀ with different ligands were calculated as described by Weiland & Molinoff (1981).

Solubilization of ¹²⁵I-SMS 204-090-receptor complexes

Membranes (35–70 μ g of protein/ml) in binding buffer were incubated for 75 min at 25 °C with 1.0 nM-¹²⁵I-SMS 204-090 in the presence (non-specific binding) or absence (total binding) of 1 μ M-SS-14. After centrifugation the membranes were suspended (1 mg of membrane protein/ml) in Tris (50 mM)/CHAPS (1%)/glycerol (10%, v/v)/CaCl₂ (0.2 mM)/bacitracin (0.5 mM)/soybean trypsin inhibitor (0.02%)/benzamidine (1 mM), pH 8.0 (solubilization buffer). Non-specifically labelled membranes were solubilized in solubilization buffer supplemented with, or devoid of, tracer at 1 nM. The mixture was gently agitated for 4 h at 4 °C. The mixture was thereafter centrifuged at 230000 *g* (50000 rev./min in a Beckman 50 TI type rotor) for 30 min in a Beckman model L5-50 ultracentrifuge. The supernatant was gently removed and kept at 4 °C for further experiments. To estimate the amount of complex-bound tracer, 200 μ l of the supernatant was loaded on to Sephadex G-25-SF columns measuring 11 cm $\times$ 0.9 cm and eluted at 4 °C at a flow rate of 0.5 ml/min in solubilization buffer supplemented with 1% bovine serum albumin. Complexes were eluted in the void volume of the column (as determined by the elution volume of Blue Dextran).

Lectin-adsorption chromatography of solubilized complexes

Wheat-germ agglutinin was coupled to activated agarose according to the supplier's instructions. Adsorption of solubilized complexes to the gel was performed by mixing 2–10 ml of solubilized material with 1 ml of the gel. The mixture was gently agitated by rotation for 1 h at 4 °C and thereafter poured into a 10 cm $\times$ 1 cm chromatographic column. The gel was washed with 10 ml of solubilization buffer at 4 °C and a flow rate of 250 μ l/min, and adsorbed complexes were eluted with 1 ml of 4 mM-triacetylchitotriose in solubilization buffer.

Gel-permeation chromatography of solubilized complexes

Complex-bound tracer obtained in the eluate after lectin-adsorption chromatography was further analysed by gel filtration on a Trisacryl GF-2000 column that measured 330 mm $\times$ 16 mm and was equilibrated and eluted at 4 °C with solubilization buffer at a flow rate of 8 ml/h. The column was calibrated with Blue Dextran, apoferritin, catalase and aldolase.

Dissociation of solubilized complexes

Dissociation of solubilized complexes recovered after lectin adsorption chromatography was followed by subjecting 200 μ l of the eluate to gel filtration on G-25-SF as described above after incubation at 25 °C in the presence or absence of GTP[S] (10⁻⁸–10⁻⁴M) for 30–120 min.

Cross-linking of ¹²⁵I-SMS 204-090 bound to membranes and to solubilized complexes

A portion (1 mg) of membrane protein equilibrated with tracer at 1.0 nM was precipitated and washed twice with 5 ml of Hepes (25 mM)/CaCl₂ (0.2 mM), pH 7.4 (cross-linking buffer). The membranes were resuspended

Fig. 1. Saturation of pancreatic acinar membranes with ^{125}I -SMS 204-090

Membranes ($0.6\text{--}\mu\text{g}$) were incubated at 25°C for 75 min in $150\text{ }\mu\text{l}$ of binding buffer with different concentrations of the tracer in the absence (●) or presence (○) of SS-14 at $0.1\text{ }\mu\text{M}$. In the inset is shown the same data transformed into a Scatchard plot (B_{max} , 5.7 pmol/mg of protein; K_D , 60 pM). Results are means $\pm$ S.E.M. ($n = 3$). This Figure is representative of three different experiments.

in 1.5 ml of cross-linking buffer. At 0°C in the dark was added $7.5\text{ }\mu\text{l}$ of ANB-NOS (10 mM in dimethyl sulphoxide). After 4 min in the dark the mixture was left for 8 min at 0°C under an HPK 125-W mercury-vapour lamp (u.v. light) at a distance of 15 cm. The reaction was stopped by addition of 1.5 ml of Tris(50 mM)/EDTA (2 mM), pH 7.4 (quenching buffer). After centrifugation the pellet was washed twice with quenching buffer and suspended in Tris (50 mM)/SDS (5%)/glycerol (5%), pH 6.8.

To 1.5 ml of the triacetylchitotriose eluate containing the solubilized complexes after lectin-adsorption chromatography were added $7.5\text{ }\mu\text{l}$ of ANB-NOS (10 mM in dimethyl sulphoxide). After 4 min at 0°C in the dark the mixture was exposed to u.v. light as described above. The reaction was stopped by addition of 1.5 ml of quenching buffer. The samples were concentrated to $300\text{ }\mu\text{l}$ in Centriscart I tubes by centrifugation at 3500 rev./min for 10 min. Quenching buffer was added to 2.5 ml , and the procedure repeated twice; the concentrated material was supplemented with sample buffer to reach final concentrations of 5% (w/v) SDS and 5% (v/v) glycerol and a pH of 6.8.

Immediately before application to polyacrylamide slab gels for electrophoresis by the method of Laemmli (1970), the samples were heated to 100°C for 3 min in the presence or absence of 1% β -mercaptoethanol.

The samples were applied to a 1 mm-thick polyacrylamide slab gel consisting of a 2 cm stacking gel (3% polyacrylamide) followed by a linear gradient gel (5–15%). After electrophoresis, gels were stained with

Fig. 2. Kinetics of binding of ^{125}I -SMS 204-090 to pancreatic acinar membranes

Membranes ($5.0\text{ }\mu\text{g}$) were incubated at 25°C with 200 pM (20000 c.p.m.) of ^{125}I -SMS 204-090 in the absence (●) or presence (○) of SS-14 ($1\text{ }\mu\text{M}$). After 60 min membranes were washed with, and reincubated in, binding buffer in the presence of SS-14 ($1\text{ }\mu\text{M}$) (▲) or of GTP[S] ($1\text{ }\mu\text{M}$) (△). Each point represents the mean of triplicate determinations. This Figure is representative of three different experiments.

Coomassie Blue (0.1%)/methanol (40%, v/v)/acetic acid (10%, v/v) and destained in methanol (40%, v/v)/acetic acid (10%, v/v). Autoradiograms of the dried gels were obtained on Kodak X AR-5 films by use of an enhancing screen and exposure for 3–14 days at -80°C .

RESULTS

Binding characteristics of ^{125}I -SMS 204-090

Fig. 1 demonstrates a saturation binding curve for ^{125}I -SMS 204-090. Transformation of the results into Scatchard plots reveals a single class of binding sites with a dissociation constant of $58 \pm 3\text{ pM}$ (mean $\pm$ S.E.M., $n = 3$) and a value of $4.7 \pm 0.8\text{ pmol/mg}$ (mean $\pm$ S.E.M., $n = 3$) for the maximal number of binding sites. With 0.2 nM -tracer, binding equilibrium was obtained after 60 min of incubation, and the displaceable binding was constant for at least another 90 min (Fig. 2). After removal of unbound tracer by washing after 60 min and subsequent incubation with SS-14 at $1\text{ }\mu\text{M}$, dissociation was found to be slow, with 85% of the original radioactivity remaining attached to the membranes after 90 min. From the results presented in Fig. 2 the rate constants of association and dissociation were calculated to be $0.057\text{ nM}^{-1}\cdot\text{min}^{-1}$ and 0.0022 min^{-1} respectively. The K_D obtained from these kinetic results is thus 39 pM . Incubation of the washed membranes with $1\text{ }\mu\text{M}$ -GTP[S] removed within 15 min about 60% of the membrane-associated radioactivity (Fig. 2). As calculated from indirect Hill plots, binding of the tracer in the presence of SMS-14 or SMS 201-995 was inhibited with IC_{50} values of 180 and 110 pM respectively (Fig. 3). Other peptides (insulin, vasoactive intestinal peptide, glucagon) as well as naloxone had no effects upon binding at concentrations up to $1\text{ }\mu\text{M}$ (results not shown).

Fig. 3. Inhibition of binding of ^{125}I -SMS 204-090 by SMS-14 and SMS 201-995

Membranes ($5.0\ \mu\text{g}$) were incubated at $25\ ^\circ\text{C}$ with $200\ \text{pM}$ - ^{125}I -SMS 204-090 for 75 min with different concentrations of SS-14 (○) or SMS 201-995 (●). Data are expressed as percentages of binding obtained in the absence of peptides. Each point represents the mean of triplicate determinations. This Figure is representative of three different experiments.

