RAPID COMMUNICATION

Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptors of human pituitary gland

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ABSTRACT. Antagonism between GH secretagogues (GHS) and somatostatin (SRIH) has been postulated and demonstrated, but SRIH does not bind to GHS receptors (GHS-R) and potent synthetic peptidyl GHS (GHRP6, hexarelin) do not displace radiolabeled SRIH from its receptors. However, non-natural SRIH octapeptide agonists (mainly lanreotide and vapreotide) displace ^{125}I-Tyr-Ala-hexarelin from pituitary binding sites suggesting that an endogenous factor related to SRIH might exist and interact with GHS-R. Our aims were to investigate the ability of different SRIH-like peptides such as various SRIH fragments (SRIH 3-14, SRIH 7-14, SRIH 3-10, SRIH 7-10, SRIH 2-9) and a natural neuropeptide that shows a high structural homology with SRIH such as cortistatin-14 (CST) to compete with ^{125}I-Tyr-Ala-hexarelin for human pituitary binding sites and to compare their binding affinity with that of hexarelin and ghrelin, a gastric-derived peptidyl GHS that has been proposed as a natural ligand of GHS-R. While the binding of ^{125}I-Tyr-Ala-hexarelin to pituitary membranes was completely displaced by unlabelled hexarelin, ghrelin and CST, none of the SRIH fragments tested inhibited this binding. Ghrelin and CST exhibited a similar affinity (4.6-5.4 x 10^{-7} mol/l) for the binding while hexarelin was more effective by about four orders of magnitude in displacing ^{125}I-Tyr-Ala-hexarelin. Our data demonstrate for the first time that cortistatin, a natural peptide related to SRIH, binds to GHS-R and suggest that this factor may play a role in modulating the activity of these receptors.

(J. Endocrinol. Invest. *24:* RC1-RC3, 2001)

INTRODUCTION

GH secretagogues (GHS) are synthetic, peptidyl [GH-releasing peptides (GHRP6, hexarelin)] and nonpeptidyl molecules that possess strong, dose-dependent, and reproducible GH-releasing activity *in vivo* in several species and in man (1). These substances act through specific receptors (GHS-R) which have been originally identified in the pituitary gland and the hypothalamus. (2-4). GHS represent the synthetic counterpart of an endogenous peptide (ghrelin) produced by the stomach (5) that potently stimulates GH in men (6) by acting on GHS-R (7). Recently, we observed that some synthetic somatostatin (SRIH) octapeptide agonists (mainly lanreotide and vapreotide), but not SRIH, displaced ^{125}I-Tyr-Ala-hexarelin from its receptors suggesting that an endogenous factor related to SRIH might exist and interact with the GHS-R (8).

Key-words: Cortistatin, somatostatin, ghrelin, hexarelin, pituitary receptors.

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Based on the foregoing, the aims of the present study were: 1) to investigate the ability of different SRIH-like peptides such as various SRIH fragments (SRIH 3-14, SRIH 7-14, SRIH 3-10, SRIH 7-10, SRIH 2-9) and a natural neuropeptide that shows a high structural homology with SRIH such as cortistatin-14 (CST) (9) to compete with ^{125}I-Tyr-Ala-hexarelin for binding sites of human pituitary gland; and 2) to compare their binding affinity with that of hexarelin and ghrelin.

MATERIALS AND METHODS

Chemicals

Hexarelin (His-D-2Me-Trp-Ala-Trp-D-Phe-Lys-NH_2), Tyr-Ala-hexarelin, human ghrelin [Gly-Ser-Ser-(O *n*-octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-NH_2], CST (Pro-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys]-Lys-NH_2), and different fragments of somatostatin-14 (SRIH): SRIH 3-14 (c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]), SRIH 3-10 (H-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-OH) , SRIH 7-14 (H-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH), SRIH 2-9 (H-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-OH) and SRIH 7-10 (H-Phe-Trp-Lys-Thr-OH) were supplied by Europeptides (Argenteuil, France). ^{125}I-

Tyr-Ala-hexarelin (SA 1800-2000 Ci/mmol) was iodinated using a lactoperoxidase method and purified by reverse-phase high performance liquid chromatography as previously described (2).

