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A Single Amino Acid Substitution in Somatostatin Receptor Subtype 5 Increases Affinity for Somatostatin-14

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SUMMARY

Four of the five somatostatin receptor (SSTR) subtypes bind the two native forms of somatostatin, i.e., somatostatin-14 (S-14) and amino-terminally extended somatostatin-28 (S-28), with comparable affinities (approximately 0.2 nM). The SSTR5 subtype exhibits 10–50-fold higher affinity for S-28 than for S-14 (0.2 and 5 nM, respectively). To determine which domains in SSTR5 are responsible for the observed pharmacological selectivity, a series of SSTR2/SSTR5 chimeras were constructed and expressed in Chinese hamster ovary cells. Saturation and competition radioligand binding studies demonstrated that the region encompassing transmembrane domain 6 (TM6) through the carboxyl terminus plays a critical role in the lower binding affinity of S-14 for SSTR5. Substitution of this region with the corresponding region of SSTR2 produced chimeric receptors with high affinity for both S-28 and S-14. Examination of amino acid sequences revealed both a specific conserved hydrophobic residue and a conserved tyrosine in TM6 of SSTR1–4. At comparable positions in SSTR5, these residues are glycine (G258) and phenylalanine (F265), respectively. Substitution of G258 with phenylalanine did not alter the preference of SSTR5 for S-28 over S-14. However, substitution of F265 with tyrosine increased the binding affinity of S-14 by 20-fold, to an affinity comparable to that observed for the SSTR2 subtype. These data indicate that replacement of phenylalanine with tyrosine at position 265 in SSTR5 can modify ligand binding selectivity and abolish the preference for S-28 over S-14. This finding suggests that the tyrosine in the predicted TM6 may be an important contact point between somatostatin and SSTR.

The diverse biological effects of somatostatin are mediated by five distinct SSTR subtypes (1–7). The five SSTR subtypes are products of different genes and are members of the G protein-coupled receptor superfamily, with seven membrane-spanning domains (1–7). The five SSTR subtypes exhibit distinct pharmacological properties (8, 9). Although many of the cellular responses of somatostatin have not been attributed to individual receptor subtypes, subtype-selective somatostatin agonists can be used to assign all five subtypes to different *in vivo* biological effects (8, 9). For example, SSTR2 mediates inhibition of growth hormone release (8, 9) and SSTR5 mediates inhibition of insulin and glucagon release (10).

Somatostatin is found in two predominant forms, i.e., a 14-amino acid, disulfide-linked, cyclic peptide (S-14) and an amino-terminally extended, 28-amino acid form (S-28), which exhibit overlapping but not identical potencies for *in vivo* responses (11). Rat SSTR subtypes 1–4 do not discriminate between S-14 and S-28. Both of these natural hormones bind with high affinity, in the 200 pM range, to SSTR1–4 (1–6). In contrast, S-28 binds with 10–50-fold higher affinity than S-14 to the SSTR5 subtype (7). Compared with rat SSTR1–4, the binding affinity of S-14 at SSTR5 is lower (5 nM), rather than S-28 having a higher affinity (7). Structural determinants conferring the lower binding affinity of S-14 for the SSTR5 subtype have not yet been described. In the current investigation a series of six SSTR2/SSTR5 chimeric receptors were used to localize the domain responsible for the pharmacological selectivity of the two forms of somatostatin at SSTR5. The region encompassing TM6, extracellular loop 3, and TM7 was found to play a critical role in the differential binding affinities of S-28 and S-14 for the SSTR5 subtype. Sequence comparisons revealed a conserved tyrosine in TM6 of SSTR1–4 but a phenylalanine in the corresponding position of SSTR5. Substitution of the phenylalanine (F265) in TM6 of SSTR5 with a tyrosine reconstituted high affinity S-14 binding comparable to that observed with SSTR1–4.

