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SOLUBILIZATION OF ACTIVE SOMATOSTATIN RECEPTORS FROM RABBIT RETINA

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Abstract—Somatostatin receptors from rabbit retinal membranes were solubilized in an active form using a mixture of the detergents *n*-octyl *b*-D-glycopyranoside (OG) and CHAPS. The binding of [^{125}I]-Tyr^{11}-somatostatin to the soluble extract was saturable and of high affinity, with an apparent affinity constant (K_d) of 0.60 ± 0.20 nM and a maximum number of binding sites (B_{max}) of 80 ± 48 fmol/mg protein. The specific binding of [^{125}I]Tyr^{11}-somatostatin was inhibited in a dose-dependent manner only by the somatostatinergic analogs. The biochemical characteristics of both the membrane-bound and soluble receptors were studied by photoaffinity labeling techniques. Analysis by SDS–PAGE and subsequent autoradiography revealed the presence of a major protein of similar relative molecular mass (M_r 54,000 and 57,000 for membrane and soluble sites, respectively). The photolabeling of this protein was specifically inhibited by somatostatin-28, somatostatin-14, SMS 201-995 (a synthetic octapeptide analog of somatostatin) but not by bombesin and somatostatin-28(1–14). The non-hydrolysable GTP analog guanosine-5′-*O*-(3-thio-triphosphate) (GTPγS) regulated the photolabeling of [^{125}I]Tyr^{11}-somatostatin to the membrane and soluble receptors. These studies describe for the first time the successful solubilization of the somatostatin receptor and the biochemical characterization of both membrane-bound and soluble receptors from rabbit retina.

The neuropeptide somatostatin is a cyclic tetradecapeptide derived from the prohormone somatostatin-28. It is distributed throughout the central nervous system and the periphery where it is believed to play an important physiological role [1]. It inhibits the release of many hormones from the pituitary and the pancreas [2, 3], whereas in the brain it influences the firing of neurons and affects the release of other neurotransmitter substances [4, 5]. To mediate its physiological responses somatostatin interacts with specific receptors found in the plasma membrane. Somatostatin receptors have been studied with radioligand binding techniques in the brain, pituitary, the pancreas and various endocrine and exocrine tumor cells [6, 7]. They are coupled to pertussis toxin sensitive G proteins ($G_{i\alpha1}$,$G_{i\alpha3}G_{o\alpha}$) and modulate the activity of adenylate cyclase, calcium channels and other intracellular systems [8–11].

The biochemical characterization of the somatostatin receptor in different tissues has been attempted using photoaffinity labeling techniques. Results from these studies suggest that the somatostatin receptor is a protein of apparent relative molecular mass 45,000–228,000 [12–16] and subtypes of the somatostatin receptors have been proposed [13, 17–19]. Recently, the cloning of a family of somatostatin receptors has been performed [20, 21]. Yamada *et al.* [21] reported that the biological effects of somatostatin are mediated by tissue-specific expression of a family of somatostatin receptors. Further studies are needed, however, to elucidate the mechanisms at the molecular level via which somatostatin exerts its diverse physiological responses.

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Shapiro *et al.* [22] first reported the presence of somatostatin in the retina in the late seventies. Recently, a number of reports have focused on somatostatin-immunoreactivity in amacrine, associational ganglion and interplexiform cells of the retina [23–25]. These reports suggested that somatostatin may be a neurotransmitter, neuromodulator or trophic factor. There are very few reports, however, that describe somatostatin's mechanism of action and its physiological role in this organ. Kossut *et al.* [26, 27] reported high affinity binding sites for somatostatin in the retina of adult mice (C57B2/6J), while Liapakis and Thermos [28] identified [^{125}I]Tyr^{11}-somatostatin binding sites with a high affinity for somatostatin and its analogs in the rabbit retina.

Knowledge of the physicochemical characteristics of both the membrane-bound and the soluble somatostatin receptor will aid our understanding of somatostatin's mechanism of action. To this end, the present study was undertaken to investigate the biochemical components of the somatostatin receptor in the retina. We report the successful solubilization of somatostatin receptors and the biochemical and pharmacological characterization of both the membrane-bound and soluble receptor from the rabbit retina.

MATERIALS AND METHODS

Materials

All biochemicals were obtained from the Sigma Chemical Co. (Deisenhofen, Germany). [^{125}I]-Tyr^{11}-somatostatin was obtained from Amersham (Germany). All peptides were obtained from Bissendorf Biochemicals (Germany).

Animals

Male rabbits (New Zealand) weighing 2–3 kg were

purchased from a local breeder and were maintained in individual cages with free access to food and water. A 10:14 hr light:dark cycle was loosely maintained and all animals were killed during the light phase under ordinary room lighting.

Preparation of rabbit retinal membranes

The eyes of male rabbits were enucleated and the cornea and lens were removed. Retinas were teased from the pigment epithelium and sclera as described previously by Liapakis and Thermos [28]. They were immersed in 50 mM Tris–HCl, pH 7.4 at 4°, containing bacitracin (20 μg/mL), EGTA (1 mM), $MgCl_2$ (5 mM), leupeptin (1 μg/mL), aprotinin (0.5 μg/mL) and phenylmethanesulfonyl fluoride (PMSF*) (0.1 mM) (buffer I) and homogenized using a Polytron (Brinkman). The homogenate was centrifuged twice at 45,000 *g* ($r_{av} = 65.7$) at 4° for 30 min. The final pellet was resuspended in buffer I and used as the retinal membrane preparation or solubilized directly. Protein determinations were performed according to the procedure of Bradford [29].

