Tissue-specific distribution of cross-linked somatostatin receptor proteins in the rat

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Pharmacological studies have suggested that the somatostatin (SS) receptor is heterogeneous and exhibits SS-14- and SS-28-selective subtypes. Whether such subtypes arise from molecular heterogeneity of the receptor protein has not been definitively established. Previous reports characterizing the molecular properties of the SS receptor by the cross-linking approach have yielded divergent size estimates ranging from 27 kDa to 200 kDa. In order to resolve this discrepancy, as well as to determine whether SS-14 and SS-28 interact with specific receptor proteins, we have cross-linked radioiodinated derivatives of [¹²⁵I-Tyr¹¹]SS-14 (T*-SS-14) and [Leu⁸,D-Trp²²,¹²⁵I-Tyr²⁵]SS-28 (LTT*-SS-28) to membrane SS receptors in rat brain, pituitary, exocrine pancreas and adrenal cortex using a number of chemical and photoaffinity cross-linking agents. The labelled cross-linked receptor proteins were analysed by SDS/PAGE under reducing conditions followed by autoradiography. Our findings indicate that the pattern of specifically labelled cross-linked SS receptor proteins is sensitive to the concentration of chemical cross-linking agents such as disuccinimidyl suberate and dithiobis-(succinimidyl propionate). Labelled high-molecular-mass complexes of cross-linked receptor–ligand proteins were observed only when high concentrations of these cross-linkers were employed. Using optimized low concentrations of cross-linkers, however, two major labelled bands of 58 ± 3 kDa and 27 ± 2 kDa were detected. These two bands were identified as specifically labelled SS receptor proteins subsequent to cross-linking with a number of photoaffinity cross-linking agents as well. We demonstrate here that the 58 kDa protein is the major SS receptor protein in the rat pituitary, adrenal and exocrine pancreas, whereas the 27 kDa moiety represents the principal form in the brain. Additionally, the presence of a minor specifically labelled band of 32 kDa was detected uniquely in the brain, and a minor labelled protein of 42 kDa was observed in the pancreas. The labelling pattern obtained with LTT*-SS-28 was identical to that observed with T*-SS-14. Labelling of the 27 kDa band by either ligand was inhibited by SS-14 and SS-28 in a dose-dependent manner. Densitometric quantification showed that SS-14 exhibited > 2-fold greater potency than SS-28 for inhibiting the labelling of the 27 kDa species. These findings emphasize the need for careful interpretation of cross-linking data obtained for SS receptors, and provide evidence for molecular heterogeneity and for a tissue-specific distribution of the two principal SS receptor proteins.

INTRODUCTION

Somatostatin (SS) is an important regulatory peptide with a wide tissue distribution and a broad spectrum of actions [1]. The two principal bioactive forms in mammals consist of SS-14 and SS-28, which are derived by differential processing of prosomatostatin and are distributed in varying concentrations in different tissues [2]. The actions of the two peptides are mediated by plasma membrane receptors which are coupled via guanine-nucleotide-binding proteins to membrane effector systems, notably adenylate cyclase and K⁺ channels [3,4]. Although both peptides exhibit similar biological activities, they show relative preference for certain actions [5–13]. Such selectivity can be explained by differential interaction of SS-14 and SS-28 with SS receptors, and suggests the existence of receptor subtypes selective for the two peptides. Additionally, SS-14 and SS-28 exert preferential effects on receptor-linked transmembrane signalling systems, e.g. K⁺ channels [14,15]. Whether these pharmacologically distinct SS receptor subtypes arise as a result of molecular heterogeneity and/or differential coupling to second messenger systems has not been resolved. We have previously reported, using the cross-linking approach, that SS-14 and SS-28

