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Somatostatin Octapeptides (Lanreotide, Octreotide, Vapreotide, and Their Analogs) Share the Growth Hormone–Releasing Peptide Receptor in the Human Pituitary Gland

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The binding affinity of somatostatin-14 (SRIF), various SRIF derivatives, and some peptides belonging to the growth hormone-releasing peptide (GHRP) family to specific receptors for SRIF and GHRP in the human pituitary gland has been measured. GHRP receptors have been identified using [^{125}I]Tyr-Ala-hexarelin, a peptide that thas been demonstrated to be a potent growth hormone (GH) releaser in humans. Tyr-Ala-hexarelin binding was displaced in a dose-dependent manner by different GHRPs (hexarelin, GHRP-2, and EP-51216). Surprisingly, some SRIF octapeptide derivatives such as vapreotide, lanreotide, octreotide, and their analogs were also able to displace the GHRP ligand. By contrast, no inhibition of Tyr-Ala-hexarelin binding was observed in the presence of SRIF or SRIF derivatives (SRIF H-2186, H-2485, and H-3382) that are known to have a weak SRIF-like activity. When [^{125}I]Tyr^{1}-SRIF-14 was used as a ligand, we observed displacement with SRIF and the octapeptide SRIF analogs but not with GHRPs and other SRIF derivatives. The results point to a sharing of the GHRP receptor with the octapeptide SRIF analogs, but not SRIF. Our data are consistent with the hypothesis that the putative natural GH secretagogue ligand may be a growth hormone release inhibiting factor that is different from SRIF and that is antagonized by GHRP.

Key Words: Somatostatin octapeptides; growth hormone–releasing peptides; receptors; human pituitary gland.

Introduction

In 1992, C. Y. Bowers *(1)*, who discovered the family of growth hormone–releasing peptides (GHRPs), presented several mechanistic models (hypothalamic, hypothalamic–pituitary, and pituitary) to explain the observed synergism between GHRP and growth hormone–releasing hormone (GHRH) on the release of growth hormone (GH) in vivo. The existence of a natural GHRP-like ligand was postulated (U-factor), which was deemed to stimulate, together with GHRH, the pituitary somatotrophs in secreting GH.

The U-factor hypothesis was strengthened when specific receptors for growth hormone secretagogues ([GHSs], encompassing peptidic and peptidomimetic substances) were isolated and cloned *(2)*. The hypothesis that the natural ligand (and all its mimics) are GHS receptor (GHS-R) agonists was never challenged; after all, a robust secretion of GH is what is observed when a GHRP or a GHS is administered to an animal.

The interactions of GHSs and somatostatin (SRIF) are not well understood. The GHRP receptor is structurally and functionally different from the known somatostatin receptors and yet partial opposition to somatostatin actions has been postulated *(3)*. Some researchers, however *(4)*, have recently provided evidence that the GH release during a 7-d continuous infusion of the GHRP hexarelin in conscious male rats does not involve suppression of functional somatostatin tone.

To elucidate further the interactions between SRIF and GHRP, in the present study we have evaluated the ability of GHRP, SRIF, and various SRIF derivatives to compete with [^{125}I]Tyr^{1}-SRIF-14 and the GHRP ligand ([^{125}I]Tyr-Ala-hexarelin) for the pituitary binding sites.

Results

To probe the functionality of the GHRP receptor, we used the ligand [I^{125}]-Tyr-Ala-hexarelin Tyr-Ala-hexarelin. (Tyr-Ala-His-D-2MeTrp-Ala-Trp-D-Phe-Lys-NH_2) has been demonstrated *(5)* to be a potent GH releaser in humans. Known GHSs (such as MK 677, GHRP-2, hexarelin) displace [I^{125}]-Tyr-Ala-hexarelin from the human pituitary receptor (vide infra). Surprisingly, well-known somatostatin analogs (octreotide, lanreotide, vapreotide) and other octapeptide analogs of somatostatin (Table 1) *(6)* are able to displace the GHRP ligand.

