RAPID COMMUNICATION

# Growth hormone-inhibiting activity of cortistatin in the rat

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**ABSTRACT. Cortistatin-14 (CST-14) is an endogenous neuropeptide with notable structural similarities to native somatostatin-14 (SS-14), but different physiological functions. Differences in the physiology of the two peptides do not provide conclusive evidence for a specific receptor for CST. To date, the effects of CST-14 on anterior pituitary hormones have never been reported. Aim of this study was to investigate the *in vivo* effects of CST-14 on GH secretion in comparison to SS-14. Our results demonstrate that CST-14 was very effective in reducing GH secretion in normal male anaesthetized rats. Its activity was similar to that of SS-14 and had a rapid onset and a slightly longer duration of action. In conclusion, we have reported for the first time that CST is a potent and effective inhibitor of GH release in rats and that its action may be mediated by the interaction with one or different SS receptor subtypes.**

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## *INTRODUCTION*

Cortistatin-14 (CST-14) is an endogenous neuropeptide endowed with many analogies to native somatostatin-14 (SS-14) but with a restricted brain cortical expression and different pharmacological profile (1). CST-14 shares 11 amino acids with SS-14 and was shown to bind with similar affinities to all five cloned human SS receptor subtypes expressed in transfected cell lines (2). However, differently from SS, CST induces slow-wave sleep, reduction of locomotor activity, and activation of cation selective currents not responsive to SS (3). Expression of mRNA encoding CST follows a circardian rhythm and is up regulated on deprivation of sleep, suggesting that CST might act as a sleep modulatory factor (4). However, the differences in SS and CST physiology do not provide conclusive evidence for CST specific receptors. Whether CST effects are mediated via SS and/or a CST specific receptor(s) still remains to be determined. Interestingly, it has recently reported that CST-14 and some synthetic SS octapeptide analogs (mainly lanreotide and vapreotide), but not SS-14, may interact with the receptor for GH secretagogues (GHS) in human pituitary membranes (5). It is therefore of importance to investigate the in vivo effects of CST-14 in comparison with SS-14 in search of a correlation between binding and biological activities of these natural neuropeptides on the endocrine system, an aspect so far neglected.

**Key-words:** Cortistatin, somatostatin, growth hormone, pituitary receptors, growth hormone secretagogues.

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## *MATERIALS AND METHODS*

### *Chemicals*

All peptides reported in this paper have been synthetized by conventional solid phase synthesis. Each peptide was purified to at least 98% purity by high-performance liquid chromatography.

### *Animals*

Male Sprague-Dawley rats weighing 300-350 g [Charles River, Calco (Lecco), Italy] were housed in our facilities under controlled conditions (22 C, 65% humidity, artificial light from 06:00-20:00 h). A standard dry diet and water were available *ad libitum*. All the experiments were performed in accordance with the Italian guidelines for the use of animals in medical research.

### *Experimental procedure*

On the day of the experiment, rats were deeply anaesthetized with ketamine hydrochloride (58 mg/kg, ip) and xylazine (12 mg/kg, ip) and then treated sc with graded doses (40, 80, 160, and 320 µg/kg) of CST-14 or SS-14 or isovolumetric amounts of physiological saline. Blood samples

were collected immediately before (T=0) and 10, 20, and 30 min after drug administration.
All blood samples were immediately centrifuged, and plasma was separated and stored at –20 C until processed for GH assay.

### *GH assay*

Plasma rat GH concentrations were determined in duplicate by double antibody RIA using materials supplied by the NHPP, NIDDK, NICHD, USDA, USA. Values are expressed in terms of rat-GH-RP-2 standard (potency 2 IU/mg) as ng/ml; intra-assay variability was 6%.

### *Statistical analysis*

Statistical evaluation of results was performed by one-way analysis of variance (ANOVA), followed by the Dunnett's *t*-test for the comparison of differences between multiple groups. A $p<0.05$ was considered to be significant.

## *RESULTS*

Graded doses of CST-14 significantly decreased GH secretion in adult male anaesthetized rats. All four doses of CST-14 significantly inhibited GH secretion 10 and 20 min after treatment (Fig. 1A). The effect of CST-14 had a rapid onset, reached its maximal activity at 10 min and persisted until 30 min post-treatment (about 50% inhibition at time 30). Calculation of the integrated GH secretion over the 30 min period ($AUC_{0\text{-}30min}$) revealed that all doses effectively inhibited GH secretion ($AUC_{0\text{-}30min}$: 3750±179, 2079±104, 1859±279, 1658±174 and 1340±196 for saline, CST-14 40, 80, 160 and 320 μg/kg, respectively; $p<0.05$).
Graded doses of native SS-14 inhibited GH release with a profile almost superimposable to that of CST-14 (Fig. 1B). Again, the maximal inhibitory activity was reached 10 min after drug administration. SS, however, inhibited GH secretion more effectively than CST at 20 min, but had lost almost completely its activity at time 30. Calculation of the $AUC_{0\text{-}30min}$ showed a nice dose-dependent inhibitory effect of SS on GH release ($AUC_{0\text{-}30min}$: 3969±154, 3002±247, 2148±248, 1523±113 and 956±72 for saline, SS-14 40, 80, 160 and 320 μg/kg, respectively; $p<0.05$).

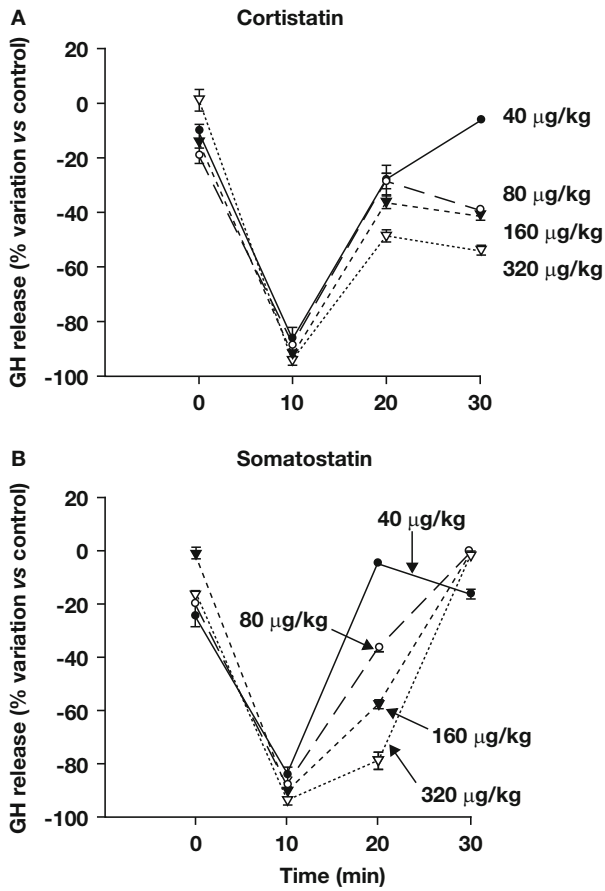


Fig. 1 - *Inhibition of GH secretion following administration of cortistatin (panel A) and somatostatin (panel B). Data are expressed as the percent variation* vs *GH values in the control group. Each point represents the mean±SE of 6 determinations. Statistics: in panel A, all doses at time 10 and 20 and the 3 higher doses at time 30 are significantly different (*p*<0.05) from respective controls. In panel B, all doses at time 10 and the 3 higher doses at time 20 are significantly lower than respective controls (*p*<0.05).*