Tissue samples

Pituitary glands were obtained at autopsy from 7 subjects (4 males and 3 females ranging in age from 39 to 64 years, median age 52 years) who died of trauma or neoplasms and were submitted to autopsy for diagnostic purposes in the Department of Pathology, University of Turin. Tissue was removed 24-28 h after death with the approval of our hospital Ethical Committee and immediately stored at -35 C for 1-2 months until processed for membrane preparation and binding studies. At histopathological examination, pituitary glands were all preserved from both an architectural and cytological point of view.

Binding studies

GHS binding assay with tissue membranes (30,000 g fraction) was performed as previously described (2) using ^{125}I-Tyr-Ala-hexarelin as ligand. This hexarelin analog has been reported to have *in vivo* the same GH-releasing potency of hexarelin (10) and to be a reliable probe for labeling GHS receptors in human tissues (7). Incubations in triplicate were kept at 0 C for 60 min in 0.5 ml assay buffer (50 mmol/l Tris-HCl, 2 mmol/l EGTA, 20 mg/l bacitracin and 1 g/l BSA) containing 100 µg membrane protein and 0.5 nmol/l ^{125}I-Tyr-Ala-hexarelin. Specific binding was defined as the total radioligand bound minus that bound in the presence of 10 µmol/l unlabelled Tyr-Ala-hexarelin. Inhibition of ^{125}I-Tyr-Ala-hexarelin binding to pituitary membranes by various competitors was obtained using 8 different concentrations (from 1 nmol/l to 10 µmol/l) of each substance. The concentration of competitor required to inhibit radiotracer binding by 50% (IC_{50}) was calculated by iterative nonlinear curve-fitting with the Prism 3 program (GraphPad Software, Inc., San Diego, CA). In all assays, the binding reaction was terminated by adding ice cold assay buffer followed by filtration through Whatman GF/B filters. The radioactivity remaining bound to the filters was measured by a Packard gamma counter.

Statistical analysis

Values are expressed as mean±SE. Significant differences between groups were assesses by one-way ANOVA followed by Duncan's multiple-range test. $P<0.05$ was chosen as the level of significance.

RESULTS

The ability of unlabeled hexarelin, ghrelin, and different peptides related to SRIH such as SRIH 3-14, SRIH 3-10, SRIH 7-14, SRIH 2-9 and SRIH 7-10 and CST to compete with ^{125}I-Tyr-Ala-hexarelin for the pituitary binding sites of male subjects is reported in Figure 1. While the binding of ^{125}I-Tyr-Ala-hexarelin to pituitary membranes was completely displaced by increasing concentrations of unlabeled hexarelin, ghrelin and CST, none of the SRIH fragments tested or SRIH (8) inhibited the binding, of radiotracer. Ghrelin and CST exhibited a similar affinity for the binding while hexarelin was more effective ($p<0.01$) by about four orders of magnitude in displacing ^{125}I-Tyr-Ala-hexarelin. The IC50 values (mean ±S.E. of four separate experiments) were $(4.6\pm0.4)\times10^{-7}$ mol/l for ghrelin, $(5.4\pm0.3)\times10^{-7}$ mol/l for CST and $(1.3\pm0.2)\times10^{-7}$ mol/l for hexarelin. Determinations performed with another batch of radiolabeled Tyr-Ala-hexarelin on membranes from autoptic pituitary specimens of three female subjects, yielded overlapping binding values (data not shown).