Experimental Procedures

Cloning of rat SSTR2 and SSTR5. The cloning of rat SSTR2 has been described (12). The rat SSTR5 coding region was amplified by PCR, utilizing the nucleotide sequence reported by O'Carroll *et al* (7). Terminal oligonucleotide primers were 5′-AAGCTTCGCATGGAGCCCCTCTCTCTGG-3′ (sense) and 5′-TCTAGAATGCATCTCAGCAAACTCTCA-3′ (antisense). The sense primer includes the trans-

ABBREVIATIONS: SSTR, somatostatin receptor; CHO, Chinese hamster ovary; S-14, somatostatin-14; S-28, somatostatin-28; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MK678, cyclo(*n*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe); ICL3, third intracellular loop; ET, endothelin; TM1–7, transmembrane domains 1–7; PCR, polymerase chain reaction.

lation start codon and the antisense primer includes the translation stop codon. The PCR was performed according to protocols provided by the reagent supplier (Perkin Elmer Cetus), using 0.15 nmol of each primer plus 20 ng of rat genomic DNA as template. Amplification was performed with a Perkin Elmer model 9600 thermal cycler, using cycles of 94.5° for 20 sec, 60° for 20 sec, and 72° for 90 sec. The PCR product was cloned into the pCRII vector (Invitrogen Corp.). Clones were confirmed by nucleotide sequencing, using reagents and equipment purchased from Applied Biosystems. All sequence analyses were performed using the MacVector software package (Kodak, New Haven, CT).

Construction of chimeric receptors. The rat SSTR2 gene contains a *Bam*HI site in the sequence encoding the predicted ICL3. A *Sal*I site was engineered in the region encoding TM3, using standard PCR techniques.[1] *Bam*HI and *Sal*I restriction sites were engineered in the SSTR5 gene by PCR methods. The changes were designed to directly correspond to the same sites in SSTR2. Oligonucleotides to create the *Sal*I site were ACAGTCGACGGCATCAACCAGTTC (positions 504–527) and GCCGTCGACTGTCATGACCAGGCG (positions 515–492). Base changes are indicated by underlining. These changes produce one amino acid change, i.e., leucine-116 to valine. Oligonucleotides to create the *Bam*HI site were CAGGGATCCGCGTAGGCTCCTCAA (positions 850–873) and CGCGGATCCCTGCAGC CTTCACCT (positions 861–838). This also changes one amino acid, i.e., methionine-232 to isoleucine. SSTR2 and SSTR5 genes containing these restriction sites were used to make all six possible combinations of the three regions (amino terminus to TM3, TM3 to ICL3, and ICL3 to carboxyl terminus) by standard molecular biology techniques (13). All changes were sequenced using an Applied Biosystems automated DNA sequencer.

Site-directed mutagenesis. Oligonucleotides were designed to change codon 258 from GGC to TTC and codon 265 from TTC to TAC in the SSTR5 coding region. These changes result in substitution of glycine by phenylalanine (R5G258F) or phenylalanine by tyrosine (R5F265Y) in TM6. The oligonucleotides were 5′-TGTTCTGCTGGC TGCCTTTCTTC-3′ and 5′-GGCAGCCAGCAGAACACAAACAC-3′ for the R5G258F change and 5′-TGCTGGCTGCCTTTCTACATTG-3′ and 5′-TGACAATGTAGAAAGGCAGCCA-3′ for the R5F265Y change. Each oligonucleotide was used with an opposing terminal primer (described above) in PCRs using the original SSTR5 clone as template. The two R5G258F or R5F265Y PCR products were then diluted 1/1000 and used at a further 1/25 dilution in secondary PCRs with only the terminal primers included. These reactions produced full-length SSTR5 coding regions with the desired mutation. Base changes were confirmed by nucleotide sequencing.

Expression of receptors. All receptor constructs were inserted into the *Hind*III and *Xba*I sites of the expression vector pRC/CMV (Invitrogen). Stable transfections of CHO (K1 strain) cells were accomplished by the calcium phosphate method (Stratagene). Six individual clones from each transfection were selected for resistance to G-418 (500 μg/ml; GIBCO-BRL). Individual clones with the highest level of receptor expression were selected for further analysis. Receptor expression levels were determined in crude plasma membrane preparations by saturation radioligand binding assays using ^{125}I-Tyr^{11}-S-14.

Crude membrane preparations. Crude membranes were prepared from cells as described by Eppler *et al.* (14).