exhibit relative selectivity for interaction with 27 kDa and 58 kDa receptor proteins respectively in GH₃ and AtT-20 tumour cells [16]. The existence of such selectivity in normal tissues has not been examined. Additionally, there is wide variation in the size of SS receptor reported, ranging from 27 kDa to 200 kDa, in cross-linking studies from different laboratories [16–28]. These studies have employed different receptor sources, ligands and cross-linking conditions, and a systematic evaluation of the molecular properties of SS receptors in these systems under identical cross-linking conditions has not been carried out. In order to explain the marked discrepancy in the reported molecular size estimates of the SS receptor(s), as well as to establish whether the selective interaction of SS-14 and SS-28 with SS receptor subtypes results from their preferential binding to one or more of these receptor proteins in normal tissues, we assessed the molecular size of SS receptor proteins cross-linked under identical conditions to radioiodinated derivatives of SS-14 and SS-28 in normal rat tissues. Four tissues, i.e. brain, pituitary gland, pancreas and adrenal gland, were selected because they represent rich sources of SS receptors which exhibit heterogeneity of the binding site [3,18,28–31]. Our results demonstrate the presence of two major specifically labelled SS receptor forms in normal tissues of the

Abbreviations used: ANBNOS, *N*-(5-azido-2-nitrobenzoyloxy)succinimide; BSOSES, bis-[2-(succinimido-oxycarbonyloxy)ethyl] sulphone; DSP, dithiobis-(succinimidyl propionate); DSS, disuccinimidyl suberate; HSAB, *N*-hydroxysuccinimidyl-4-azidobenzoate; SANPAH, *N*-succinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate; SS-14, somatostatin-14; SS-28, somatostatin-28; T*-SS-14, [¹²⁵I-Tyr¹¹]SS-14; LTT*-SS-28, [Leu⁸,D-Trp²²,¹²⁵I-Tyr²⁵]SS-28.

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rat: a 58 kDa protein in the pituitary, adrenal cortex and exocrine pancreas, and a 27 kDa protein in the rat brain which exhibits relative selectivity for SS-14.

MATERIALS AND METHODS

Materials

Synthetic cyclic SS-14 was obtained from Ayerst Laboratories (Montreal, Quebec, Canada). [^{125}I]SS-14 and [^{125}I]-D-Trp²², Tyr²⁵]SS-28 were purchased from Bachem Fine Chemicals (Marina Del Ray, CA, U.S.A.). The cross-linking reagents DSS, DSP, BSOSES, HSAB, ANBNOS and SANPAH were purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). BSA and prelabelled molecular mass markers for SDS/PAGE were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of analytical grade and were obtained from various commercial sources. Radioiodinated derivatives of the tyrosinated peptides were prepared with Na^{125}I by the chloramine T method and purified by gel filtration or reverse-phase h.p.l.c. [32]. The calculated specific radioactivities for the two radioligands were 1650 Ci/mmol for [^{125}I]-Tyr¹¹]SS-14 (T*-SS-14) and 1050 Ci/mmol for [^{125}I]-D-Trp²², Tyr²⁵]SS-28 (LTT*-SS-28).

Preparation of membrane receptors

Synaptosomal membranes were prepared from freshly obtained cerebral cortices of adult male Sprague-Dawley rats as previously described [28]. Membrane fractions from pituitary, adrenal cortex and pancreatic exocrine cells of these rats were prepared as previously reported from our laboratory [18,29,30].

Receptor-binding and cross-linking of receptor-ligand complex

Binding of T*-SS-14 and LTT*-SS-28 to membrane SS receptors was carried out at 30 °C as previously described, by incubating 50–200 μg of membrane protein with 1 nM radioligand in 50 mM-Hepes/KOH buffer (pH 7.5) containing BSA (0.2 $\text{mg}\cdot\text{ml}^{-1}$), 5 mM- Mg^{2+} , Trasylol (Aprotinin, 200 kallikrein-inhibitory units $\cdot\text{ml}^{-1}$) and 0.02 $\mu\text{g}\cdot\text{ml}^{-1}$ each of bacitracin and phenylmethanesulphonyl fluoride (RRA buffer) in the absence or presence of 100 nM-SS-14 [28–30]. For binding studies with pancreatic acinar cell membranes Mg^{2+} was replaced by Ca^{2+} (0.5 mM) in the RRA buffer [18]. After equilibrium binding was attained, the radioligand bound to the membranes was separated by centrifugation (2500 g , 5 min) followed by washing with $2\times 1\text{ ml}$ of RRA buffer from which BSA was omitted, and recentrifugation.

