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Cortistatin-8, a synthetic cortistatin-derived ghrelin receptor ligand, does not modify the endocrine responses to acylated ghrelin or hexarelin in humans

F. Prodam [a,b,1], A. Benso [a,1], E. Gramaglia [a], B. Lucatello [a], F. Riganti [a], A.J. van der Lely [c], R. Deghenghi [a], G. Muccioli [d], E. Ghigo [a], F. Broglio [a,*]

[a] *Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Corso Dogliotti 14, 10126 Turin, Italy*
[b] *Department of Medical Sciences, University of Piemonte Orientale, Novara, Italy*
[c] *Division of Endocrinology, Department of Internal Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands*
[d] *Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, Turin, Italy*

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Abstract

Cortistatin (CST), a neuropeptide with high structural homology with somatostatin (SST), binds all SST receptor (SST-R) subtypes but, unlike SST, also shows high binding affinity to ghrelin receptor (GHS-R1a). CST exerts the same endocrine activities of SST in humans, suggesting that the activation of the SST-R might mask the potential interaction with ghrelin system. CST-8, a synthetic CST-analogue devoid of any binding affinity to SST-R but capable to bind the GHS-R1a, has been reported able to exert antagonistic effects on ghrelin actions either in vitro or in vivo in animals. We studied the effects of CST-8 (2.0 μg/kg iv as a bolus or 2.0 μg/kg/h iv as infusion) on both spontaneous and ghrelin- or hexarelin- (1.0 μg/kg iv as bolus) stimulated GH, PRL, ACTH and cortisol secretion in 6 normal volunteers. During saline, no change occurred in GH and PRL levels while a spontaneous ACTH and cortisol decrease was observed. As expected, both ghrelin and hexarelin stimulated GH, PRL, ACTH and cortisol secretion ($p < 0.05$). CST-8, administered either as bolus or as continuous infusion, did not modify both spontaneous and ghrelin- or hexarelin-stimulated GH, PRL, ACTH and cortisol secretion. In conclusion, CST-8 seems devoid of any modulatory action on either spontaneous or ghrelin-stimulated somatotroph, lactotroph and corticotroph secretion in humans in vivo. These negative results do not per se exclude that, even at these doses, CST-8 might have some neuroendocrine effects after prolonged treatment or that, at higher doses, may be able to effectively antagonize ghrelin action in humans. However, these data strongly suggest that CST-8 is not a promising candidate as GHS-R1a antagonist for human studies to explore the functional interaction between ghrelin and cortistatin systems.

Keywords: Cortistatin; Cortistatin-8; Hexarelin; GHS-R1a; GH; PRL; ACTH; Cortisol

1. Introduction

Cortistatin (CST) is a recently described neuropeptide cloned from rat, mouse and human, the prepro-peptide cDNA of which shows high structural homology with prepro-somatostatin (SST), particularly in the carboxyl terminus from which SST-14 and SST-28 are enzymatically processed (Spier and de Lecea, 2000). Like pro-SST, pro-CST generate different mature products, i.e. CST-14 and CST-29 in rat and CST-17 and CST-29 in human (Spier and de Lecea, 2000; Fukusumi et al., 1997).

As their prepro-molecules, also the CST and SST mature products share a very high structural homology

* Corresponding author. Tel.: +39 011 6334317; fax: +39 011 6647421.
E-mail address: fabio.broglio@unito.it (F. