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Synthesis and biological activities of potent peptidomimetics selective for somatostatin receptor subtype 2

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ABSTRACT **A series of nonpeptide somatostatin agonists which bind selectively and with high affinity to somatostatin receptor subtype 2 (sst2) have been synthesized. One of these compounds, L-054,522, binds to human sst2 with an apparent dissociation constant of 0.01 nM and at least 3,000-fold selectivity when evaluated against the other somatostatin receptors. L-054,522 is a full agonist based on its inhibition of forskolin-stimulated adenylate cyclase activity in Chinese hamster ovary-K1 cells stably expressing sst2. L-054,522 has a potent inhibitory effect on growth hormone release from rat primary pituitary cells and glucagon release from isolated mouse pancreatic islets. Intravenous infusion of L-054,522 to rats at 50 μg/kg per hr causes a rapid and sustained reduction in growth hormone to basal levels. The high potency and selectivity of L-054,522 for sst2 will make it a useful tool to further characterize the physiological functions of this receptor subtype.**

Somatostatin is widely distributed throughout the central nervous system and various endocrine tissues (1–3). Two biologically active forms of somatostatin are known, a 14-amino acid peptide and an N-terminal extended peptide with 28 amino acids (4–6). Somatostatin has multiple functions, including modulation of growth hormone, insulin, glucagon, and gastric acid secretion (3, 7–10). Five somatostatin receptors (sst1–5) have been cloned and characterized (11–14). All five receptors are members of the G protein-linked receptor family (15). Structure–function studies with a large number of peptidal analogs have shown that the Trp^8-Lys^9 dipeptide of somatostatin is necessary for high-affinity binding (16) and have facilitated the development of potent analogs, including SMS 201-955 (Sandostatin or octreotide) which is clinically used for the treatment of acromegaly and certain endocrine tumors (17–19). We describe here strategies that were successful in designing small molecule subtype 2-selective agonists whose potencies on this receptor exceed somatostatin. Studies utilizing one of these subtype-selective somatostatin peptidomimetics, L-054,522, in both *in vivo* and *in vitro* experiments demonstate that somatostatin receptor subtype 2 (sst2) mediates the inhibition of growth hormone release from the rat anterior pituitary as well as glucagon from the rat pancreas. Insulin release is only inhibited at significantly higher concentrations of the compound. These results suggest possible therapeutic applications of selective sst2 agonists.

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MATERIALS AND METHODS

Chemicals. Somatostatin-14, leupeptin, aprotinin, and pepstatin were purchased from Sigma. Bacitracin was obtained from Bachem. The radiolabeled somatostatin (3-[^{125}I]iodotyrosyl^{25} somatostatin-28(Leu^8, D-Trp^{22}, Tyr^{25}) was purchased from Amersham.

Compound **4** was initially synthesized in a combinatorial library consisting of 20 diamines, 20 amino acids, and 79 amines. The diamines were linked to 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid functionalized Tentagel resin via urethane chemistry in a regiorandom way. 9-Fluorenylmethoxycarbonyl amino acids were coupled to the amine resins using standard 1,3-diisopropylcarbodiimide coupling. The 9-fluorenylmethoxycarbonyl groups were removed with piperidine and activated with *p*-nitrophenyl chloroformate before urea formation with the final amine. With the inclusion of regioisomers and stereoisomers, the library contained $38 \times 35 \times 99 = 131{,}670$ compounds. Details will be reported elsewhere.

The other compounds shown in Fig. 1 were prepared by coupling *N*-benzyloxycarbonyl-D-Trp (Cbz-D-TrpOH) with mono *N-tert*-butoxycarbonyl (Boc) diamines ($BocNH(CH_2)_5NH_2$ and *N*-ε-BocLys methyl ester using 1-hydroxybenzotriazole and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride under standard peptide synthesis conditions. The Cbz-protecting groups were removed by palladium-catalyzed hydrogenolysis. Urea linkages were formed by treatment of the amine with one equivalent of *N*,*N*′-disuccinimidyl carbonate in tetrahydrofuran followed by spiro(indene-1,4′-piperidine) and *N*,*N*-diisopropylethylamine. Finally, the Boc-protecting groups were removed with gaseous HCl in ethyl acetate. The Lys *t*-butyl ester analogs shown in Fig. 2 were prepared similarly but with a different protection scheme. Thus, Boc-protected amino acids were coupled to *N*-ε-Cbz-Lys *t*-butyl ester followed by selective deprotection of the Boc group with methanesulfonic acid. After formation of ureas with 4-(2-keto-1-benzimidazolinyl)piperidine, the Cbz-protecting groups were removed by hydrogenolysis to provide the final products. Boc-2*R*,3*R*-β-MeTrp and Boc-2*R*,3*S*-β-MeTrp were prepared according to Huang *et al.* (20). Spectra were acquired in CD_3OD on a Varian Unity 400-MHz spectrometer. Chemical shifts are expressed in ppm relative to the CD_2H signal set at 3.30 ppm.

Computational Studies. A three-dimensional structure of the cyclic hexapeptide **9** was generated from NMR studies of the corresponding Phe^7 cyclic peptide (16, 21) using distance geometry methods to include the following features: a βII′

Abbreviations: CHO, Chinese hamster ovary; sst2, somatostatin receptor subtype 2; GH, growth hormone; GBSS, Gey's balanced salt solution.

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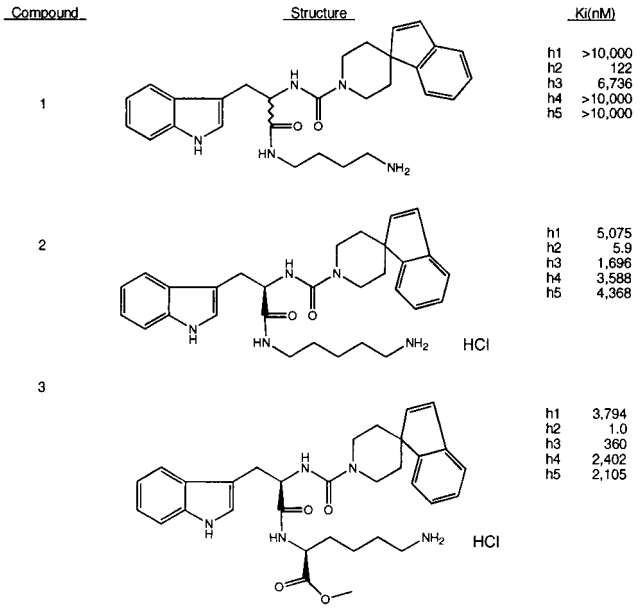
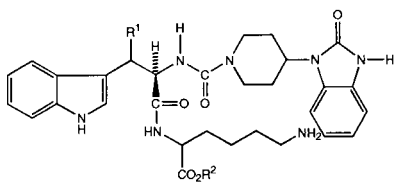


FIG. 1. sst 2-Selective leads.

turn around Tyr^7–D-Trp^8–Lys^9–Thr^{10} (nuclear Overhauser effect data); proximate distances between the side chains of D-Trp^8-Lys^9 (upfield shift of Lys^9 γ protons); proximate distances between the aromatic side chains of Phe^7 and D-Trp^8 (temperature-dependent upfield shift of aromatic protons), and a cis amide bond at Pro^6 (nuclear Overhauser effect and C^{13} shifts in the proline ring). A series of CHARMm dynamics (22) were run and the model was minimized to produce the hexapeptide conformation which is depicted in Fig. 3. Although not shown, further refinements have been made to the model to reflect additional structure–activity relationships and structural information made available since its first development.