Solubilization of the membrane receptor

The final retinal pellet was suspended in buffer I containing *n*-octyl *b*-D-glycopyranoside (OG) (0.6%; w/v), glycerol (20%; w/v) and phospholipids (lecithin from soy bean; Serva; 0.65 mg/mL) previously sonicated (Vibracell, Bioblock Scientific) for 2 min in 50 mM Tris–HCl, pH 7.4 at 4°. The mixture was gently agitated for 60 min on ice and subsequently CHAPS (0.6%; w/v) was added. After an additional 45 min incubation the mixture was centrifuged at 100,000 *g* for 60 min. The supernatant was removed and used immediately as the soluble extract.

Equilibrium binding assays

Soluble extracts (100 μg) were added to polystyrene tubes containing [^{125}I]Tyr^{11}-somatostatin (2000 Ci/mmol; 1.5 nM), bovine serum albumin (BSA) (0.2%; w/v), glycerol (16%; v/v) and non-radioactive drug or buffer I, in a total volume of 0.25 mL. The mixture was equilibrated for 90 min at 25° and the binding reaction was terminated by vacuum filtration over GF/F filters that were previously soaked for 90 min in polyethyleneimine (PEI) (0.5%; w/v). The filters were washed with 2 × 4 mL 50 mM Tris–HCl buffer, pH 7.6 and counted for radioactivity in a gamma counter (LKB; efficiency 75%). Specific binding was determined in the presence of D-Trp^8-somatostatin (1 μM). To obtain the desired concentration, the iodinated ligand was diluted with unlabeled Tyr^{11}-somatostatin. For the competition studies the results were analysed using a log–logit plot, and the IC_{50} values obtained at logit = 0.

Photoaffinity labeling

Membranes. Retinal membranes (500 μg) were incubated with [^{125}I]Tyr^{11}-somatostatin (1.5 nM) in the presence or absence of somatostatin-28, somatostatin-14 and its analogs, and guanosine 5′-*O*-(3-thio-triphosphate) (GTPγS) for 90 min at 25°. Subsequently the mixture was washed three times by centrifugation (35,000 *g* for 5 min at 4°) and the final pellet was resuspended in phosphate-buffered saline (PBS) buffer and irradiated with UV light (450 W, ACE Hanova) for 15 min on ice under constant stirring in the presence of the photocrosslinking agent *N*-hydroxysuccinimidyl-4-azidobenzoate (HSAB) (0.1 mM). The covalently labeled proteins were examined using SDS–PAGE and subsequent autoradiography.

Soluble preparation. The soluble extract (580 μg) was incubated with [^{125}I]Tyr^{11}-somatostatin (1.5 nM) in the presence or absence of somatostatin analogs for 90 min at 25°. Immediately the mixture was photolabeled as mentioned for the retinal membranes. Subsequently the photocrosslinked soluble mixture was examined using gel filtration chromatography.

Gel filtration chromatography

The soluble mixture photoaffinity labeled with [^{125}I]Tyr^{11}-somatostatin was separated on a Sephadex G-50 column (9 cm × 35 cm) previously equilibrated with 50 mM Tris–HCl buffer, pH 7.4, containing 0.03% CHAPS at 4°. Blue Dextran 2000 was used to define the void volume. Fractions (1 mL) were collected at a constant flow rate of 1.2 mL/min at 4°. The optical density at A_{280} and radioactivity were measured for each fraction. Fractions eluted at the void volume were pooled, concentrated on Amicon-30 filters,and run on SDS–PAGE.

SDS–PAGE

Gel electrophoresis was performed according to Laemmli [30] using 1.5 mm thick slab gels containing 10% acrylamide, and run at constant voltage (200 V). After electrophoresis the gels were fixed overnight in glacial acetic acid (10%) and dried using a Hoeffer model slab dryer. Autoradiograms were obtained from the dried gels after exposure for 2–14 days to KODAK XAR-5 film with a Dupont Cronex Lightening Plus intensifying screen.

RESULTS

Solubilization of somatostatin receptors

Several detergents were examined for solubilization of [^{125}I]Tyr^{11}-somatostatin binding sites from rabbit retina. The per cent of protein and [^{125}I]-Tyr^{11}-somatostatin binding sites solubilized was determined and shown in Table 1. The most effective solubilization was achieved when a mixture of OG and CHAPS was employed, solubilizing a high percentage of binding sites (55%), as afforded by one-point binding (1.5 nM [^{125}I]Tyr^{11}-somatostatin).

A range of concentrations of CHAPS was tested. The optimal concentration for maximizing the yield of solubilized protein and [^{125}I]Tyr^{11}-somatostatin binding was 0.6% (data not shown), and was used in subsequent studies.