Radioligand binding assays. Radioligand binding assays were performed as described by Hadcock *et al.* (15), with the bound ligand being separated by rapid filtration on an Inotech cell harvester. All competition curves were performed using ^{125}I-S-14 only (250 pM in 200-μl final volume) in binding buffer composed of 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, and 0.25% bovine serum albumin, with the protease inhibitors 5 μg/ml leupeptin, 5 μg/ml aprotinin, 100 μg/ml bacitracin, and 100 μg/ml benzamidine. These four protease inhibitors were used in all membrane preparations.

[1] J. R. Hadcock, unpublished observations.

cAMP accumulations. Inhibition of forskolin (10 μM)-stimulated cAMP accumulation by S-14 or S-28 was measured in intact cells as described (15, 16). Cells were detached from plates and suspended in Kreb's Ringers phosphate buffer containing 2 mM $CaCl_2$ and 100 μM isobutylmethylxanthine. Reactions (50,000 cells/assay tube) were allowed to proceed for 15 min at 37° and were terminated by the addition of 1 N HCl, followed by heating of the samples for 3 min at 100°. The samples were then neutralized with 1 N NaOH. Measurements of cAMP accumulations were performed as described by Hadcock *et al.* (16).

Analysis of data. Data analyses were performed using the INPLOT or PRIZM programs (GraphPAD), and values are expressed as the mean ± standard deviation.

Results

Rat SSTR5 cDNA was expressed in CHO cells. In agreement with a previous report (7), S-14 bound SSTR5 with a 50-fold lower affinity than did S-28 (Fig. 1). The IC_{50} values for S-14 and S-28 binding to SSTR5 were calculated to be 5 and 0.1 nM, respectively. In contrast, the rat SSTR2 subtype exhibits equivalent binding affinities for S-14 and S-28 (Fig. 1). To determine which domains in SSTR5 are responsible for the poor binding affinity of S-14 for this subtype, a series of six SSTR2/SSTR5 chimeric receptors were constructed and expressed in CHO cells (Fig. 2). Each receptor subtype cDNA was partitioned into three domains by addition of unique restriction endonuclease sites. The A domain extends from the amino terminus to the start of TM3. The B domain contains the remainder of TM3 to the end of TM5. The C domain contains ICL3 through the carboxyl terminus. The six chimeric receptors, designated CHM 1–6, represent all possible combinations of the three domains. Chimeric receptors were expressed in CHO cells and binding affinities for S-14 and S-28 were determined using membrane preparations of each clone. All six chimeric receptor constructs were expressed at levels equivalent to those of wild-type SSTR2 and SSTR5 (Table 1). Saturation binding with ^{125}I-S-14 indicated that CHM 1, 2, and 3 all bound with affinities comparable to that of the SSTR5 subtype (3–5 nM). CHM 1, 2, and 3 share the carboxyl-terminal domain of SSTR5. CHM 4, 5, and 6 bound ^{125}I-S-14 with affinities comparable to that of the SSTR2 subtype (0.2–0.5 nM). CHM 4, 5, and 6 all share the carboxyl-terminal domain of SSTR2. Further analysis was performed by S-14 and S-28 competition with ^{125}I-S-14 binding (Table 1). The IC_{50} values for S-14 with the chimeric receptors were equivalent to the K_d values determined in saturation binding assays. The IC_{50} values for S-28 with the chimeric receptor subtypes were all calculated to be equivalent to those with the wild-type SSTR2 and SSTR5 (0.06–0.13 nM). Although long range effects cannot be ruled out, the observation that values for S-28 were unchanged suggests that the nanomolar affinities of CHM 1, 2, and 3 for S-14 are not due to conformational changes in the chimeric receptors but are due to the poorer intrinsic affinity for S-14 in this region of SSTR5. Thus, the region of SSTR5 encompassing ICL3 to the carboxyl terminus includes a critical determinant for preferential binding of S-28 to this subtype.