The conformation of L-054,522 shown in Fig. 3 was generated using a distance geometry algorithm with the following NMR-derived constraints: an upper distance of 5.5 Å between Cγ of the Lys and the Cδ2/Cε2 of the β-Me-D-Trp (upfield shift of Lys γ-methylene) and an upper distance of 3 Å between HC7 of the benzimidazolone and HC4 of the piperidine (nuclear Overhauser effect data). A modified Karplus equation (23) was used to predict a 170 ± 10° torsion angle for HCα



Compound	R1	R2	Ki(nM) h1	h2	h3	h4	h5
4	CH$_3$	CH$_3$	5,505	0.46	1,208	4,167	4,658
5	H	C(CH$_3$)$_3$	1,701	0.1	226.4	397	1,184
6	CH$_3$	C(CH$_3$)$_3$	4,766	2.1	515.2	379	1,193
7 (L-054,522)	CH$_3$	C(CH$_3$)$_3$	2,392	0.01	31	81	163

FIG. 2. Important analogs of L-054,522.

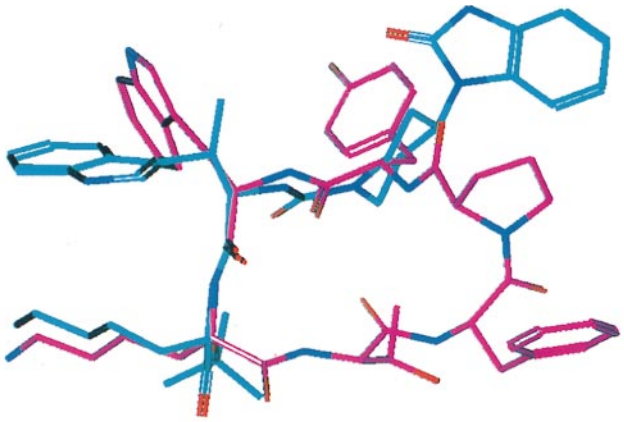


FIG. 3. Superposition of modeled conformations of compound **7** (L-054,522, green) upon cyclic peptide *9* (magenta). Both structures incorporate the available NMR data and have been energy minimized with MMFF94(s).

and HCβ of (*S*)-β-Me-D-Trp. Conformations were energy minimized using MMFF94(s) (24) and that depicted in Fig. 3 is one of the lowest energy conformations which meets the above criteria.

Preparation of Cells Stably Expressing Somatostatin Receptors. Chinese hamster ovary- (CHO) K1 cells were obtained from American Type Culture Collection and grown in α-MEM containing 10% fetal calf serum. Cells were stably transfected with DNA for all five ssts using Lipofectamine. Neomycin-resistant clones were selected and maintained in medium containing G418 (400 μg/ml). Fragments of genomic DNA carrying the various human somatostatin receptors were inserted into the multiple cloning site of pcDNA3 (Invitrogen). The fragments used were a 1.5-kb *Pst*I-*Xmn*I fragment for sst1, a 1.7-kb *Bam*HI-*Hin*dIII fragment for sst2, a 2.0-kb *Nco*I-*Hin*dIII fragment for sst3, a 1.4-kb *Nhe*I-*Nde*I fragment for sst4, and a 3.2-kb *Xho*I-*Eco*RI fragment for sst5.

Receptor Ligand-Binding Assays. All receptor-binding assays were performed with membranes isolated from CHO-K1 cells expressing the cloned human somatostatin receptors. All five receptor assays were adapted for high throughput screening in a 96-well format. The assay buffer, previously described (25), consisted of 50 mM Tris·HCl (pH 7.8) with 1 mM EGTA, 5 mM $MgCl_2$, leupeptin (10 μg/ml), pepstatin (10 μg/ml), bacitracin (200 μg/ml), and aprotinin (0.5 μg/ml). CHO-K1 cell membranes, radiolabeled somatostatin, and unlabeled test compounds were resuspended or diluted in this assay buffer. All assays were performed in 96-well polypropylene plates. The final concentration of the radiolabeled ligand was 0.1 nM for all receptor assays. Unlabeled test compounds were examined over a range of concentrations from 0.01 nM to 10,000 nM. The K_i values for compounds were determined as described by Cheng and Prusoff (26).