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Table 1
Potency of Somatostatin-14 (SRIF), SRIF Derivatives and Growth Hormone-Releasing Peptides (GHRP) in Inhibiting [^{125}I]Tyr-SRIF-14 and [^{125}I]Tyr-Ala-Hexarelin Binding to Human Pituitary Membranes[a]

Compound	Chemical structure[b]	[^{125}I]Tyr^{1}-SRIF-14 K_{05} (nmol/L)	[^{125}I]Tyr-Ala-hexarelin K_{05} (nmol/L)
SRIF	Ala-Gly-**Cys**-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-**Cys**-OH	61 ± 08	>10,000
SRIF derivatives			
SRIF H-3382	D-2-Nal-cyclo(-γ-Glu-Tyr-D-Trp-Lys-Val-L-α,γ-diaminobutyryl)-Thr-NH_2	>10,000	>10,000
SRIF H-2186	D-Phe-**Cys**-Tyr-D-Trp-Orn-Thr-**Pen**-Thr-NH_2	>10,000	>10,000
SRIF H-2485	cyclo(-7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)) acetate	>10,000	>10,000
Octreotide	D-Phe-**Cys**-Tyr-D-Trp-Lys-Thr-**Cys**-NHCH(CH_2OH)CHOHCH_3	300 ± 40	16,330 ± 193[c]
EP-41251	D-Mrp-**Cys**-Tyr-D-Trp-Lys-Thr-**Cys**-Mrp-NH_2	423 ± 49	513 ± 33[c]
Lanreotide	D-βNal-**Cys**-Tyr-D-Trp-Lys-Thr-**Cys**-Thr-NH_2	383 ± 54	1350 ± 61
EP-41252	D-βNal-**Cys**-Tyr-D-Trp-Lys-Thr-**Cys**-Mrp-NH_2	477 ± 50	560 ± 27[c]
Vapreotide	D-Phe-**Cys**-Tyr-D-Trp-Lys-Val-**Cys**-Trp-NH_2	317 ± 26	430 ± 29[c]
EP-41253	D-Phe-**Cys**-Tyr-D-Trp-Lys-Val-**Cys**-Mrp-NH_2	320 ± 26	550 ± 45[c]
EP-80874	D-Mrp-**D-Cys**-Pal3-D-Trp-Lys-Val-**Cys**-Mrp-NH_2	223 ± 35	510 ± 80[c]
EP-90089	D-Mrp-**D-Cys**-Pal3-D-Trp-Lys-Tle-**Cys**-Mrp-NH_2	257 ± 19	653 ± 38[c]
GHRP			
Hexarelin	His-D-Mrp-Trp-Ala-Trp-D-Phe-Lys-NH_2	>10,000	983 ± 32
GHRP2	D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH_2	>10,000	1030 ± 84
EP-51216	Gab-D-Mrp-D-Mrp-Phe-Lys-NH_2	>10,000	1060 ± 32

[a]Values are means ± SEM of three separate experiments. [b]Two Cys, or Cys and Pen in bold, symbolize a disulfide bond. Mrp, 2-methyl-Trp; Pal, pyridylalanyl; Tle, tertleucyl; Nal, β-2-naphtylalanyl; Pen, L-penicillamine; Bzl, benzyl; Gab, γ-aminobutyryl. [c]$p < 005$ vs hexarelin.

SRIF does not, however, displace the GHRP ligand and likewise peptides H-3382, H2186, and H2485 (Table 1) do not displace it. When [I^{125}]-Tyr-SRIF-14 is used as a somatostatin ligand (human pituitary membranes), we observe displacement with SRIF and the octapeptide SRIF analogs but not with known GHRPs such as hexarelin, GHRP-2, and EP51216 (and others; data not shown).

The ability of SRIF, several SRIF derivatives, and various GHRPs to compete with [^{125}I]Tyr^1-SRIF-14 and [^{125}I]Tyr-Ala-hexarelin (GHRP) for the pituitary binding sites is reported in Figs. 1 and 2. Both SRIF and SRIF-derived octapeptides such as octreotide, lanreotide, and vapreotide completely displaced [^{125}I]Tyr^1-SRIF-14 from pituitary binding sites (Fig. 1A). A significant inhibition of radiolabeled Tyr^1-SRIF-14 (Fig. 1B) was also observed in the presence of various SRIF agonists (EP-41251, EP-41252, EP-41253) that have been derived from octreotide, lanreotide, and vapreotide, respectively, or using other analogs (EP-80874 and EP-90089) with mixed agonist-antagonist properties on release of GH in rats (V. Locatelli, personal communication). No inhibition of [^{125}I]Tyr^1-SRIF-14 binding was observed in the presence of peptides belonging to the GHRP family (hexarelin, GHRP2, EP-51216) or SRIF derivatives that are well known to have a weak SRIF-like activity. They are SRIF H-2186, a highly selective ligand for μ-opioid receptors *(7)*; SRIF H-2485, an SRIF antagonist *(8)*; and SRIF H-3382, a peptide that possesses a strong inhibitory effect on vascular smooth muscle cell proliferation without inhibiting GH secretion *(9)* (Fig. 1C).