Cross-linking of receptor-bound ligand

Subsequent to binding, the membrane pellet was resuspended in RRA buffer without BSA and the cross-linking agents, dissolved in dimethyl sulphoxide as 100-fold concentrates, were added to give the required final concentration. Chemical cross-linking was carried out on ice for 5–20 min, followed by quenching with 1 ml of 1 M-Tris and centrifugation. For photocross-linking, the reaction mixture was exposed to u.v. light from a General Electric GE-275 sunlamp at a distance of 14 cm. The cross-linked receptor-ligand complex was then washed with 0.1 M-acetate buffer (pH 5.5) to remove non-covalently bound ligand.

Analysis of cross-linked receptor-ligand by SDS/PAGE

The cross-linked receptor preparation was dissolved in 60 mM-Tris/HCl buffer, pH 6.5, containing 10% (w/v) glycerol, 0.001% Bromophenol Blue, 3% SDS and 50 mM-dithiothreitol. After heating at 100 °C for 5 min in a boiling-water bath, the samples were centrifuged at 10000 g for 10 min and applied to 5–15%

continuous gradient polyacrylamide slab (1.5 mm thick) gels and electrophoresed according to Laemmli [33]. The gels were then fixed, dried and exposed for 3–10 days at $-80\text{ }^{\circ}\text{C}$ to Kodak XAR X-o-matic films with intensifying screens. Autoradiograms were scanned at 600 nm using a Quickscan densitometer (Helena Laboratories, Beaumont, TX, U.S.A.). Molecular masses of radioactively labelled protein bands representing covalently bound receptor-ligand complexes were obtained from linear regression plots of log (molecular mass) versus the mobility of the following protein markers: α -macroglobulin (subunit, 180 kDa), β -galactosidase (116 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (subunit, 58 kDa), fumarase (subunit, 48 kDa), lactate dehydrogenase (subunit, 36.5 kDa) and triosephosphate isomerase (subunit, 26.6 kDa).

RESULTS

Optimization of cross-linking conditions

To optimize the cross-linking conditions and to establish the specificity of the labelled cross-linked receptor proteins, studies were carried out initially with brain and adrenocortical tissues. Cross-linking of T*-SS-14 to membranes prepared from these two tissues in the presence of 1 mM-DSS revealed labelling of a prominent 200 kDa band in both tissues, a major 180 kDa band in brain which was only weakly visualized in the adrenal gland (Fig. 1, lanes 1 and 5), and a minor 60 kDa band seen only in brain (Fig. 1, lane 1). When the concentration of DSS was reduced to 0.1 mM, a strikingly different pattern of labelled receptor proteins was observed. In brain, labelling was seen predominantly in a $27\pm 2\text{ kDa}$ region; a faint band corresponding to $32\pm 2\text{ kDa}$ was also detected (Fig. 1, lane 3). By contrast, adrenocortical membranes showed a dominant labelled band of $58\pm 3\text{ kDa}$ (Fig. 1, lane 7). Inclusion of unlabelled SS-14 (0.1 μM) inhibited the labelling of all these bands (Fig. 1, lanes

Fig. 1. Chemical cross-linking of T*-SS-14 to rat brain synaptosomal (lanes 1–4) and adrenocortical (lanes 5–8) membranes

Membranes (150 μg) were incubated with 1 nM-T*-SS-14 in buffer containing BSA (0.2 $\text{mg}\cdot\text{ml}^{-1}$) in the absence or presence of excess SS-14. After cross-linking of the receptor-bound radioligand with DSS at the concentrations shown, the samples were electrophoresed on a 5–15% gradient SDS/polyacrylamide gel under reducing conditions and analysed autoradiographically. Cross-linking with 1 mM-DSS resulted in the formation of labelled bands of 200 kDa, 180 kDa and 70 kDa in the brain (lane 1) and of 200 kDa and 180 kDa in adrenal gland (lane 5). By contrast, cross-linking with 0.1 mM-DSS revealed the presence of a single intensely labelled band of 27 kDa and a minor band of 32 kDa in brain (lane 3), and a single band of 58 kDa in adrenal gland (lane 7). The specificity of labelling under these conditions was demonstrated by the inhibition of labelling by 0.1 μM -SS-14 in lanes 2, 4, 6 and 8.