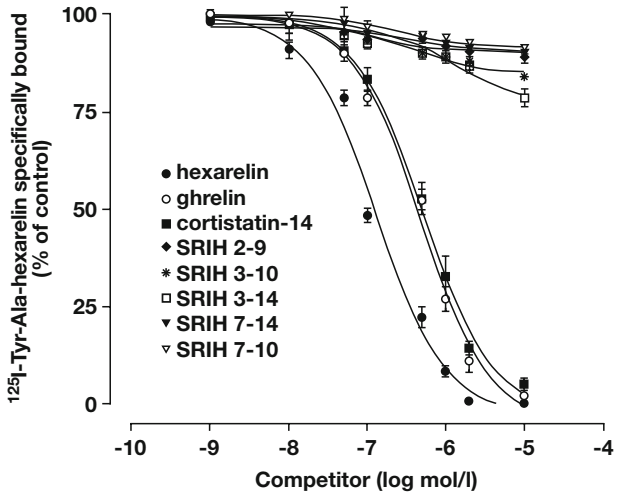

Fig. 1 - *Displacement of ^{125}I-Tyr-Ala-hexarelin from pituitary membranes of male subject by hexarelin, ghrelin, and different peptides related to somatostatin-14: SRIF 3-14, SRIF 3-10, SRIF 7-14, SRIF 2-9 and SRIF 7-10 and cortistatin-14. Each point represents the mean±SE of four separate experiments.*

DISCUSSION

It has been clearly shown that synthetic peptidyl GHS such as hexarelin act through GHS-R, which have been originally identified in the pituitary gland and the hypothalamus (2-4). Ghrelin, a 28 amino acid peptide isolated from the stomach (5), is a natural ligand of GHS-R and, in fact, it has been shown to displace ^{125}I-Tyr-Ala-hexarelin from pituitary binding sites (7). The existence of GHS-R subtypes has been already reported (3, 4, 7) and the possibility that more than one single ligand exists has been suggested (8, 11).

We have recently reported that some synthetic somatostatin (SRIH) octapeptide agonists (mainly lanreotide and vapreotide), but not native SRIH, displace ^{125}I-Tyr-Ala-hexarelin from its receptors (8). This evidence suggested the working hypothesis that an endogenous factor related to SRIH might exist and interact with the GHS-R, thus representing another natural ligand of these receptors. To clarify this hypothesis, we investigated the ability of different SRIH-like peptides such as various SRIH fragments (SRIH

3-14, SRIH7-14, SRIH 7-10, SRIH 2-9) and a putative natural neuropeptide such as CST, recently described in rat and human brains (12), to compete with ^{125}I-Tyr-Ala-hexarelin binding sites of human pituitary gland comparing their binding affinity with that of hexarelin and ghrelin. The different SRIH fragments were chosen as potential metabolites (13), whereas CST, though it shares 11 of its 14 amino acids with SRIH (9) and binds all five SRIH-receptor subtypes (14), possesses several effects that do not parallel those of SRIH. Our findings showing that CST, like hexarelin and ghrelin, but not various fragments of native SRIH, displaces ^{125}I-Tyr-Ala-hexarelin from its pituitary receptors, suggest that this factor may play a role in modulating the activity of GHS-R. The evidence that CST, like hexarelin and ghrelin, binds to GHS-R suggests that this peptide could represent another endogenous GHS ligand; this hypothesis could, in turn, imply that GHS-R is another specific receptor for CST. The observed actions of CST strongly depend on the N-terminal proline and the C-terminal lysine amide residue present on its structure (9). On the other hand, some of most active non-natural GHS such as GHRP6 and hexarelin also have a lysine amide group in C-terminal position.
This preliminary study shows for the first time a functional link between cortistatin and GHS-R in humans, which will be clarified in future studies on the endocrine effects of CST.

ACKNOWLEDGMENTS

The authors wish to thank Dr. I.C.A.F. Robinson for suggesting CST as a potential ligand and Dr. C. Ghè and Dr. F. Catapano for their skilful technical assistance. This work was supported in part by grants (ex-60% to G.M. and E.G.) from the Ministero dell'Università e Ricerca Scientifica e Tecnologica (MURST); by a grant from Foundation for the Study of Endocrine and Metabolic Disease (SMEM) SMEM; and by a grant from Europeptides.

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