A 20-μl aliquot of 1 nM ^{125}I-labeled somatostatin-28 was added to each well of the plate, followed by a 20-μl aliquot of the unlabeled test compound and 160 μl of the CHO-K1 cell membrane suspension. The amount of membrane protein used for each of the receptor subtypes was adjusted so that the number of binding sites was approximately equivalent in each assay. The mixtures were incubated for 45 min at room temperature and then harvested onto Packard Unifilter GF/C plates pretreated with 0.1% polyethyleneimine. The plates were washed with ice-cold 50 mM Tris·HCl (pH 7.8) and dried overnight at room temperature. Microscint-20 scintillation fluid was added before the plates were sealed and radioactivity was quantitated using a Packard Topcount scintillation counter.

Inhibition of Forskolin-Stimulated cAMP Accumulation. The assay was based on a mouse L cell line that contains a

cAMP response element fused to the *Escherichia coli* β-galactosidase gene (*lacZ*) (27). These cells were independently and stably transfected with sst2. Cells were grown in T-75 flasks containing 20 ml of DMEM with 4.5 mg/ml glucose, 0.584 mg/ml L-glutamine, and 25 mM Hepes supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 5% fetal bovine serum (heat inactivated), 5% calf serum (heat inactivated), 250 units/ml hygromycin, and 500 μg/ml G418 and were refed every 2 days. The cells were passaged with trypsin once per week at 1:10 to 1:20 dilution. Confluent cells were detached by incubation with 0.25% trypsin for 4 min, washed once in growth medium, resuspended in half the original volume (10 ml/T-75 flask) of growth medium, and diluted with an equal volume of macrophage–serum-free medium (GIBCO/BRL). The cell suspension (70 μl/well) was inoculated into 96-well flat-bottomed tissue culture plates. The plates were incubated at 37°C in 6% CO_2 and >95% humidity for 2–3 days. Spent medium was removed by aspiration and cAMP accumulation medium (65 μl/well) containing test compounds was added. Accumulation medium consisted of macrophage–serum-free medium supplemented with 100 nM forskolin, protease inhibitors (5 mg/liter leupeptin, 10 mg/liter benzamidine, 40 mg/liter bacitracin, 5 mg/liter trypsin inhibitor), and 0.1 μM phosphodiesterase inhibitor (Ro 20–1724). The plates were incubated for 6 hr at 37°C in 6% CO_2 and >95% humidity. The medium was removed by aspiration and the plates were stored at −75°C.

For assay of β-galactosidase, 100 μl of β-galactosidase assay buffer [prepared by mixing 200 ml of 0.5 M sodium phosphate (pH 8), 20 ml of 0.1 M $MgSO_4$, 10 ml of 10 mM $MnCl_2$; 5 ml of Triton X-100, and 3.1 ml of β-Me) was added to each well. After 10 min at room temperature, the reaction was initiated by the addition of 50 μl of *o*-nitrophenyl-β-D-galactopyransoside (2 mg/ml) in the β-galactosidase assay buffer. The rate of increase in absorbance at 405 nm was determined. Results were expressed as percentage of the activity obtained in the absence of drug.

Inhibition of Growth Hormone (GH) Release. *In Vitro Studies.* Functional activity of the various compounds was evaluated by measuring growth hormone secretion from primary cultures of rat anterior pituitary cells. Cells were isolated from rat pituitaries by enzymatic digestion with 0.2% collagenase and 0.2% hyaluronidase in Hanks' balanced salt solution. The cells were suspended in culture medium and adjusted to a concentration of 1.5×10^5 cells/ml and 1.0 ml of this suspension was placed in each well of a 24-well tray. Cells were maintained in a humidified 5% CO_2/95% air atmosphere at 37°C for 3–4 days. The culture medium consisted of DMEM containing 0.37% $NaHCO_3$, 10% horse serum, 2.5% fetal bovine serum, 1% nonessential amino acids, 1% glutamine, 1% nystatin, and 0.1% gentamicin. Before testing compounds for their capacity to inhibit GH release, cells were washed twice 1.5 hr before and once more immediately before the start of the experiment with the above culture medium containing 25 mM Hepes (pH 7.4). Compounds were tested in quadruplicate by adding them in 1 ml of fresh medium to each well and incubating them at 37°C for 2 hr followed by centrifugation at 2000 × *g* for 15 min to remove any cellular material. The supernatant fluid was assayed for GH by a double antibody radioimmunoassay.

In Vivo Studies. Intact male Sprague Dawley rats were anesthetized and using aseptic technique, chronic indwelling catheters were inserted into the femoral artery (for blood sampling) and into the femoral vein (for drug infusion). Infusion of a 2% solution of heparinized saline was initiated to keep the catheters patent. Rats were housed individually in isolation chambers for at least 72 hr to recover from surgery. On the day of experiment, the animals were attached to an automatic blood microsampler through the catheter inserted into the femoral artery. Blood samples of 30 μl were withdrawn every 10 min for a total period of 24 hr. L-054,522 dissolved in dimethyl sulfoxide and diluted with saline (final dimethyl sulfoxide concentration, 2%) was infused at a rate of 50 μg/kg per hr via the venous indwelling catheter for the second 12-hr period. After the completion of the experiment, blood samples were centrifuged at 12,000 × *g* for 15 min. Plasma was removed and assayed for GH content by a double antibody RIA procedure.

Inhibition of Glucagon and Insulin Release from Rat Pancreatic Islets. The pancreata of male 8- to 10-wk-old C57BL/6J mice (The Jackson Laboratory) were removed immediately after death and minced in ice-cold Gey's balanced salt solution (GBSS) supplemented with 0.2% bovine serum albumin and washed several times with cold GBSS to remove the fat. The tissue was digested with collagenase type IV (10 mg/1 gm of wet tissue) for 20 min at 37°C on a wrist action shaker (120 cycles/min). After digestion, collagenase was removed by washing four times. The pancreatic islets freed from connective tissue were individually selected under a stereomicroscope. Approximately 400 islets were selected for each experiment and these islets were maintained at 37°C (in a humidified atmosphere of 5% CO_2 in air) in 10 ml of RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. After 24 hr, the media were removed by washing several times with GBSS and the islets were maintained in GBSS at 37°C for 45 min.

Islets (10/ml GBSS) were incubated with L-arginine (20 mM) and KCl (20 mM) in the presence or absence of the test compounds for 2 hr at 37°C. An aliquot of the media (900 μl) was then removed and centrifuged (800 × *g* for 5 min) at 4°C. The amount of insulin and glucagon was then determined using a double antibody RIA technique.

RESULTS AND DISCUSSION

The lead structure **1** from which potent sst2-selective somatostatin agonists were designed was selected from the Merck sample collection. The compound originated in a project to synthesize compounds for broad screening by derivatizing "privileged structures" with capped amino acids. The rationale for this lead discovery approach was reported earlier in a paper describing the design of the GH secretagogue MK-0677 (28). The concept that there are recurring structural units in many receptor ligands originated with Ariens *et al.* (29), who noted the presence of double-ring structures in many biogenic amine antagonists which they suggested might bind in conserved accessory binding sites of receptors. More recently, Evans *et al.* (30) termed these recurring units privileged structures and suggested their derivatization as a strategy to discover receptor ligands. In the progenitor of MK-0677 and in lead compound **1**, closely related spiroindanylpiperidine and spiroindenylpiperidine frameworks were derivatized with capped amino acids to achieve peptidomimetic character.

The selection of compound **1** for testing in somatostatin receptor assays was based on the three-dimensional structure of compound **9** c[-Pro^6–Tyr^7–D-Trp^8–Lys^9–Thr^{10}–Phe^{11}-] (16), in which the superscript numbers indicate the location of amino acids in somatostatin. This potent agonist is the Tyr^7 analog of the corresponding Phe^7 cyclic peptide which has been the subject of extensive structure–activity relationships and conformational studies (16, 21, 31, 32) which identified a βII′ turn around D-Trp^8-Lys^9 and essential amino acid side chains of Phe^7, D-Trp^8, Lys^9, and Phe^{11}. It was also known from this work that the peptide backbone is not required for activity (33) and this fact is illustrated in carbohydrate- (34–36) and benzodiazepinone- (37) derived somatostatin ligands. Using the side chains of the Tyr^7–D-Trp^8–Lys^9 in the modeled hexapeptide as the probe, our proprietary three-dimensional similarity search engine, SQ (38), was employed to search a database (39) of three-dimensional models of compounds in the Merck

chemical collection. By testing less than a hundred compounds, the lead activity of compound **1** was identified.