## *DISCUSSION*

For the first time we report herein that CST-14 is active *in vivo* to reduce GH secretion in normal male anaesthetized rats. The inhibitory activity of CST is clear-cut and dose-related. Comparison with the inhibitory effect evoked by graded doses of native SS-14 in the same experimental conditions indicates that both neuropeptides share an overlapping GH-reducing activity, likely mediated by an action at the five cloned SS receptor subtypes. This finding might suggest a minor physiological relevance, as CST mRNA expression is relatively low in the hypothalamus (1). However, the known different pharmacological profile of SS and CST opens a new attractive window in the biology of the statin family. The reported capability of CST-14 to bind to receptors for natural and synthetic GHS (5-7) is an exciting possibility to develop selective antagonists to these receptors.
The GHS receptors are widely distributed within the body (8) where they participate in the regulation of different endocrine and non-endocrine functions (9, 10). It will be therefore relevant to extend the comparison between CST and SS, and their interactions, also to the other known activities regulated by the GHS system.
The reported inhibitory activity of CST on GH secretion and its similarity with SS cannot exclude a different mechanism of action. SS has its principal site of action on its pituitary receptors and CST, in addition to the latter, might have also acted antagonizing the "physiological" stimulatory tone of endogenous GHS. In line with this hypothesis, CST-14 and the SS analog vapreotide, but not SS-14, have

been shown to effectively displace the binding of $^{125}$I-human ghrelin, the endogenous GHS labeled for binding purposes, to human pituitary and hypothalamic membranes (7), suggesting that a SS-like agent might exist as an endogenous ligand. This hypothesis received confirmation when it was shown that CST, but not SS or SS-fragments, bind to the GHS-receptor in human pituitary tissues (6). Studies in progress in our laboratories will hopefully clarify this intriguing event in the forthcoming future.

## *REFERENCES*


1. Spier A.D., de Lecea L. Cortistatin: a member of somatostatin neuropeptide family with distinct physiological functions. Brain Res. Rev. 2000, *33:* 228-241.
2. Siehler S., Seuwen K., Hoyer D. $^{125}$I Tyr10-cortistatin-14 labels all five somatostatin receptors. Naunyn Schmiedebergs Arch. Pharmacol. 1998, *357:* 483-489.
3. Bell G.I., Reisine T. Molecular biology of somatostatin receptor. Trends Neurosci. 1993, *16:* 34-38.
4. de Lecea L., Criado J.R., Prospero-Garcia O., Gautvik K.M., Schweitzer P., Danielson P.E., Dunlop C.L., Siggins G.R., Henriksen S.J., Sutcliffe J.G. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. Nature 1996, *381:* 242-245.
5. Deghenghi R., Papotti M., Ghigo E., Muccioli G., Locatelli V. Somatostatin octapeptides (lanreotide, octreotide, vapreotide, and their analogs) share the growth hormone releasing peptide receptor in the human pituitary gland. Endocrine 2001, *14:* 29-33.
6. Deghenghi R., Papotti M., Ghigo E., Muccioli G. Cortistatin, but not somatostatin, binds to growth hormone secretagogue receptors of human pituitary gland. J. Endocrinol. Invest. 2000, *23:* 1-3.
7. Muccioli G., Papotti M., Locatelli V., Ghigo E., Deghenghi R. Binding of $^{125}$I-labeled ghrelin to membranes from human hypothalamus and pituitary gland. J. Endocrinol. Invest. 2001, *24:* RC7-RC9.
8. Papotti M., Ghe C., Cassoni P., Catapano F., Deghenghi R., Ghigo E., Muccioli G. Growth hormone secretagogue binding sites in peripheral human tissues. J. Clin. Endocrinol. Metab. 2000, *85:* 3803-3807.
9. Müller E.E., Locatelli V., Cocchi D. Neuroendocrine control of growth hormone secretion. Physiol. Rev. 1999, *79:* 511-607.
10. Ghigo E., Arvat E., Giordano R., Broglio F., Gianotti L., Maccario M., Bisi G., Graziani A., Papotti M., Muccioli G., Deghenghi R., Camanni F. Biologic activities of growth hormone secretagogues in humans. Endocrine 2001, *14:* 87-93.