We examined sequence alignments of domain C for all five SSTR subtypes. Two amino acids, i.e., a hydrophobic amino acid and a tyrosine in TM6, are conserved in SSTR1–4 but not SSTR5 (Fig. 3). In SSTR5, glycine (G258) and phenylalanine (F265) are found in the respective positions. Site-directed mutagenesis was performed on the SSTR5 cDNA to substitute the two conserved amino acids. The receptor containing the sub-

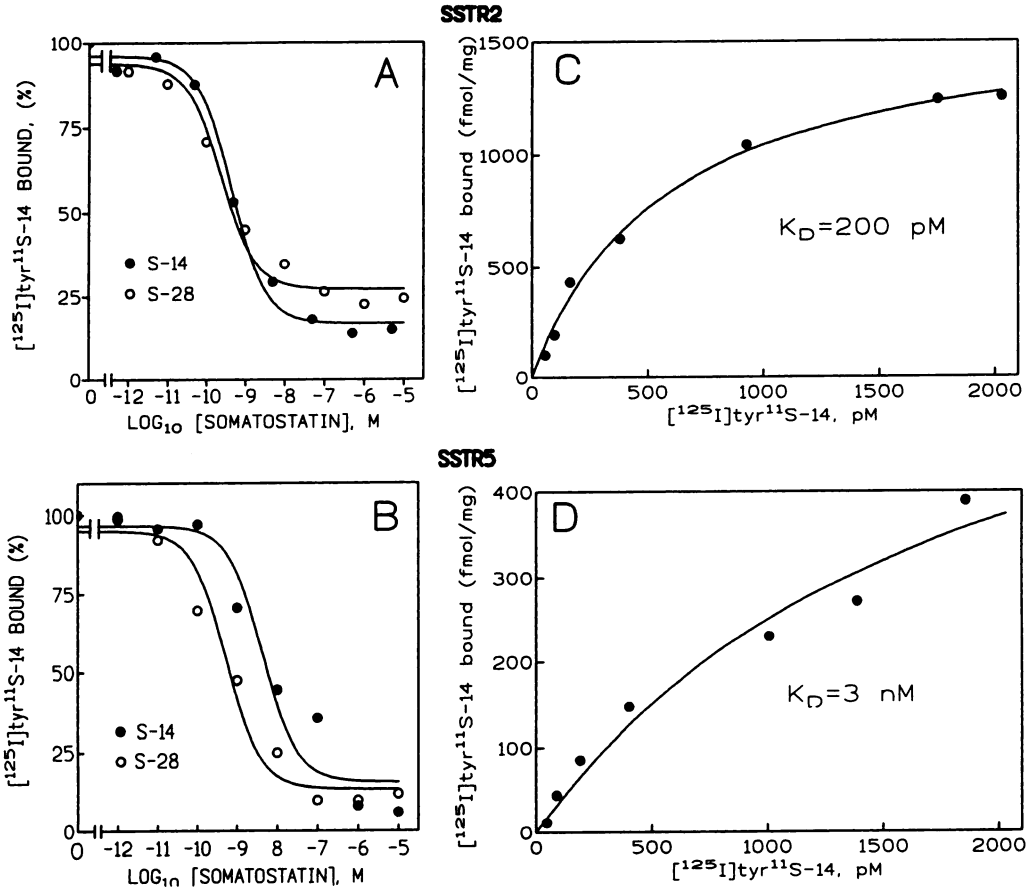

Fig. 1. Competition by S-14 or S-28 with ^{125}I-S-14 binding and saturation binding of ^{125}I-S-14 to SSTR2 or SSTR5. A and B, The ability of increasing concentrations of S-14 or S-28 (10^{-12} M to 10^{-5} M) to displace ^{125}I-S-14 (50 fmol, 250 pM) was examined in membranes prepared from CHO cells expressing the SSTR2 or SSTR5 subtype. C and D, Saturation binding was performed with 0–2000 pM ^{125}I-S-14. Nonspecific binding for each point (determined as cpm bound in the presence of 5 μM unlabeled S-14) ranged from 10 to 25%. For each point, 3 μg of protein/tube were used for SSTR2 and 10 μg of protein/tube were used for SSTR5. Displayed are representative experiments, which were performed three times with comparable results.