* Abbreviations: PEI, polyethyleneimine; PMSF, phenylmethanesulfonyl fluoride; PBS, phosphate-buffered saline; HSAB, *N*-hydroxysuccinimidyl-4-azido-benzoate, OG, *n*-octyl *b*-D-glycopyranoside; SMS 201-995, synthetic octapeptide analog of somatostatin; GTPγS, guanosine-5′-*O*-(3-thio-triphosphate).

Table 1. Percentage of solubilization of membrane proteins and [^{125}I]Tyr^{11}-somatostatin binding sites by various detergents

Detergent	Detergent concn (%)	Protein	[^{125}I]Tyr^{11} somatostatin binding
CHAPS	0.6	24 ± 4	30 ± 8
OG	0.6	9 ± 4	21 ± 5
OG + CHAPS	0.6 ± 0.6	50 ± 8	55 ± 1
Digitonin	1.0	13 ± 7	28 ± 5

The values presented in columns 2 and 3 represent % protein (mg/mL) and % specific binding (fmoles) in the soluble extracts as compared to the total protein and specific binding, respectively, detected in the membrane preparation prior to solubilization.

Membrane pellets were suspended in buffer I containing glycerol (20% v/v), phospholipids (0.65 mg/mL) and the above detergents. For CHAPS, OG and digitonin the mixtures were agitated on ice for 60 min, and subsequently centrifuged at 100,000 *g* for 60 min. When OG + CHAPS was utilized, the mixture was initially agitated with OG for 60 min on ice and subsequently CHAPS was added, agitated for 45 min longer and centrifuged as above. In all instances the supernatant was used immediately as the soluble preparation.

The values presented represent the mean ± SD. Protein concentrations were determined according to Bradford [29]. Binding of [^{125}I]Tyr^{11}-somatostatin to the soluble extracts was performed as described in Materials and Methods using 1.5 nM [^{125}I]Tyr^{11}-somatostatin and somatostatin-14 (1 μM) was used to define specific binding.

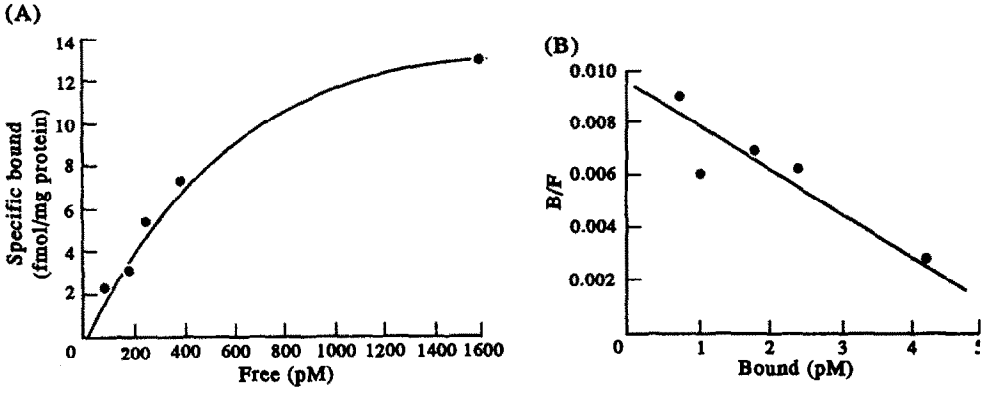

Fig. 1. (A) Saturation isotherm of [^{125}I]Tyr^{11}-somatostatin to soluble extracts from rabbit retina. Soluble extracts (40 μg) were incubated with increasing concentrations of [^{125}I]Tyr^{11}-somatostatin in a final volume of 100 μL for 90 min at 25°. Specific binding is defined as the difference between total binding and that in the presence of D-Trp^8-somatostatin. (B) Scatchard analysis of the saturation data. The line drawn represents the best fit to the data as determined by linear regression analysis. For this experiment the K_d is 0.43 nM and the B_{max} 20 fmol/mg protein ($r = 0.90$).

Characterization of the binding properties of solubilized receptors

Binding of [^{125}I]Tyr^{11}-somatostatin to the soluble extract measured at the concentration range of 0.1–1.5 nM [^{125}I]Tyr^{11}-somatostatin was saturable at 25° (Fig. 1A). Scatchard analysis of the binding data [31] suggest a population of soluble sites with an apparent affinity constant of 0.60 ± 0.20 nM and a B_{max} of 80 ± 48 fmol/mg protein (N = 6; Fig. 1B). The pharmacological profile of the soluble receptors was studied by means of competition binding experiments. As shown in Fig. 2, somatostatin-28, somatostatin-14 and its analogs inhibited the binding of [^{125}I]Tyr^{11}-somatostatin to the soluble extracts from rabbit retina in a dose-dependent manner. All agents were active in the nanomolar range. With the exception of Tyr^{11}-somatostatin the other three competition ligands displayed Hill coefficients less than unity. This would suggest that [^{125}I]Tyr^{11}-somatostatin is labeling more than one binding site in the soluble extracts of the retina. The inactive peptide somatostatin-28(1–14) and bombesin had no effect on the [^{125}I]Tyr^{11}-somatostatin binding at