Solubilization of the ^{125}I -SMS 204-090-receptor complexes

To study the solubilized receptor, we chose to prelabel membranes with tracer at $1\ \text{nM}$. At this concentration, displaceable binding represented 80% or more of total binding capacity (B_{max}), and non-specific binding never exceeded 2–4% of total binding. The 4 h solubilization with 1% CHAPS released 70% of the proteins from the preparation to the supernatant after ultracentrifugation. At the same time, 50–60% of the bound radioactivity was removed from the membranes. Of the solubilized radioactivity, 20–35% was recovered in the void volume after gel filtration on small Sephadex G-25-SF columns. Preliminary results showed that changes in concentrations of detergent or glycerol diminished the yield of solubilized complexes, and prolongation of the time of solubilization did not significantly increase the amount of solubilized complex-bound radioactivity (results not shown). Most of the radioactivity was found to be eluted in the same position as unbound tracer, signifying a considerable amount of dissociation of complexes during solubilization (Fig. 4). Solubilization of membranes labelled in the presence of $1\ \mu\text{M}$ -SS-14 resulted in release to the supernatant of all bound radioactivity, but the radioactivity was found exclusively in the elution volume of free tracer after gel filtration on Sephadex G-25-SF columns, and supplementing this solubilization mixture with $1\ \text{nM}$ -tracer during the 4 h incubation resulted in the occurrence of a very small peak of radioactivity in the void volume (Fig. 4). The recovery of applied radioactivity on these columns was 95–98%.

Fig. 4. Separation of complex-bound and free solubilized ^{125}I -SMS 204-090 on Sephadex G-25-SF

After ultracentrifugation of membranes labelled with $1\ \text{nM}$ - ^{125}I -SMS 204-090 in the absence (●) or presence (○) of SS-14 at $1\ \mu\text{M}$ and solubilized with 1% CHAPS as described in the Experimental section, $200\ \mu\text{l}$ of the supernatant was applied to small Sephadex G-25-SF columns. Δ , Elution profile of the radioactivity from membranes labelled in the presence of $1\ \mu\text{M}$ -SS-14 followed by solubilization in the presence of the tracer at $1\ \text{nM}$. The arrowhead marked 'a' represents the elution volume of Blue Dextran, and that marked 'b' the elution volume of free tracer.

Chromatographic characterization of the solubilized complexes

After adsorption of the solubilized material to agarose-immobilized wheat-germ agglutinin, 8–15% of the prebound radioactivity was eluted with triacetylchitotriose (Fig. 5), comprising, thus, 55–75% of the applied solubilized complexes, as estimated by gel filtration of the crude solubilised material on Sephadex G-25-SF. After solubilization of 10 mg of membrane proteins followed by lectin-affinity chromatography, 140–200 μg of protein was found in the chitotriose eluate, this representing between 1.4 and 2% of the original membrane proteins. Since 8–15% of the originally membrane bound radioactivity was eluted with this amount of protein, a purification factor in the order of 5 was obtained, despite the dissociation occurring during solubilization.

After gel permeation on Trisacryl GF-2000 of the complex-bound radioactivity recovered in the triacetylchitotriose eluate, the radioactivity occurred as a peak with a distinct maximum between the elution volumes of apoferritin and catalase. Recovery of the applied radioactivity was 72–75% (Fig. 6).

Effect of GTP[S] on dissociation rate for the complexes

Incubation of solubilized complexes recovered in the triacetylchitotriose eluate at $25\ ^\circ\text{C}$ in the presence of

Fig. 5. Lectin-adsorption chromatography of solubilized ^{125}I -SMS 204-090-receptor complexes

After ultracentrifugation of ^{125}I -SMS 204-090-prelabelled solubilized membranes the supernatant was incubated with WGA-coupled agarose as described in the Experimental section. The gel was washed (fractions 1–18) with solubilization buffer and complexes were eluted (at 'a') with 1 ml of 4 mM-triacetylchitotriose in the same buffer. Each fraction had a volume of 0.75 ml.

Fig. 6. Gel-permeation profile of solubilized ^{125}I -SMS 204-090-receptor complexes on Trisacryl GF-2000

^{125}I -SMS 204-090-receptor complexes obtained in the triacetylchitotriose eluate were applied to a Trisacryl GF-2000 column measuring 33 cm $\times$ 1.6 cm and eluted in solubilization buffer at 4 $^{\circ}\text{C}$ and at a flow rate of 1 ml/10 min. Fractions (0.8 ml) were collected. On the Figure is furthermore marked: a, the elution volume of the first appearing peak of Blue Dextran; b, apoferritin (M_r 670 000); c, catalase (240 000); and d, aldolase (158 000). The Figure is representative of three experiments.