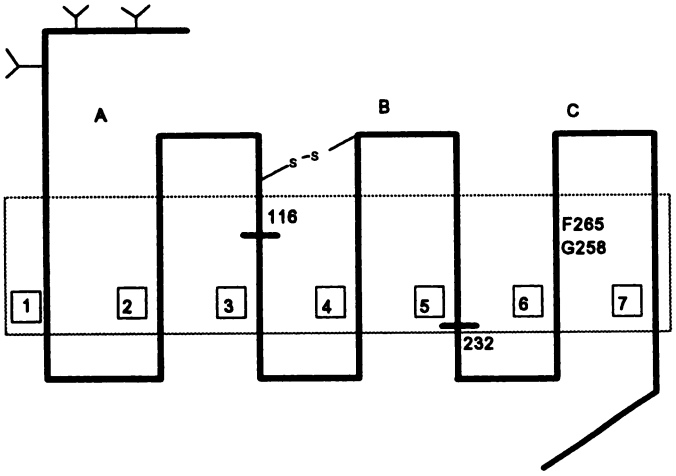

Fig. 2. Structure of SSTR2/SSTR5 chimeric receptors. A representation of a SSTR is shown with extracellular domains oriented toward the top of the page. *Boxed numbers*, TM1–7. Amino acid 116 of SSTR5 is indicated to illustrate the position of the chimeric receptor junction corresponding to the *Bam*HI site in the cDNA. Similarly, amino acid 232 in ICL3 corresponds to the *Sal*I site. The three domains of the receptor produced by these unique restriction sites are designated A, B, and C. Amino acids G258 and F265 in SSTR5 are indicated.

stitution of G258 with phenylalanine (R5G258F) displayed SSTR5 pharmacology, with S-28 binding exhibiting 50-fold higher affinity than S-14 (data not shown). The receptor containing the substitution of F265 with tyrosine (R5F265Y), however, did not exhibit SSTR5 pharmacology. Analysis of the R5F265Y mutant receptor revealed saturable binding of ^{125}I-S-14 with a K_d of 0.5 ± 0.12 nM (three experiments), an affinity similar to that of SSTR2 rather than SSTR5 (Fig. 4). Further analysis of R5F265Y by S-14 or S-28 competition with ^{125}I-S-14 binding revealed equivalent IC_{50} values for the two hormones. Although the IC_{50} value for S-28 was essentially unaltered, the affinity for S-14 was increased 25-fold, compared with wild-type SSTR5 (Fig. 4). The IC_{50} values for S-14 with SSTR5 and R5F265Y were calculated to be 5 and 0.2 nM, respectively. The affinity of the SSTR2-selective agonist ^{125}I-MK678 at R5F265Y was found to be unaltered, with a K_d value equivalent to that of the SSTR5 subtype rather then the SSTR2 subtype (Table 2).

The functional consequence of the increased affinity of S-14 for R5F265Y was examined in cAMP accumulation assays. Both SSTR2 and SSTR5 mediate inhibition of cAMP accumulation (7, 12). In agreement with the binding data, S-14 inhibits cAMP accumulation with an EC_{50} 50-fold lower than that of S-28 in CHO cells expressing SSTR5 (7). Inhibition of forskolin-stimulated cAMP accumulation by S-14 or S-28 was examined in CHO cells expressing SSTR5 or R5F265Y (Fig. 5). The EC_{50} for S-28-mediated inhibition was equivalent for SSTR5 and R5F265Y (0.8 and 0.9 nM, respectively). In accordance with the increased binding affinity of S-14 for the R5F265Y mutant, the EC_{50} for S-14 was shifted from 15 nM with SSTR5 to 2 nM by this single amino acid substitution.

Discussion

The recent cloning and pharmacological analyses of five SSTR subtypes allow for the elucidation of the molecular determinants of ligand/receptor interactions. Much progress

TABLE 1

Pharmacology of chimeric SSTR2/SSTR5 SSTRs

The pharmacology of the six SSTR2/SSTR5 chimeras was compared with that of wild-type SSTR2 and SSTR5. Competition binding (^{125}I-S-14 versus S-14 of S-28) and saturation binding (^{125}I-S-14) were both performed in membranes from CHO cells expressing each construct. The data shown are the means ± standard deviations of three separate experiments.