In accord with its method of selection, the potency of compound **1** was increased by resolution and by extending the amine chain length to that of Lys in the D-Trp analog **2** (40). Completion of the Lys structure as its methyl ester **3** provided both high affinity and selectivity for sst2. Extensive variations were then undertaken of the spiroindenylpiperidine-privileged structure component of compound **3** using both combinatorial and single compound synthesis. Components in the combinatorial library included (2*R*,3*S*)-β-MeTrp, (L)-Lys-OCH_3, and *N*-(4-piperidinyl)benzimidazol-2-one, which when combined in compound **4** afforded an increase in potency and selectivity. Based on the high activity of **5** for sst2, a final increase in potency and selectivity was achieved in the *t*-butyl ester **7** (L-054,522).

Inclusion of all four possible isomers of β-MeTrp in our library was made to investigate the effect of conformational restriction upon potency. Compound **7** was more potent and somewhat more selective than compound **5** in which Trp is unsubstituted by an (*S*)-β-Me group (Fig. 2). This result is in accord with previously described results (20) in which D-Trp^8 was replaced by (2*R*,3*S*)-β-MeTrp in the cyclic peptide c[-Pro^6–Phe^7–D-Trp^8–Lys^9–Thr^{10}–Phe^{11}-] with an attendant increase in binding to AtT20 cells. Also in agreement with these earlier studies, the (2*R*,3*R*)-isomer **6** bound much less tightly to sstr2. In their cyclic peptide the (2*R*,3*S*)-β-MeTrp adopts a transrotamer configuration and our modeling studies of compound **7** are in agreement with this conformation which directs the Trp indole group close to the Lys side chain. NMR studies further support this orientation. A highly shielded Lys γ-methylene in L-054,522 is compelling evidence for proximity of the Lys and the β-MeTrp side chains. The chemical shift of 0.7 ppm in the NMR spectrum of L-054,522 is comparable to that in the cyclohexapeptide MK-678 and represents a 0.6–0.7 ppm upfield displacement from a typical unperturbed value. Further evidence for the preferred orientation can be inferred from a low temperature study in which the γ-methylene protons are displaced progressively upfield and become increasingly nonequivalent as the temperature is reduced. This orientation has long been considered to be important for somatostatin agonist activity (41) and is strongly supported by comparison to the less active desmethyl analog **5** and the poorly active epimeric methyl analog **6** whose chemical shifts are at 1.10 ppm and 1.32 ppm, respectively.

A modeling comparison of L-054,522 with cyclic peptide **9** is shown in Fig. 3. Hand-fitted overlay of the (D)-Trp-Lys components of these molecules results in placement of the *N*-(4-piperidinyl)benzimidazol-2-one-privileged structure in the vicinity of the Tyr^7 in **9**. Thus, the privileged structure can apparently take up an amino acid side chain position in the turn region of a peptide and need not be in an accessory binding site as proposed by Ariens *et al.* (29) for some biogenic amine antagonists. A related correspondence has been proposed for the spiroindolinylpiperidine part structure of MK-0677 with Trp^4 of the GH secretagogue GHRP-6 (42).