Fig. 2. Autoradiogram of rat brain membrane SS receptor proteins chemically cross-linked to T*-SS-14

Binding and cross-linking were performed in BSA-free buffer in the presence of DSS (0.1 mM) for different times as indicated prior to analysis by SDS/PAGE. The 27 kDa and 32 kDa bands were detected as the major and minor labelled SS receptor protein components at all time points. Cross-linking for up to 20 min did not result in the formation of high-molecular-mass labelled complexes.

Fig. 3. Autoradiogram showing the detection in brain of the 27 kDa and 32 kDa bands as the major and minor SS receptor proteins respectively labelled with T*-SS-14

Cross-linking was with 0.1 mM concentrations of a variety of chemical (DSS, DSP, BSOCES) and 0.3 mM concentrations of photoreactive (ANBNOS, HSAB, SANPAH) cross-linking agents. Binding and cross-linking in BSA-free buffer and SDS/PAGE were carried out as described in the Materials and methods section. Cross-linkers used: lanes 1 and 2, DSS; lanes 3 and 4, DSP; lanes 5 and 6, BSOCES; lanes 7 and 8, ANBNOS; lanes 9 and 10, SANPAH; lanes 11 and 12, HSAB.

Fig. 4. Effect of GTP on the labelling of SS receptor proteins in rat brain (a) and adrenal cortex (b)

Membranes were incubated with T*-SS-14 in the presence or absence of 0.1 mM-GTP followed by cross-linking with 0.1 mM-DSS and analysis by SDS/PAGE and autoradiography. Labelling of the 27 and 32 kDa proteins in brain and of the 58 kDa band in adrenal gland was inhibited by GTP.

2, 4, 6 and 8). At intermediate concentrations of DSS the intensity of the high-molecular-mass band seen at 1 mM-DSS decreased progressively and was accompanied by the appearance

Fig. 5. Comparison of labelling of brain SS receptor proteins by T*-SS-14 and LTT*-SS-28

(a) Autoradiogram illustrating the dose-dependent inhibition by SS-14 (lanes 1–5) and SS-28 (lanes 6–10) of the labelling of SS receptor proteins in rat brain membranes by T*-SS-14 (top panel) and LTT*-SS-28 (bottom panel). Binding and cross-linking of T*-SS-14 and LTT*-SS-28 to brain membranes and cross-linking with 0.1 mM-DSS was carried out in BSA-free buffer in the absence or presence of unlabelled peptides. Both radioligands revealed labelling of one major (27 kDa) and one minor (32 kDa) band which was inhibited by SS-14 and SS-28 over a concentration range of 0.1–100 nM. Data are representative of three experiments. (b) Densitometric quantification of the labelling of the 27 kDa band by T*-SS-14 and LTT*-SS-28 in rat brain depicted in (a). The intensity of labelling by the radioligand at each concentration of the unlabelled peptides is expressed as a percentage of labelling intensity observed with the radioligand alone. SS-14 (●) was > 2-fold more potent than SS-28 (+) in inhibiting the labelling by T*-SS-14 (top panel) as well as by LTT*-SS-28 (bottom panel).

of smaller labelled bands, the intensity of which increased as the cross-linker concentration was lowered. Since BSA was added as a carrier protein in the RRA buffer, and since BSA has been reported to produce non-specifically labelled bands during cross-linking [34], we suspected that the labelling observed in the high-

Fig. 6. Comparison of SS receptor proteins chemically cross-linked to receptor-bound T*-SS-14 (a) and LTT*-SS-28 (b) in rat tissues