SSTR	B_{max}	K_D (^{125}I-S-14)	IC_{50}		Relative affinity[a]
			S-14	S-28	
	pmol/mg	nM	nM		
SSTR5	0.7 ± 0.2	4.3 ± 1.1	5.2 ± 1.9	0.1 ± 0.05	52
SSTR2	1.5 ± 0.15	0.2 ± 0.1	0.2 ± 0.03	0.15 ± 0.03	1.3
CHM1 (R2/R5/R5)	0.8 ± 0.2	2.6 ± 1.7	9.2 ± 4.2	0.4 ± 0.5	23
CHM 2 (R2/R2/R5)	2.6 ± 1	5.1 ± 3.1	4.0 ± 2.6	0.08 ± 0.02	50
CHM 3 (R5/R2/R5)	0.9 ± 0.3	2.2 ± 0.6	3.1 ± 2.6	0.11 ± 0.07	28
CHM 4 (R2/R5/R2)	2.6 ± 1.3	0.48 ± 0.4	0.14 ± 0.07	0.07 ± 0.03	2
CHM 5 (R5/R5/R2)	1.0 ± 0.4	0.57 ± 0.15	0.28 ± 0.28	0.22 ± 0.10	1.3
CHM 6 (R5/R2/R2)	1.4 ± 0.37	0.33 ± 0.21	0.3 ± 0.2	0.31 ± 0.34	1

[a] The relative affinity is the ratio of IC_{50} values for S-14/S-28.

SSTR1 (269-294)	I	T	L	M	V	M	M	V	V	M	V	F	V	I	C	W	M	P	F	Y	V	V	Q	L	V	N	V
SSTR2 (254-279)	V	T	R	M	V	S	I	V	V	A	V	F	I	F	C	W	L	P	F	Y	I	F	N	V	S	S	V
SSTR3 (264-289)	V	T	R	M	V	V	A	V	V	A	L	F	V	L	C	W	M	P	F	Y	L	L	N	I	V	N	V
SSTR4 (253-278)	I	T	R	L	V	L	M	V	V	T	V	F	V	L	C	W	M	P	F	Y	V	V	Q	L	L	N	L
SSTR5 (246-271)	V	T	R	M	V	V	V	V	V	L	V	F	V	Q	C	W	L	P	F	F	I	V	N	I	V	N	L

Fig. 3. Sequence alignment of TM6 of SSTR subtypes. Amino acids replaced in SSTR5 are *boxed*.

has been made in establishing the structure-activity relationships of somatostatin agonists and assigning subtype-selective ligands to cloned receptors (8, 9). However, little is known regarding the contact sites between native somatostatin ligands and SSTRs. Of the five rat SSTR subtypes cloned, only SSTR5 displays preferential affinity for S-28 over S-14. A comparison of S-28 and S-14 binding affinities for a series of SSTR2/SSTR5 chimeric receptors indicated that the domain encompassing ICL3 to the carboxyl terminus, including TM6 and TM7, determined pharmacological selectivity of SSTR5 for S-28. The amino acid substitution F265Y converted the S-28-preferring SSTR5 to a receptor that does not discriminate between the natural ligands S-14 and S-28. This was accomplished by an increase in the affinity for S-14, rather than a decrease in the affinity for S-28. In fact, no significant alteration in the affinity for S-28 was observed. By analogy, these data suggest that the tyrosine conserved in TM6 (Fig. 3) in SSTR1–4 is a possible contact site for S-14.

A plausible explanation for the observed differences in somatostatin binding affinities at SSTR5 is that there are additional receptor contact sites in S-28 that are absent in S-14. The absence of one S-14 contact site in SSTR5, compared with the other SSTRs, may be masked by additional contact sites for S-28. This results in equivalent affinities for S-28 at all SSTRs, even though one S-14 contact site is not present in the SSTR5 subtype. Additional contact sites in SSTR5 for S-28 could be unique to this subtype. Interestingly, the IC_{50} value for S-28 competition with ^{125}I-S-14 binding at either SSTR2 or SSTR5 is decreased 5-fold in the presence of the amino-terminal, 14-amino acid fragment of S-28 (1 μM).[2] No competition is observed with the amino-terminal fragment alone. These data support the inference that the amino-terminal domain of S-28 may have contact sites other than the S-14 contact sites contributing to the high affinity binding of S-28 to its receptors.