The receptor affinities of somatostatin and various analogs were determined by competition binding experiments. Membranes were prepared from CHO-K1 cell lines stably expressing the various human ssts and then incubated with [^{125}I]–Tyr^{11}–somatostatin-28 in the presence of increasing concentrations of the competitor. No specific binding was detected in nontransfected cells. Somatostatin-14 binds with high affinity to sst1–5 whereas the other peptidal analogs in Fig. 4 do not bind well to sst1 and 4. Octreotide is relatively selective for sstr2, and the hexapeptide MK-678 is relatively selective for sst2 with lower affinity for sst3 and 5. As shown in Fig. 2, L-054,522 has a higher affinity for sst2 than any compound tested. L-054,522 demonstrates >3,000-fold selectivity for sst2

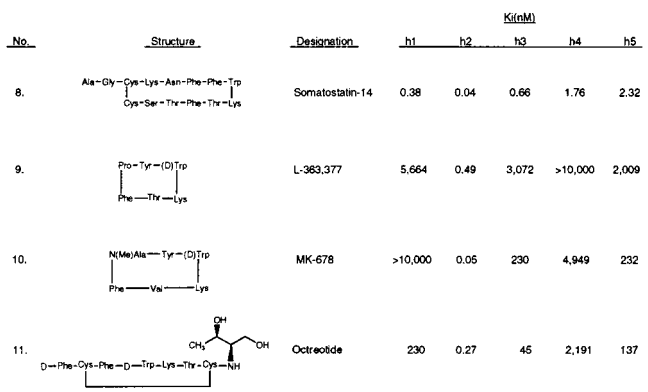


FIG. 4. Receptor subtype potencies of somatostatin and several important analogs.

compared with sst3 and is even more selective when compared with the other ssts.

The functional activity of somatostatin analogs was determined from their inhibition of forskolin-stimulated cAMP production in L cells stably expressing sst2 and a cAMP-responsive reporter construct. In this assay using 100 nM forskolin, somatostatin-14 inhibits cAMP levels 70–95% with an IC_{50} value of 0.15 nM (Fig. 5). Nontransfected cells do not respond to somatostatin. L-054,522 has full agonist activity when tested against L cells expressing sst2 with an IC_{50} value of 0.1 nM. L-054,522 had no effect on cAMP levels in the absence of forskolin. These data suggest that L-054,522 is a full agonist and its potency is consistent with results obtained in the receptor-binding assays.

The use of L cells cotransfected with both sst2 and the cAMP-responsive construct permits the rapid evaluation of a large number of compounds, but need not predict *in vivo* activity. More physiological approaches include inhibition of GH from anterior pituitary cells and inhibition of glucagon release from pancreatic islets. As shown in Table 1, somatostatin-14 is a potent inhibitor of GH release from rat primary anterior pituitary cells with an IC_{50} value of 0.1 nM. In parallel experiments, it was demonstrated that L-054,522 had a similar inhibitory effect on GH release with an IC_{50} value of 0.05 nM and maximal inhibition at 2 nM.

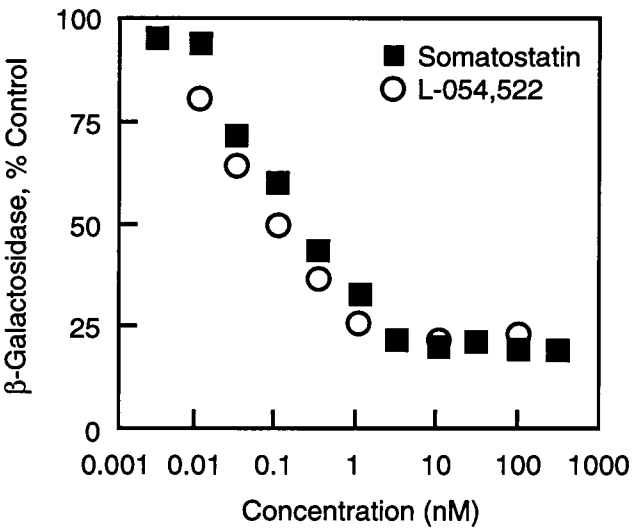


FIG. 5. Inhibition of forskolin-stimulated cAMP accumulation. cAMP accumulation in forskolin-stimulated mouse L cells stably transfected with sst2 was measured in the presence of increasing concentrations of somatostatin-14 or L-054,522. Each point is the average of three determinations, replicate experiments gave similar results.