Surprisingly, octreotide, lanreotide, vapreotide (Fig. 2A), and their analogs (Fig. 2B) caused a substantial displacement of [^{125}I]Tyr-Ala-hexarelin from pituitary GHRP binding sites. These compounds, with the exception of octreotide and lanreotide, inhibited Tyr-Ala-hexarelin binding at concentrations lower than those of hexarelin. By contrast, SRIF (Fig. 2C) and other SRIF derivatives were all weak competitors of [^{125}I]Tyr-Ala-hexarelin. The inhibitory affinity constants of the compounds studied for [^{125}I]Tyr^1-SRIF-14 and [^{125}I]Tyr-Ala-hexarelin binding to pituitary membranes are given in Table 1.

Note that the ligand is in micromolar concentration. This relatively low specific activity in vitro has prevented its use in the identification of a GHS-R by the Merck Group *(2)*, who, however, reported similar binding affinities and biologic potencies for hexarelin and MK-0677. In vivo, both hexarelin and Tyr-Ala-hexarelin are highly potent *(5)*.

Discussion

We have shown, for the first time, that SRIF octapeptide analogs such as vapreotide, lanreotide, and octreotide share the GHRP receptor, which, however, does not accommodate SRIF. Our finding opens a new venue of research for such analogs hitherto thought to act only on one or more of the five cloned SRIF receptors *(15)*.

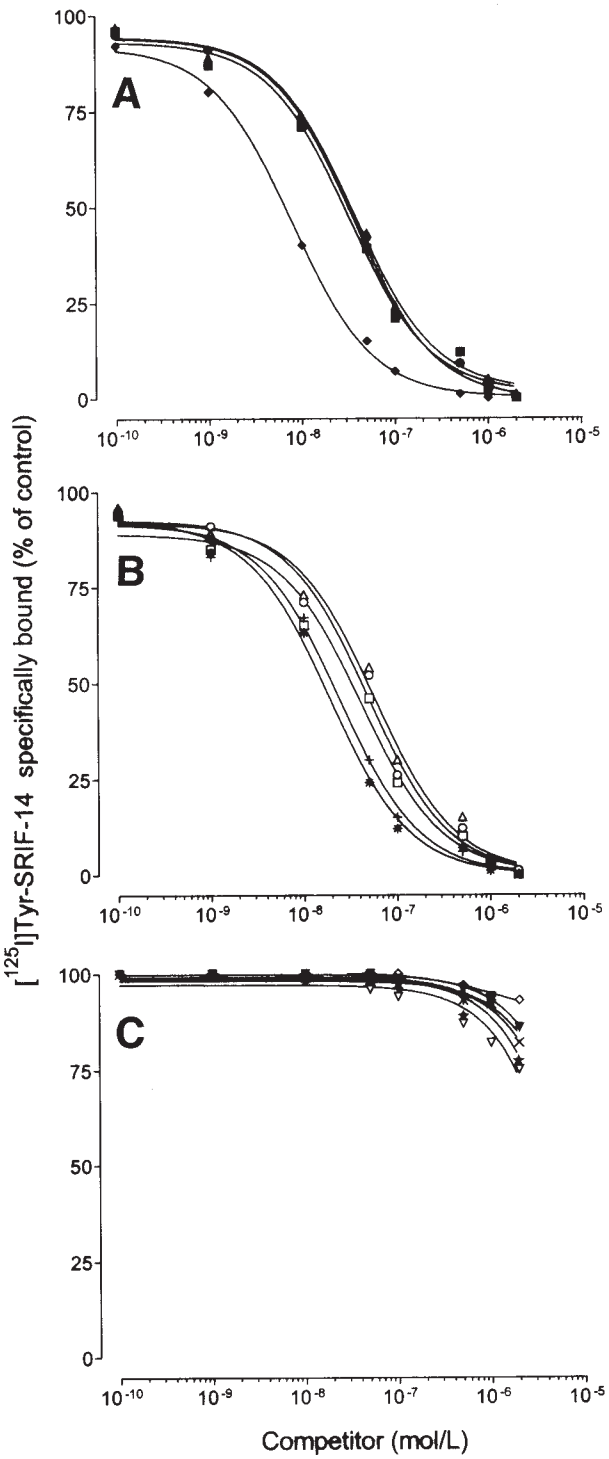

Fig. 1. Inhibition of [^{125}I]Tyr^1-SRIF-14 binding to pituitary membranes by **(A)** (◆) SRIF-14, (●) octreotide, (▲) lanreotide, (■) vapreotide; **(B)** (○) EP-41251, (△) EP-41252, (□) EP-41253, (*) EP-80874, (+) EP-90089; and (C) (×) hexarelin, (▼) GHRP2, (◇) EP-51216, (▽) SRIF H-3382, (★) SRIF H-2186, (▭) SRIF H-2485. The representative inhibition curves shown were taken from the same data sets used to produce the values given in Table 1.