Portions of 50–200 μ g of membrane proteins from rat brain, pituitary, exocrine pancreas and adrenal cortex were cross-linked with DSS (0.1 mM) to receptor-bound T*-SS-14 (a) and LTT*-SS-28 (b). In brain both radioligands specifically labelled a major protein band of 27 ± 2 kDa and a minor band of 32 ± 2 kDa. By contrast, the major band identified with T*-SS-14 and LTT*-SS-28 in pituitary, adrenal cortex and exocrine pancreas corresponded to 58 ± 3 kDa. In exocrine pancreas an additional band corresponding to 42 kDa was also observed.

molecular-mass regions could be an artifact resulting from complexes between the ligand associated with BSA and the receptor proteins. To rule out the possibility that inclusion of BSA was contributing to the appearance of spuriously labelled bands in the presence of high cross-linker concentrations, we incubated brain membranes with T*-SS-14 and cross-linked using BSA-free buffer. Although BSA increased the intensity of high-molecular-mass bands marginally, even in its absence specific labelling of these bands could be detected subsequent to cross-linking with 1 mM- but not 0.1 mM-DSS (results not shown). Based on these findings, all subsequent binding and cross-linking reactions were performed in the absence of BSA using optimized low concentration of DSS. Fig. 2 shows the effect of prolonged incubation with 0.1 mM-DSS in the absence of BSA on the pattern of T*-SS-14-labelled cross-linked SS receptor proteins in brain membranes. Specifically labelled protein bands of 27 kDa and 32 kDa only were seen. In particular, the formation of high-molecular-mass complexes was not observed even after incubation of the receptor-bound ligand with 0.1 mM-DSS for up to 20 min. Finally, the pattern of labelling of both brain and adrenal SS receptor proteins observed under these optimized conditions for cross-linking was unaffected by the inclusion of proteolytic inhibitors at the time of binding and/or cross-linking (results not shown).

Effect of different chemical and photoreactive cross-linkers

To further authenticate the 27 kDa, 32 kDa and 58 kDa proteins, the pattern of T*-SS-14-labelled SS receptor proteins in brain and adrenals cross-linked with 0.1 mM-DSS was compared with that obtained with two other chemical cross-linkers, DSP and BSOCOES, and the three photoreactive cross-linkers ANBNOS, HSAB and SANPAH (Fig. 3). At this concentration, chemical cross-linking with DSP and BSOCOES also revealed

specific labelling of the 27 kDa band as a major component and the 32 kDa band as a minor component. As illustrated in Fig. 3, only the 27 kDa and 32 kDa labelled proteins were detected in brain subsequent to photocross-linking with ANBNOS, HSAB and SANPAH. Likewise, only the 58 kDa protein was identified by all cross-linkers in the adrenal (results not shown).

Effect of GTP on the labelling of SS receptor proteins

To further validate the specificity of the labelled bands, the effect of GTP on the labelling pattern of SS receptor proteins in brain and adrenal membranes cross-linked in the presence of 0.1 mM-DSS was analysed. GTP lowers the affinity of G-protein-coupled receptors such as the SS receptor(s) for ligand interaction [4]. As shown in Fig. 4, GTP (0.1 mM) inhibited the labelling of the principal 58 kDa and 27 kDa bands in the two tissues respectively, as well as the minor 32 kDa band in brain. Unlike GTP, similar concentrations of ATP, ADP and GDP did not significantly decrease the labelling of these bands by T*-SS-14.

Comparison of the labelling of brain SS receptor proteins by T*-SS-14 and LTT*-SS-28

Cross-linking of LTT*-SS-28 to cerebrocortical membranes showed that this ligand labelled the identical protein bands as those detected with T*-SS-14. As seen in Fig. 5, the 27 kDa and 32 kDa bands appeared as the major and minor bands respectively with both radioligands. Furthermore, labelling of these two bands by T*-SS-14 and LTT*-SS-28 was inhibited by SS-14 and SS-28 in a dose-dependent manner. Densitometric quantification and comparison of the dose-dependent inhibition of the labelling of the 27 kDa band by the peptides ($n = 6$) revealed that SS-14 was more potent than SS-28, reflecting the previously documented 3-fold greater potency of SS-14 for receptor binding in brain [31].