Like biogenic amine receptors, SSTRs appear to have important ligand binding determinants within transmembrane domains (17). For many other peptide hormone receptors, many of the binding determinants have been localized to extracellular domains (18, 19). A notable exception is the ET receptor family, in which a critical subtype-selective binding determinant has

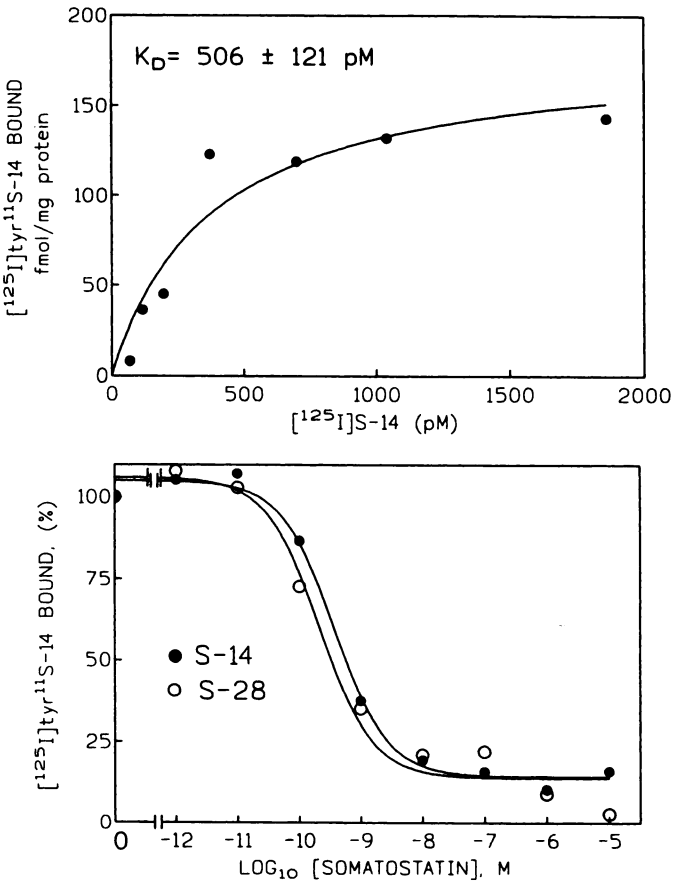

Fig. 4. Saturation binding of ^{125}I-S-14 and competition by S-14 or S-28 with binding of ^{125}I-S-14 to R5F265Y. *Top*, the ability of increasing concentrations of S-14 or S-28 (10^{-12} M to 10^{-5} M) to displace ^{125}I-S-14 (50 fmol, 250 pM) was examined in membranes (10 μg/point) prepared from CHO cells expressing the R5F265Y mutant. *Bottom*, saturation binding was performed with 0–2000 pM ^{125}I-S-14. Nonspecific binding for each point (determined as cpm bound in the presence of 1 μM unlabeled S-14) ranged from 10 to 35%. For each point, 10 μg protein/tube were used. Displayed are representatives of three independent experiments performed in triplicate.

[2] J. R. Hadcock, unpublished observations.

TABLE 2

Comparison of ^{125}I-MK678 saturation binding to SSTR2, SSTR5, and SSTR5 F265Y SSTRs

Saturation binding of ^{125}I-MK678 was performed in membranes from CHO cells expressing each construct. The data shown are the means ± standard deviations of two or three separate experiments.

SSTR	K_d	B_{max}
	nM	pmol/mg
SSTR2	0.1 ± 0.02	1.6 ± 0.26
SSTR5	1.85 ± 0.35	0.93 ± 0.1
R5F265Y	1.6 ± 0.4	0.24 ± 0.01

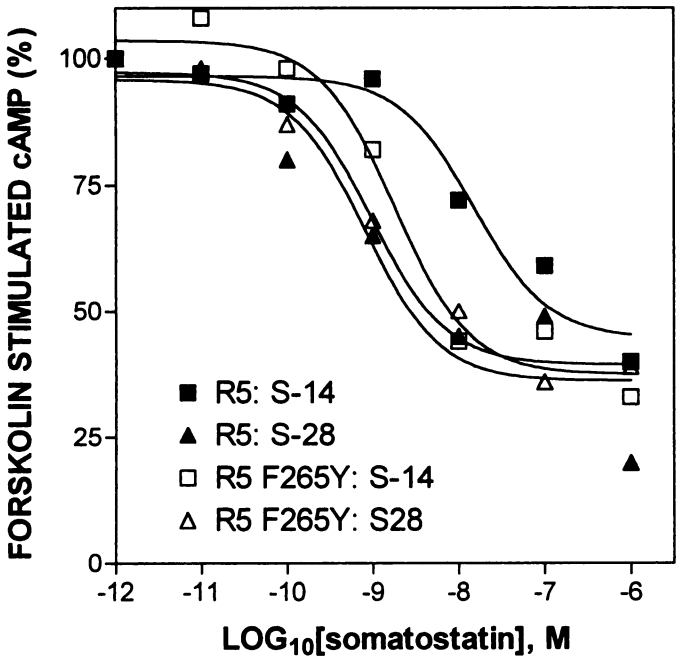

Fig. 5. Comparison of S-14- or S-28-mediated inhibition of cAMP accumulation in CHO cells expressing SSTR5 or R5F265Y. The ability of S-14 or S-28 to inhibit forskolin-stimulated (10 μM) cAMP accumulation in intact CHO cells expressing SSTR5 or R5F265Y was examined. Displayed is a representative experiment performed twice with equivalent results. Forskolin-stimulated cAMP accumulation (10 μM) was 97 pmol/10^6 cells in cells transfected with SSTR5 and 88 pmol/10^6 cells in cells transfected with R5F265Y. Basal cAMP levels were 6 and 4 pmol/10^6 cells for SSTR5 and R5F265Y, respectively.

been localized to TM2 (20). Mutation of tyrosine-129 to alanine in TM2 of the ET_A subtype switches the binding from ET_A to ET_B receptor pharmacology. Like many biogenic amine receptors, all SSTR subtypes contain conserved aspartates in TM2 and TM3 (1–7). It has been reported that the TM2 aspartate is involved in Na^+ modulation of somatostatin binding (21). The TM3 aspartate conserved in all SSTR subtypes appears to be a critical determinant for somatostatin binding to SSTR2.[3] The aspartate in TM3 and the tyrosine/phenylalanine in TM6 would be predicted to localize to approximately the same level of the cell membrane. The amino acids at these two positions may lie in close proximity to each other. Modeling of other G protein-linked receptors indicates that TM3 and TM6 are positioned opposite each other in the plasma membrane (22). Many amine receptors contain a tyrosine or phenylalanine in the equivalent position of TM6 (23). For the rat m3 muscarinic receptor, the tyrosine in TM6 plays a critical role in the hydrogen bonding with the ester side chain of acetylcholine (24). Tetramethylammonium, a weak muscarinic agonist that does not contain the ester side chain, does not interact with the TM6 tyrosine of the m3 muscarinic receptor, so this ligand binds with much lower affinity (10–50-fold), compared with ligands containing the ester side chain (24). The affinity of tetramethylammonium for the m3 muscarinic receptor is not altered when the tyrosine is substituted with a phenylalanine. In an analogous fashion, we suggest that S-14 (possibly threonine-10) forms a hydrogen bond with the TM6 tyrosine of SSTR1–4. The lower affinity for S-14 at SSTR5, which contains a phenylalanine at this position, may be due to the loss of hydrogen bonding at this position. The substitution of tyrosine in SSTR5 increased the binding affinity for S-14 by 10-fold, to a value comparable to that for the high affinity S-14 binding to SSTR1–4. In contrast to the increased affinity of S-14 at R5F265Y, the affinity of the SSTR2-selective analog MK678 was comparable to that observed for SSTR5, rather than for SSTR2. MK678 contains a valine in place of a threonine at the equivalent position of S-14. Thus, some contact sites for MK678 and S-14 may involve different amino acids within the SSTR subtypes. In fact, a recent report has localized MK678 binding determinants to the regions encompassing extracellular loops 2 and 3 of SSTR2 (25). Although the current work does not rule out the possibility of S-14 binding sites in the extracellular domains, at least some S-14 contact sites are localized to transmembrane domains.

[3] J. Strnad and J. R. Hadcock, role of conserved acidic amino acids in the high-affinity binding of somatostatin to the somatostatin receptor subtype 2, manuscript in preparation.

Acknowledgments

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