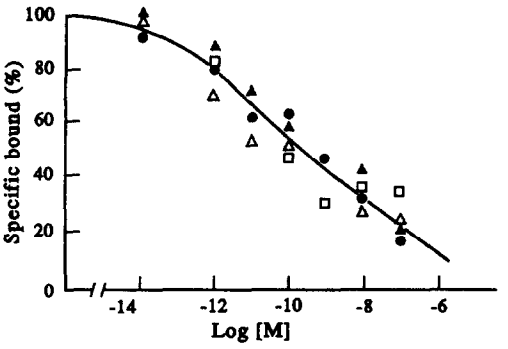

Fig. 2. Inhibition of specific [^{125}I]Tyr^{11}-somatostatin binding to soluble extracts from rabbit retina. [^{125}I]Tyr^{11}-somatostatin (1.5 nM) binding to soluble extracts (100 μg) was inhibited by various concentrations of somatostatin-28 (□), somatostatin-14 (●), D-Trp^{8}-somatostatin (▲), Tyr^{11}-somatostatin (△). This is a representative experiment repeated three to eight times.

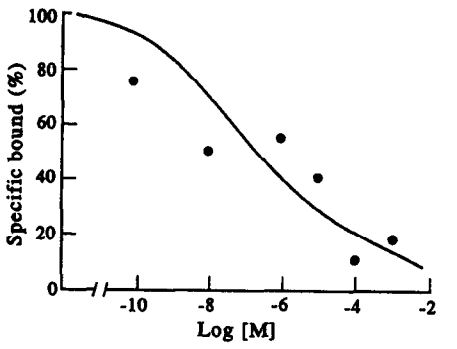

Fig. 3. GTPγS inhibition of specific [^{125}I]Tyr^{11}-somatostatin binding to soluble retinal receptors. [^{125}I]Tyr^{11}-somatostatin (1.5 nM) was incubated for 90 min at 25° with soluble extracts (100 μg) from rabbit retina and increasing concentrations of GTPγS. This is a representative experiment repeated twice.

Table 2. Inhibition of [^{125}I]Tyr^{11}-somatostatin binding to soluble extracts from rabbit retina by somatostatin and its analogs

Ligand	IC_{50} (nM)
Somatostatin-14	0.75 ± 0.22
D-Trp^{8}-somatostatin	0.82 ± 0.28
Somatostatin-28	0.54 ± 0.10
Tyr^{11}-somatostatin	0.38 ± 0.22
Somatostatin-28(1–14)	>1000
Bombesin	>1000

Somatostatin-28, somatostatin-14 and its analogs at concentrations of 10^{-14}–10^{-6} M were incubated with [^{125}I]-Tyr^{11}-somatostatin (1.5 nM) and soluble extracts (100 μg) from rabbit retina for 90 min at 25°. The IC_{50} values are presented as the mean ± SEM. Somatostatin-28(1–14) and bombesin were examined at the concentration of 1 μM.

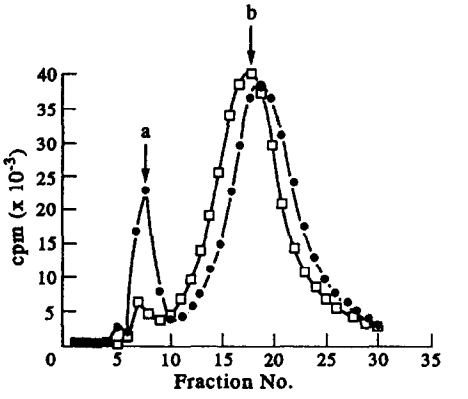

Fig. 4. Gel filtration chromatography of [^{125}I]Tyr^{11}-somatostatin photocrosslinked to soluble receptors from rabbit retina. Soluble extracts (580 μg) were incubated with [^{125}I]Tyr^{11}-somatostatin (1.5 nM) in the presence (□) or absence (●) of somatostatin-28 for 90 min at 25°. The mixture was irradiated in the presence of HSAB as described in Materials and Methods and subsequently separated at 4° on a Sephadex G-50 column, previously equilibrated with 50 mM Tris–HCl buffer containing 0.03% CHAPS, pH 7.4 at 4°. Fractions of 1 mL were collected. The arrowhead marked 'a' represents the elution volume of Blue Dextran and that marked 'b' the elution volume of free ligand.

concentrations below 1.0 μM (Table 2). Furthermore, the non-hydrolysable GTP analog GTPγS was found to inhibit the binding of [^{125}I]Tyr^{11}-somatostatin to the soluble receptors in a dose-dependent manner (Fig. 3).

These results provide, for the first time, evidence for the successful solubilization of somatostatin receptors from rabbit retina.

Biochemical characterization of somatostatin receptors

To study the biochemical characteristics of the soluble receptor, [^{125}I]Tyr^{11}-somatostatin was photocrosslinked to the soluble extract (as mentioned in Materials and Methods) and the complex studied with gel filtration and SDS–PAGE. Chromatography of the photolabeled soluble extract on a Sephadex G-50 column afforded a radioactivity peak in the void volume, while the remaining radioactivity coeluted with free [^{125}I]Tyr^{11}-somatostatin. When somatostatin-28 (1 μM) was added together with [^{125}I]Tyr^{11}-somatostatin in the incubation mixture the resulting elution profile showed a dramatic decrease of the radioactivity eluted only in the void volume of the Sephadex G-50 column (Fig. 4).