GTP[S] at 1 μM augmented the dissociation of the complexes to the extent of about 30 % after 30 min as compared with controls without GTP[S] (Fig. 7). This effect was dose-dependent, as was found also for the membrane-associated radioactivity (Fig. 8).

Fig. 7. Dissociation of solubilized ^{125}I -SMS 204-090-receptor complexes with and without GTP[S] (1 μM)

Solubilized ^{125}I -SMS 204-090-receptor complexes recovered in the triacetylchitotriose eluate after adsorption to WGA-agarose were incubated at 25 $^{\circ}\text{C}$ in the presence (●) or absence (○) of 1 μM -GTP[S]. Separation of bound and free tracer was obtained by gel filtration on small Sephadex G-25-SF columns as described in the Experimental section. Data are presented as means $\pm$ S.E.M. ($n = 3$).

Cross-linking of ^{125}I -SMS 204-090 to membrane-associated and solubilized complexes

In Fig. 9 are shown the autoradiograms of SDS/polyacrylamide slab gels after electrophoresis of ANB-NOS-cross-linked membrane-bound and solubilized complex-bound radioactivity. A broad band is evident, with a maximal intensity at an M_r of ~ 90 000, both in the membranes as well as in the solubilized complexes. Neither the intensity nor the position of the band was influenced by 1 % β -mercaptoethanol (results not shown). There was no darkening of the autoradiographic film when membranes were incubated with 1 μM -SS-14 (i.e. the unlabelled SMS displaced the labelled analogue).

DISCUSSION

Binding characteristics of ^{125}I -SMS 204-090

The present results demonstrate the specific binding of radioiodinated 204-090 to receptors of somatostatin on rat pancreatic membranes. The reaction between ligand and receptor is characterized by a K_D of 58 pM, a value comparable with, yet lower than, values obtained with tyrosine-substituted analogues of SMS-14 ([Tyr¹]- and [Tyr¹¹]-SMS-14) (Sakamoto *et al.*, 1984; Srikant & Patel, 1986). The number of revealed binding sites is very high (4.7 pmol/mg of protein). The value obtained is 5–10 times higher than the value reported by Sakamoto *et al.* (1984) and by Srikant & Patel (1986). The reason for this augmentation in the number of sites as compared with

Fig. 8. Dissociation of complex-bound ^{125}I -SMS 204-090 from intact or solubilized membranes in the presence of different concentrations of GTP[S]

Prelabelled membranes (○) or solubilized complexes (●) after lectin-adsorption chromatography were incubated at 25 °C for 30 min in the presence of different concentrations of GTP[S]. Separation of bound and free tracer was performed as described in the Experimental section. Data are means $\pm$ S.E.M. ($n = 3$).

Fig. 9. Autoradiogram of SDS/polyacrylamide-gel electrophoretograms of ^{125}I -SMS 204-090 covalently cross-linked to intact pancreatic membranes or to solubilized complexes

Membranes prelabelled with 1 nM- ^{125}I -SMS 204-090 in the absence (track b) or presence (track a) of 1 μM -SS-14 or solubilized complexes (track c) obtained after WGA-adsorption chromatography were treated with ANB-NOS as described in the Experimental section, and the cross-linked products were subjected to SDS/polyacrylamide-slab-gel electrophoresis and autoradiography.

findings with other tracers is unclear, but the increased stability of our tracer is a likely explanation. It is furthermore possible that the hydrophobic nature of our tracer makes it possible for it to gain access to receptors buried within the membranes or, perhaps, oriented towards the interior of the membranous vesicles. Thirdly, the small size of the molecule might contribute to its capacity to reach the binding sites. Our finding that this tyrosine-substituted SMS analogue reveals a very large number of sites is in accordance with similar findings in rat brain (Reubi, 1985). The notion that the tracer simply binds to receptors other than the SS-14 one is refuted by the fact that SS-14 displaced practically all membrane-associated radioactivity, by the homogeneity of the binding sites and by the lack of effect of several tested peptides on the binding. Although SMS 201-995 and its analogues have been suggested to interact with the μ subclass of the opiate receptors (Maurer *et al.*, 1982), naloxone did not influence binding of the tracer at all. Noteworthy also was the extremely low level of non-specific binding observed even when membranes were incubated with high concentrations of the tracer. All these features of the tracer might prove of interest in future attempts to characterize pancreatic SS-14 receptors.