Comparison of labelled cross-linked SS receptor proteins in peripheral tissues

We next compared SS receptors cross-linked with 0.1 mM-DSS to T*-SS-14 and LTT*-SS-28 in peripheral tissues. As with brain, the pattern of labelling of the SS receptor proteins in pituitary, adrenal and exocrine pancreas was comparable for the two radioligands (Fig. 6). Autoradiograms of cross-linked receptor preparations from pituitary and adrenal cortex demonstrated a single specifically labelled protein (58 kDa) detectable with both T*-SS-14 (Fig. 6a), and LTT*-SS-28 (Fig. 6b). In the exocrine pancreas, the main labelled band was in the 58 kDa region, but appeared as a doublet. The size estimates of the major SS receptor proteins detected in peripheral tissues using other cross-linking agents were identical to those obtained with DSS (results not shown). It can be seen from Fig. 6 that the major receptor protein in pituitary, pancreas and adrenal cortex detected with T*-SS-14 and LTT*-SS-28 is larger (58 kDa) than the principal SS receptor protein constituent (27 kDa) identified in cerebral cortex by the same ligands.

DISCUSSION

Somatostatin receptors occur at high concentrations throughout the central nervous system, as well as in the interior pituitary, exocrine pancreas and adrenal cortex [18–20,28–30,35–37]. These receptors have also been localized in GH₃, GH₃C₁ and AtT-20 pituitary tumour cells [3,14,27,38], in hamster insulinoma and RINm5f cells [22,39] and in human leukaemic (Jurkat T-cell) and myeloma (U 266) cell lines [40]. The molecular properties of plasma membrane SS receptors have been characterized in a number of these tissues using the cross-linking approach [16–27,41]. These studies have investigated different tissues, different SS ligands (both chemical and photo-cross-linkers), and have reported single or multiple labelled SS receptor proteins of widely divergent sizes. In the present study we have shown that high concentrations of DSS, a commonly used chemical cross-linker, produce very high molecular mass aggregates of the cross-linked receptor–ligand complex as previously reported for other receptors, e.g. the growth hormone-releasing factor receptor [34]. When an optimized low concentration of DSS was employed, however, two principal specifically labelled cross-linked SS receptor proteins of 58 kDa and 27 kDa were identified in the different tissues examined. The 58 kDa and 27 kDa forms were also the only major bands seen with a wide range of other chemical and photoreactive cross-linkers used under carefully optimized conditions. Labelling of each of these two major proteins was sensitive to inhibition by SS-14 and SS-28, attesting to their specificity as putative SS receptor proteins. Based on the present findings, some of the reported high-molecular-mass estimates, including our own previously reported estimate of 200 kDa [17,18,22], represent overestimates and should be revised.

Our data clearly show the presence of two major and two minor SS receptor forms, of molecular mass 58 kDa, 42 kDa, 32 kDa and 27 kDa, distributed in a tissue-specific manner. The radioligand LTT*-SS-28 did not differ from T*-SS-14 in the pattern of labelling of cross-linked receptor proteins in these tissues. Although LTT*-SS-28 is widely used as an SS-28 ligand (because it is the only putative SS-28 ligand available), its selectivity for binding is demonstrable only in autoradiographic studies of fixed tissue [42–45]. In direct binding and cross-linking studies with membranes or whole cells, LTT*-SS-28 appears to be indistinguishable from T*-SS-14, as reported previously for AtT-20 and GH₃ cells [3,14,16]. In the pituitary, adrenal and exocrine pancreas the major SS receptor species identified by the two ligands was of the 58 kDa size. This is in agreement with the previously reported size estimate of the SS receptor in AtT-20