The fractions collected in the void volume of the Sephadex G-50 column were pooled, concentrated using Amicon-30 filters, and separated on 10% polycrylamide gels. As shown in Fig. 5A a band of M_r 57,000 ± 3000 was obtained. A lower relative molecular mass protein (48,000) and a broad band from 31,000 to 45,000 were also observed but in

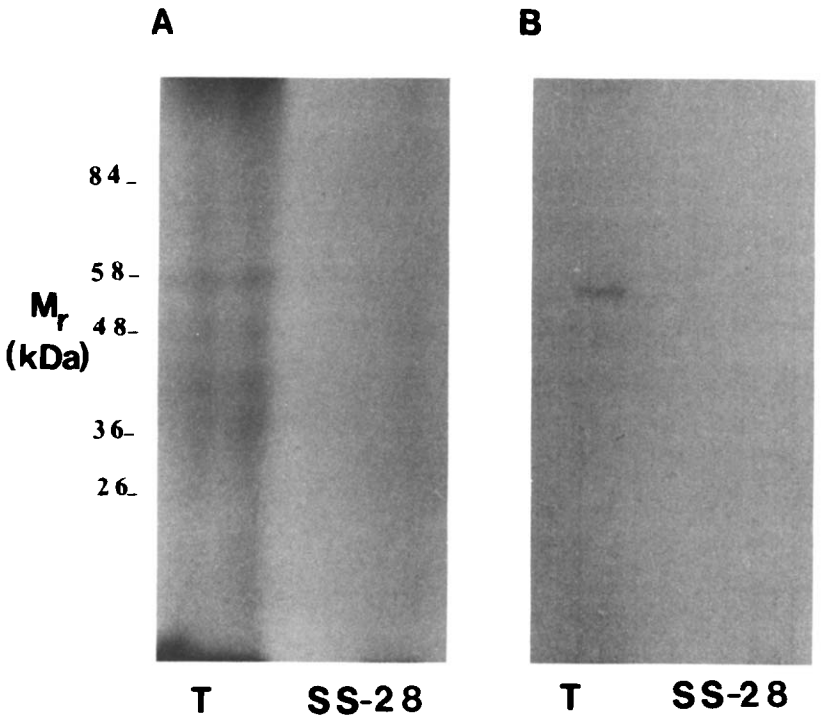

Fig. 5. (A) Autoradiogram of [^{125}I]Tyr^{11}-somatostatin photocrosslinked to soluble receptors from rabbit retina. Samples of photocrosslinked soluble extracts previously separated on a Sephadex-G50 column at 4° and eluted in the void volume were pooled, concentrated on Amicon-30 filters and analysed by SDS–PAGE and autoradiography. (B) Autoradiograms of [^{125}I]Tyr^{11}-somatostatin photocrosslinked to membrane rabbit retinal receptors. Retinal membranes (500 μg) were incubated with [^{125}I]Tyr^{11}-somatostatin (1.5 nM) in the presence or absence of somatostatin-28 for 90 min at 25°. The mixture was photocrosslinked as described in Materials and Methods. The covalently labeled proteins were studied by SDS–PAGE and autoradiography.

more experiments were of lower density, as assessed by densitometry of the autoradiograms (data not shown).

To compare the biochemical characteristics of the soluble receptors to those of the membrane bound receptors, retinal membranes were incubated with [^{125}I]Tyr^{11}-somatostatin and subsequently photolabeled in the presence of HSAB (0.1 mM). The mixture was studied using SDS–PAGE and a major band of M_r 54,000 ± 4000 was obtained. The photolabeling of this biological entity was inhibited by somatostatin-28 (Fig. 5B) but not by bombesin (data not shown). To provide more evidence that the photolabeled protein in the soluble extracts (M_r 57,000) represents the somatostatin receptor from rabbit retina, somatostatin-28, somatostatin-14, SMS 201-995 (a synthetic octapeptide analog of somatostatin) and bombesin at concentrations of 1 μM were examined for their ability to inhibit the photolabeling of the [^{125}I]Tyr^{11}-somatostatin binding sites. The photolabeling was inhibited specifically by all the somatostatinergic analogs but not by bombesin (Fig. 6). Somatostatin-28 and the SMS 201-995 analog also appear to inhibit the photolabeling of the smaller molecular mass components in the soluble extract.

The presence of GTPγS in the incubation mixture reduced the photolabeling of [^{125}I]Tyr^{11}-somatostatin to both membrane and soluble receptors from rabbit retina (data not shown).

DISCUSSION

The present study was undertaken to investigate the biochemical components of the somatostatin receptor in rabbit retina as a means of elucidating somatostatin's mechanism of action in this organ. Solubilization of the receptor is a prerequisite for its biochemical characterization. Although somatostatin receptors are known to be distributed widely in various tissues, there have been very few studies on their solubilization and purification [32–35].

Using a mixture of the non-ionic detergent OG and the non-denaturing zwitterionic detergent CHAPS, we have successfully solubilized the somatostatin receptor from rabbit retina in an active form. Either of the above detergents alone or digitonin were not able to yield high amounts of soluble protein and [^{125}I]Tyr^{11}-somatostatin binding sites, respectively.