Solubilized ligand-receptor complexes

Solubilization of the prelabelled pancreatic membranes with 1% CHAPS caused a release of complexes to the supernatant after ultracentrifugation that comprised 12–25% of the total membrane-associated binding (as judged by the amount of radioactivity occurring in the void volume after gel filtration on Sephadex G-25-SF). This yield is quite comparable with those obtained using the same detergent with opiate receptors (Simonds *et al.*, 1980) and for cholecystokinin (Lambert *et al.*, 1985). After adsorption of the solubilized material to WGA-agarose, complexes could be eluted with triacetylchitotriose, yielding 8–15% of the total membrane-bound radioactivity. This finding is in accordance with the notion of the SS-14 receptor being a glycoprotein (Susini *et al.*, 1987) and underlines the interpretation that the solubilized radioactivity has not simply accumulated within micelles. Upon gel permeation on Trisacryl GF-2000 the complexes appeared as a distinct peak between the elution volumes of apoferritin and catalase, suggesting that the major part of the complexes possess an M_r of $\sim 400\,000$ and signifying that they are truly solubilized. Since about 30% of the solubilized complex-bound radioactivity obtained after lectin-adsorption chromatography could be dissociated by incubation with GTP[S], it is reasonable to assume that at least some of the complexes are in functional connection with a GTP-binding regulatory protein, one presumably preserved from the membrane-associated state of the receptors. An interaction between the binding site of SS-14 and a GTP-binding regulatory protein has previously been suggested on the basis of the ability of GTP to inhibit binding of the ligand to pancreatic (Koch & Schonbrunn, 1984; Srikant & Patel, 1986; Susini *et al.*, 1987) as well as to gastric (Reyl-Desmars *et al.*, 1987), pituitary (Lewis & Williams, 1987) and cerebral (Czernick & Petrack, 1983) membranes. It has been suggested that this GTP-binding unit might be the G_i protein (Reyl-Desmars *et al.*, 1987; Sakamoto *et al.*, 1987; Viguerie *et al.*, 1988), since pertussis toxin completely removes the inhibitory effects

of somatostatin on histamine and vasoactive-intestinal-peptide-stimulated adenylate cyclase activity. Since pertussis toxin has been shown to phosphorylate several membrane proteins with M_r values differing by only 1000–2000 Da (Toutant *et al.*, 1987), it remains to be established whether the SS-14 receptor interacts solely with the G_i protein.

The association of the solubilized receptor with a G-protein might, furthermore, after identification of this G-protein, represent a basis for at least a partial purification of the complexes by the use of immunoaffinity chromatography directed towards this particular G-protein.

Covalent cross-linking with ANB-NOS, of the tracer, on to the membrane-associated and to the solubilized WGA-lectin-chromatographed complexes resulted in the occurrence, after SDS/polyacrylamide-gel electrophoresis and autoradiography, of a broad band exhibiting maximal intensity in the area of 90 kDa. The considerable broadness of the band might be due to proteolytic degradation or changes in glycosylation induced during our processing of the membranes. Srikant & Patel (1986) reported two bands with M_r values of 70000 and 80000 respectively occurring after cross-linking of [Tyr^{11}]SS-14 tracer to rat pancreatic membranes with the homobifunctional compound disuccinimidyl suberate. Since our protein seems to exhibit a somewhat higher M_r , it remains doubtful whether our weak broad band represents the same cross-linking products as those found with disuccinimidyl suberate by Srikant & Patel. The discrepancy could be due to use of different cross-linkers, but of interest also is the lack of soybean trypsin inhibitor in the incubation medium of Srikant & Patel (1986), as well as the fact that those authors used a membrane preparation less purified than ours. Furthermore, Lewis & Williams (1987) reported a weak band representing an M_r of 90000 occurring after cross-linking with disuccinimidyl suberate in pituitary membranes. Our results on the cross-linking are in good agreement with what has previously been reported in purified pancreatic membranes after use of [Tyr^1]- or [Tyr^{11}]SS-14 tracers cross-linked by ANB-NOS or the closely related compound *N*-hydroxysuccinimidyl 4-azidobenzoate (Sakamoto *et al.*, 1984; Matozaki *et al.*, 1986; Susini *et al.*, 1987; Lewis & Williams, 1987).

In conclusion, this is the first report of binding of [^{125}I]-[Tyr^3]SMS to pancreatic acinar membranes. The high efficacy of the tracer in demonstrating SS-14 receptors has enabled us to monitor the solubilized receptor complexes after treatment with 1% CHAPS, both after lectin-adsorption chromatography as well as after gel filtration. We also, for the first time, demonstrate an association between the solubilized receptors and G-proteins, and, finally, have succeeded in covalently cross-linking the tracer to a binding subunit with an M_r of 90000 *in situ* in the membrane, this same subunit being found within solubilized complexes cross-linked after lectin chromatography.

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