and GH₃ tumour cells by Thermos & Reisine [27] and in AtT-20 cells from our laboratory [16]. In contrast, the major SS receptor protein in brain was the 27 kDa form. The 32 kDa and 42 kDa protein bands were uniquely observed in the brain and pancreas respectively, where they were detected as minor components. In parallel studies, we have established that the radioiodinated derivative of the octapeptide SS analogue, [Tyr³]SMS 204 090, labels the same proteins as the two radioligands used in the present study (C. B. Srikant & Y. C. Patel, unpublished work). However, a newly synthesized *N*-azidophenyl analogue of [Tyr³]SMS 204 090 (EE 581), which could be cross-linked by direct photoactivation, labelled the 32 kDa band but not the 27 kDa band in the brain, although an additional band of 58 kDa was labelled by this ligand [46–49]. In contrast to the different sized receptor proteins identified by the cross-linking approach, the solubilized affinity-purified SS receptor from rat brain membranes has recently been shown to consist of a single protein of 60 kDa [26]. On the other hand, a recently reported affinity purified human gastric cell SS receptor has been shown to be a somewhat larger protein of 90 kDa [50]. Lower molecular mass forms were not identified in these studies. This raises the question of whether the 27 kDa cross-linked receptor protein detected as the major component in brain represents a cleavage product of the 58 kDa form. Several lines of evidence, however, argue against simple degradation as an explanation for the detection of this and the minor components of 32 kDa and 42 kDa in the present study, as follows: (i) cross-linking of SS receptors in the absence or presence of protease inhibitors resulted in identical autoradiographic patterns; (ii) the tissue-specific distribution of the 58 kDa and 27 kDa forms and, in particular, the virtually exclusive presence of the 27 kDa form in the brain, cannot be explained by degradation, and (iii) the relative proportions of the 27 kDa and 58 kDa forms detectable in a given tissue have been shown to be ligand-dependent [46,48,49]. On the basis of all this evidence, it would appear that the 27 kDa form in particular is an important form of the SS receptor. Although its size is small in comparison with other G-protein-linked membrane receptors which have been cloned [51–54], it is consistent with a reported photolabelled 32 kDa form of the histamine H₂ receptor, a G-protein-linked receptor [55].

A number of pharmacological studies have established heterogeneity of SS receptors [3] and their coupling to two G-proteins, G_i and G_q [4]. SS-14 and SS-28 bind to SS receptors with differential affinities [3,14,22,29,31,32,48]. Binding of the two peptides to their receptors induces changes in two key intracellular mediators, cyclic AMP and intracellular Ca²⁺, as a result of Ca²⁺ receptor-linked effects on membrane adenylate cyclase and ionic channels [4,55,56]. In general, the potency of SS-14 and SS-28 for receptor binding correlates with the biological response in all of these systems, as well as with the ability of the two ligands to activate post-receptor events, e.g. changes in cyclic AMP and Ca²⁺ in AtT-20 and GH₃ cells [14,27]. Other studies, however, have demonstrated that the two peptides exert opposite effects on K⁺ conductance in cultured cerebrocortical neurons [57], raising the additional possibility of SS-14- and SS-28-selective receptor subtype proteins preferentially coupled to transmembrane signalling systems as described for the muscarinic receptor [51]. In previous studies using AtT-20 and GH₃ tumour cells, we have demonstrated a relative preference of the 58 kDa receptor protein for SS-28 and of the 27 kDa protein for SS-14 binding [16]. Competitive inhibition studies, however, clearly showed a high interactive potency of SS-14 for the dominant 27 kDa SS receptor form in brain. As in the case of AtT-20 and GH₃ cells, the selectivity for inhibition of the labelled bands by both radioligands in brain by SS-14 and SS-28 was relative rather than absolute. It is likely that the 58 kDa receptor form in

pancreas and adrenal cortex will differ from the 58 kDa pituitary receptor, since direct binding studies have previously shown a comparable receptor binding affinity for SS-14 and SS-28 in pancreas and adrenal cortex [18,30], in contrast with SS-28 selectivity in the pituitary [31]. Additionally, the unique Ca^{2+} -dependence of the pancreatic SS receptor may account for further heterogeneity of the 58 kDa form [18].

In summary, these studies provide clear evidence for molecular heterogeneity of SS receptor proteins. The two major forms of the SS receptor of 58 kDa and 27 kDa size exhibit tissue-specific distribution. Additionally, these findings emphasize the need for careful interpretation of cross-linking data obtained for SS receptors.

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