The properties of the somatostatin receptor were not altered after its solubilization. The binding of [^{125}I]Tyr^{11}-somatostatin to the soluble extract was saturable and of high affinity. The binding of [^{125}I]-

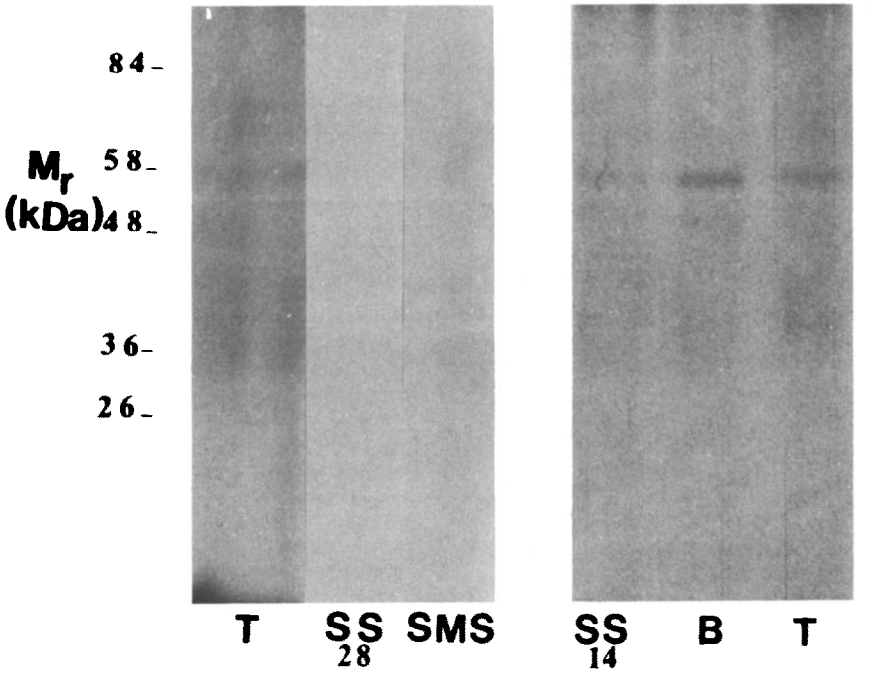

Fig. 6. Specificity of the photolabeling of soluble somatostatin receptors from rabbit retina. Soluble extracts (580 μg) were incubated with [^{125}I]Tyr^{11}-somatostatin in the presence of somatostatin-28 (SS-28), somatostatin-14 (SS-14), SMS 201–995(SMS) and bombesin (B), at concentrations of 1 μM, for 90 min at 25°. The mixtures were photocrosslinked with HSAB, separated by gel filtration and analysed by SDS–PAGE and autoradiography.

Tyr^{11}-somatostatin to retinal membranes afforded a K_d value of 0.90 ± 0.2 nM and a B_{max} value of 104 ± 52 fmol/mg protein [28], whereas the binding of [^{125}I]Tyr^{11}-somatostatin to the soluble receptor afforded a K_d value of 0.60 ± 0.20 nM and a B_{max} value of 80 ± 48 fmol/mg protein. Knuhtsen *et al.* [33] reported one binding site for [^{125}I]Tyr^3 SMS in soluble pancreatic extracts with a K_d value of 0.3 nM and a B_{max} value of 210 fmol/mg protein. He *et al.* [35], however, reported the solubilization of two sites for the rat brain receptor. Using a different radioligand they found [^{125}I]MK678 binding afforded K_d values of 1.1 nM and 35 nM and B_{max} values of 452 and 1875 fmol/mg protein for the high and low affinity site, respectively.

The pharmacological profile of the soluble receptor is very similar to that of the membrane-bound receptors from rabbit retina [28]. Somatostatin-28, somatostatin-14 and its analogs displayed IC_{50} values in the nanomolar range, whereas bombesin and somatostatin-28(1–14), were inactive in inhibiting [^{125}I]Tyr^{11}-somatostatin binding to the soluble receptors.

These data support the successful solubilization of the somatostatin receptor from rabbit retina.

Furthermore, the non-hydrolysable GTP analog GTPγS regulated the binding of [^{125}I]Tyr^{11}-somatostatin to soluble receptors, suggesting that the receptor-associated G proteins can be solubilized with the somatostatin receptor. Nakamura and Rodbell [36] have reported that G proteins remain stable after extraction with octyl glycoside.

The biochemical properties of the receptor have been studied by gel filtration and SDS–PAGE after photoaffinity labeling of the receptor with [^{125}I]Tyr^{11}-somatostatin in the presence of the photocrosslinking agent HSAB. Analysis of the photocrosslinked soluble extracts by gel filtration and SDS–PAGE revealed a protein of M_r 57,000 ± 3000. In retinal membranes a protein of similar molecular mass (M_r 54,000) was obtained. In the soluble extract smaller molecular mass components were also observed but their density varied in the different experiments and the density measured never exceeded that of the M_r 57,000 protein, but was usually much lower. All somatostatinergic ligands examined inhibited the photolabeling of [^{125}I]Tyr^{11}-somatostatin to both membrane and soluble receptors, whereas bombesin had no effect. These data provide strong evidence that the somatostatin receptor in the rabbit retina is a protein of approximately M_r 57,000. Even though the pharmacological profile of the lower molecular mass components (48,000 and 31,000–45,000) is indicative of a somatostatin receptor, further studies are essential to assess the nature of these proteins as possible subtypes of the receptor.