In addition, our findings challenge the hypothesis that the natural GHS ligand (Bowers's U-factor) is a GHS-R agonist. Additional studies with GHS-R knockout mice or

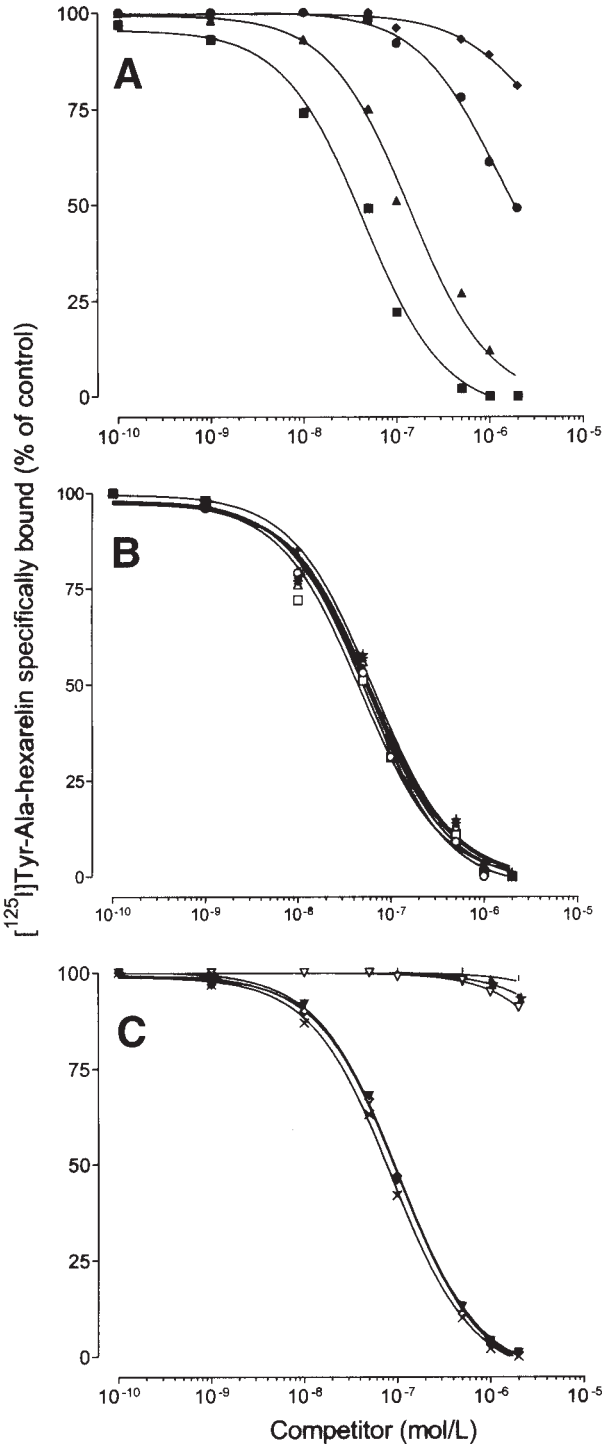

Fig. 2. Inhibition of [^{125}I]Tyr^1-Ala-hexarelin binding to pituitary membranes by **(A)** (◆) SRIF-14, (●) octreotide, (▲) lanreotide, (■) vapreotide; **(B)** (○) EP-41251, (△) EP-41252, (□) EP-41253, (*) EP-80874, (+) EP-90089; and **(C)** (×) hexarelin, (▼) GHRP2, (◇) EP-51216, (▽) SRIF H-3382, (★) SRIF H-2186, (▭) SRIF H-2485. The representative inhibition curves shown were taken from the same data sets used to produce the values given in Table 1.

suitable GHSR mutants should clarify this point. Note, however, that the pituitary hexarelin receptor subtype, identified by photoaffinity labeling by Ong et al. *(13)*, has not yet been cloned or transfected, and, therefore, it is not available for isolated studies at this time.

It is conceivable that GHRP "antagonists," such as D-Lys^3-GHRP-6, are the true mimics of the elusive natural ligand, which appears to be a modulator of GHRH activity owing to its SRIF-like action. If this hypothesis is confirmed, Bowers's U-factor would be a growth hormone release inhibiting factor, and GHSs would act by inhibiting it *(16)*.