Table 1. Inhibition of GH, insulin, and glucagon release

Compound	Inhibition of release, IC_{50}		
	GH	Insulin	Glucagon
Somatostatin-14	0.1 nM	0.3 nM	0.04 nM
L-054,522	0.05 nM	12 nM	0.05 nM

Primary cultures of rat anterior pituitary cells were used to measure inhibition of GH release and glucagon and insulin release were determined using isolated rat pancreatic islets as described in *Materials and Methods*.

Intravenous infusion of L-054,522 (50 μg/kg per hr) into conscious intact male rats immediately lowered circulating GH to basal level (Fig. 6). GH levels remained depressed throughout the duration of the experiment (12 hr). The results of both the *in vivo* and *in vitro* experiments are consistent with previous reports of relatively selective sst2 peptidal somatostatin analogs inhibiting GH release *in vivo* (1, 43–45), and with reports that potent relatively selective agonists for either sstr2 or sstr5 cause GH lowering in pituitary cell cultures in accord with the presence of both receptors on somatotrophs (46–49).

Somatostatin is also an important physiological regulator of insulin and glucagon release from the pancreatic islets. *In vivo* studies measuring the release of rat pancreatic insulin and glucagon after infusion of sst selective peptidal somatostatin agonists suggest that sst2 regulates glucagon release and sst5 inhibits insulin release (50). This is consistent with reports that the glucagon-containing cells (α cells) are positively labeled by sstr2-selective antibodies, whereas the insulin-containing β cells are selectively stained by sst5 antibodies (51) (S.W.M., unpublished data). As shown in Table 1, somatostatin-14 is a potent antagonist of glucose-stimulated glucagon and insulin release (IC_{50} = 0.04 and 0.3 nM). L-054,522 is a potent inhibitor of glucagon release in this system (IC_{50} 0.05 nM) and is a less potent inhibitor of insulin release (IC_{50} = 12 nM). These data further suggest a physiological role of sst2 in glucagon regulation.

In summary, we report here a small peptidomimetic agonist with high potency and selectivity for sst2. Using this receptor, functional agonist activity was achieved with a privileged structure dipeptide equivalent to that of the somatostatin tetradecapeptide. At the same time more than 3,000-fold selectivities toward sst2 were realized. The potency and selectivity of L-054,522 toward sst2 compared with sst3 and sst5 exceed that of octreotide and its potency on sst2 and selectivity toward sst5 are greater than those of MK-678 (Fig. 4). The synthesis of L-054,522 again demonstrates the effectiveness of the privileged structure dipeptide strategy in designing remarkably small but potent peptidomimetic agonists (28, 42). The functional activity of L-054,522 should make it useful in the evaluation of potential therapeutic targets, including ischemia-induced retinal neovascularization which may be inhibited by decreasing GH release (52).

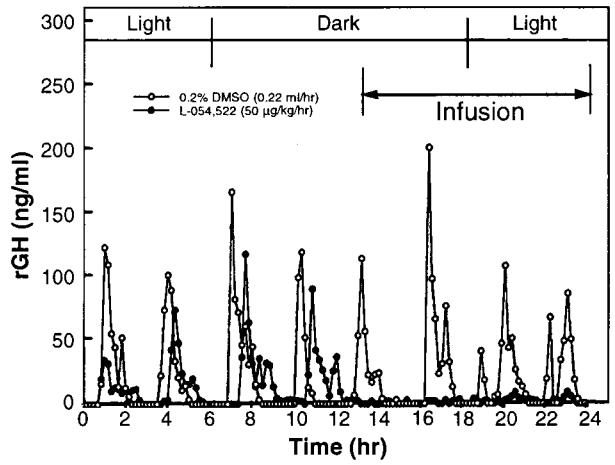


FIG. 6. Plasma GH levels were measured every 10 min for a 24-hr period. At 1300 hr the animals were infused with either L-054,522 (50 μg/kg per hr) or vehicle (0.25% dimethyl sulfoxide) for 11 hr. Replicate experiments gave similar results.

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