Previous studies using photoaffinity labeling techniques have provided different molecular masses for the somatostatin receptor in different tissues. The M_r 57,000 protein identified in our studies is in agreement with values reported by Bruno and Berelowitz [37] and Murthy *et al.* [15], using the same radioligand and photocrosslinking agent, for the somatostatin receptors in membranes from rat

pituitary and pituitary tumor cells, respectively. Thermos and Reisine [38] reported a protein of M_r 55,000 covalently labeled to [^{125}I]CGP23996 in the presence of HSAB in AtT-20 and GH3 cells. A protein of similar molecular mass was detected in membranes of rat brain and of pancreatic b-cells [13, 18].

There are very few studies depicting the successful solubilization of somatostatin receptors. Knuhtsen *et al.* [33] and He *et al.* [35] solubilized somatostatin receptors in an active form and reported a relative molecular mass of about 400,000 for soluble receptors from rat pancreas and rat brain, respectively. In denaturing gel electrophoresis, photoaffinity labeling of the soluble rat pancreas receptor with [^{125}I]Tyr^3-SMS revealed three components of M_r 100,000, 56,000 and 21,000. In addition Marie *et al.* [16] covalently labeled membranes from beta cells of hamster insulinomas with [^{125}I]Leu^8,D-Trp^{22},[Tyr^{25}]-somatostatin-28 and subsequently solubilized the mixture with Triton X-100. Analysis by SDS–PAGE revealed three components of relative molecular mass 228,000, 128,000 and 45,000.

The apparent discrepancies in the reported molecular masses may be a result of the different tissues, radioligands and detergents used. Subtypes of the somatostatin receptor have been proposed, based on binding studies [17, 38]. More recently, however, cloning experiments have shown the amino acid sequence and molecular mass of the somatostatin receptor to vary (369–391 amino acids, M_r 41,000–43,000) [20, 21, 39, 40].

In the present study, we have solubilized the somatostatin receptor from rabbit retina in an active form. Its pharmacological and biochemical characteristics are similar to those of the membrane-bound receptor. This is the first report of the characterization of the physicochemical properties of the somatostatin receptor in retina. The cloning of the somatostatin receptor(s) in different tissues will provide very important information regarding the basis of somatostatin receptor heterogeneity. Such studies will be instrumental in the elucidation of the mechanism of action of somatostatin and its functional role in all target organs, including the retina.

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REFERENCES

1. Epelbaum J, Somatostatin in the central nervous system: physiology and pathological modifications. *Prog Neurobiol* **27**: 63–100, 1986.
2. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J and Guillemin R, Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **179**: 77–79, 1983.
3. Mandarino L, Stenner D, Blanchard W, Nissen S, Gerich J, Ling N, Brazeau P, Esch F and Guillemin R, Selective effects of somatostatin-14, -25, -28, on *in vitro* insulin and glucogen secretion. *Nature* **291**: 76–78, 1981.
4. Pitman QJ and Siggins JR, Somatostatin hyperpolarized hippocampal pyramidal neurons *in vitro*. *Brain Res* **121**: 402–408, 1981.
5. Chesselet M-F and Reisine T, Somatostatin regulates dopamine release in rat striatal slices and cat caudate nucleus. *J Neurosci* **3**: 232–236, 1983.
6. Epelbaum J, Tapia-Arancibia L, Kordon C and Enjalbert A, Characterization, regional distribution and subcellular distribution of [^{125}I]Tyr^1-somatostatin binding sites in rat brain. *J Neurochem* **38**: 1515–1523, 1982.
7. Srikant CB and Patel Y, Somatostatin receptors on rat pancreatic acinar cells. *J Biol Chem* **261**: 7690–7696, 1986.
8. Lewis D, Weight F and Luini A, A guanine nucleotide-binding protein mediating the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. *Proc Natl Acad Sci USA* **83**: 9035–9039, 1985.
9. Mahy N, Woolkalis M, Thermos K, Carlsson K, Manning D and Reisine T, Pertussis toxin modifies the somatostatin receptor in anterior pituitary tumor cells. *J Pharmacol Exp Ther* **246**: 779–785, 1988.
10. Wang H, Bogen C, Reisine T and Dichter M, Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons. *Proc Natl Acad Sci USA* **86**: 9616–9620, 1989.
11. Law SF, Manning D and Reisine T, Identification of the subunits of GTP-binding proteins coupled to somatostatin receptors. *J Biol Chem* **266**: 17885–17897, 1991.
12. Sussini C, Bailey A, Szecowka J and Williams J, Characterization of covalently cross-linked pancreatic somatostatin receptors. *J Biol Chem* **261**: 16738–16743, 1986.
13. Thermos K, He H-T, Wang H, Margolis N and Reisine T, Biochemical properties of brain somatostatin receptors. *Neuroscience* **31**: 131–142, 1989.
14. Sakamoto C, Nagao M, Mazotaki T, Nishizaki H, Konda Y and Baba S, Somatostatin receptors on rat cerebrocortical membranes. *J Biol Chem* **263**: 14441–14445, 1988.
15. Murthy KK, Srikant CB and Patel YC, Evidence for multiple protein constituents of the somatostatin receptor in pituitary tumor cells: affinity crosslinking and molecular characterization. *Endocrinology* **125**: 948–956, 1989.
16. Marie JC, Cotroneo P, de Chasseval R and Rosselin G, Solubilization of somatostatin receptors in hamster pancreatic beta cells. *Eur J Biochem* **186**: 181–188, 1989.
17. Tran V, Beal M and Martin J, Two types of somatostatin receptors differentiated by cyclic somatostatin analogs. *Science* **228**: 492–495, 1985.
18. Thermos K, Meglasson MD, Nelson J, Lounsbury KM and Reisine T, Pancreatic b-cell somatostatin receptors. *Am J Physiol* **259**: E216–E224, 1990.
19. Reubi JC, Evidence for two somatostatin-14 receptor types in rat brain cortex. *Neurosci Lett* **49**: 259–263, 1984.
20. Kluxen F-W, Bruns C and Lubbert H, Expression cloning of a rat brain somatostatin receptor cDNA. *Proc Natl Acad Sci USA* **89**: 4618–4622, 1992.
21. Yamada Y, Post SR, Wang K, Tager HS, Bell GI and Seino S, Cloning and functional characterization of a family of human mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. *Proc Natl Acad Sci USA* **89**: 251–255, 1992.
22. Shapiro B, Kronheim S and Pinistone B, The presence of immunoreactive somatostatin in the rat retina. *Horm Metab Res* **11**: 79–80, 1979.