Materials and Methods

Chemicals

Somatostatin-14 (SRIF), SRIF H-3382, SRIF H-2186, and SRIF H-2485 were purchased from Bachem (Feinchemikalien AG Bubendorf, Switzerland). Somatostatin octapeptides such as octreotide, lanreotide, vapreotide, and their analogs (EP-41251, EP-41252, EP-41253, EP-80874, EP-90089), as well as various GHRPs (hexarelin, GHRP2, EP 51216), were provided by Europeptides (Argenteuil, France). [^{125}I]Tyr-Ala-hexarelin (specific activity 2200 Ci/mmol, 814 TBq/mmol) was iodinated using a lactoperoxidase method and purified by reverse-phase high-performance liquid chromatography as previously described *(10)*. [^{125}I]Tyr^1-somatostatin-14 (specific activity 2200 Ci/mmol, 814 TBq/mol) was purchased from NEN (Boston, MA).

Tissues

Pituitary glands were obtained at autopsy from three male subjects (ranging in age from 56 to 74 yr) who had died from cardiovascular diseases and were submitted to autopsy for diagnostic purposes. Tissue was removed 24–32 h after death with the approval of our hospital ethical committee and was immediately frozen at –80°C for 2–4 wk until processed for membrane preparation. At histopathologic examination, pituitary glands were all preserved from both an architectural and cytologic point of view.

Preparation of Membrane

Pituitary membranes were prepared by differential centrifugation as previously described *(10)*. The membrane pellet was resuspended in ice-cold buffer (50 mmol/L of Tris, 1 mmol/L of EDTA, 20 mg/L of bacitracin, 20 mg/L of phenylmethylsulfonyl fluoride titrated with HCl to pH 7.4) and immediately used for protein content *(11)* and somatostatin and Tyr-Ala-hexarelin (GHRP) binding studies.

Radioligand Binding Assays

Somatostatin binding studies were performed according to the method of Koenig et al. *(12)* with minor modifications. Aliquots of the membranes (50 µg of protein) were incubated (2 h at 20°C) in triplicate with 0.005 nmol/L of [^{125}I]Tyr^1-somatostatin-14 ([^{125}I]Tyr^1-SRIF-14) in a final volume of 0.5 mL of assay buffer containing 50 mmol/L of Tris (pH 7.4), 1 mmol/L of EDTA, 10 mmol/L of $MgCl_2$,

1 g/L of bovine serum albumin, 20,000 KIU/L of Trasylol, 20 mg/L of bacitracin, and 20 mg/L of PMSF. Specific binding was defined as the total radioligand bound minus that bound in the presence of 25 mmol/L of unlabeled SRIF-14.

GHRP binding studies were performed as previously described *(10)* using [^{125}I]Tyr-Ala-hexarelin as a ligand. This hexarelin analog has been reported to have the same GH-releasing potency of hexarelin in rats *(13)* and humans *(5)* and to be a reliable probe for labeling GHRP receptors in tissue membranes. Incubations in triplicate were kept at 0°C for 60 min in 05 mL containing 50 mmol/L of Tris (pH 73), 2 mmol/L of EGTA, 20 mg/L of bacitracin, 1 g/L of BSA, 50 µg of membrane protein, and 0.5 nmol/L of [^{125}I]Tyr-Ala-hexarelin. Specific binding was defined as the total radioligand bound minus that bound in the presence of 25 µmol/L of unlabeled Tyr-Ala-hexarelin.

Inhibition of [^{125}I]Tyr^{1}-SRIF and [^{125}I]Tyr-Ala-hexarelin binding to pituitary membranes by various SRIF derivatives and GHRP was obtained using eight different concentrations of each competitor. Displacement binding curves were subjected to iterative nonlinear analysis with the GraphPad Prism program (GraphPad Software, San Diego, CA), and the inhibitory affinity constant values were determined using the Cheng and Prusoff equation *(14)* and expressed as K_{05}. In all radioligand assays, the reaction was terminated by rapid filtration (Millipore apparatus) of the incubation mixture through Whatman GF/B glass fiber filters followed by three rinses with ice-cold assay buffer. Filters were presoaked in 05% polyethyleneimine to prevent nonspecific filter binding. Radioactivity bound to membranes was measured in a Packard auto–gamma counter.

Statistical Analyses

Significant differences between groups were assessed by one-way analysis of variance followed, when appropriate, by Duncan's multiple range test.

Note added in proof: The hypothetical SRIF-like ligand has been identified as cortistatin *(16)*. The recently identified ligand "ghrelin" has similar binding affinity to the GHRP receptor.

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