23. Zalutsky RA and Miller R, The physiology of somatostatin in the rabbit retina. *J Neurosci* **10**: 383–393, 1990.
24. White CA, Chalupa LM, Johnson D and Brecha NC, Somatostatin-immunoreactive cells in the adult cat retina. *J Comp Neurol* **293**: 134–150, 1990.
25. Sagar SM, Somatostatin-like immunoreactive material in the rabbit retina: immunohistochemical staining using monoclonal antibodies. *J Comp Neurol* **266**: 291–299, 1987.
26. Kossut M, Yamada T, Aldrich LB and Pinto LH, Localization and characterization of somatostatin binding sites in the mouse retina. *Brain Res* **476**: 78–84, 1989.
27. Kossut M, Aldrich LB, Yamada T and Pinto LH, The binding of somatostatin to the mouse retinal is altered by the pearl mutation. *Brain Res* **522**: 235–240, 1990.
28. Liapakis G and Thermos K, Characterization of [^{125}I]-Tyr11-somatostatin binding sites in the rabbit retina. *Neuropeptides* **21**: 13–19, 1992.
29. Bradford MM, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
30. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* **227**: 680–685, 1970.
31. Scatchard G, The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* **51**: 660–667, 1949.
32. Knuhtsen S, Esteve J, Bernadet B, Vaysse N and Sussini C, Molecular characterization of the solubilized receptor of somatostatin from rat pancreatic membranes. *Biochem J* **254**: 641–647, 1988.
33. Knuhtsen S, Esteve J, Cambillau C, Colas B, Sussini C and Vaysse N, Solubilization and characterization of active somatostatin receptors from rat pancreas. *J Biol Chem* **265**: 1129–1133, 1990.
34. He H-T, Johnson K, Thermos K and Reisine T, Purification of a putative brain somatostatin receptor. *Proc Natl Acad Sci USA* **86**: 1480–1484, 1989.
35. He H-T, Rens-Domiano S, Martin J-M, Law S, Borislow S, Woolkalis M, Manning D and Reisine T, Solubilization of somatostatin receptors from rat brain. *Mol Pharmacol* **37**: 614–621, 1990.
36. Nakamura S-I and Rodbell M, Octyl glucoside extracts GTP-binding regulatory proteins from rat "synaptoneurosomes" as large polydisperse structures devoid of $\beta\gamma$ complexes and sensitive to disaggregation by guanine nucleotides. *Proc Natl Acad Sci USA* **87**: 6413–6417, 1990.
37. Bruno JF and Berelowitz M, Covalent labeling of the somatostatin receptor in rat anterior pituitary membranes. *Endocrinology* **124**: 831–837, 1989.
38. Thermos K and Reisine T, Somatostatin receptor subtypes in the clonal anterior pituitary cell lines AtT-20 and GH3. *Mol Pharmacol* **33**: 370–377, 1988.
39. Li X-J, Forte M, North RA, Ross CA and Snyder SH, Cloning and expression of a rat somatostatin receptor enriched in brain. *J Biol Chem* **267**: 21307–21312, 1992.
40. Bruno JF, Xu Y, Song J and Berelowitz M, Molecular cloning and functional expression of a brain-specific somatostatin receptor. *Proc Natl Acad Sci USA* **89**: 11151–11155, 1992.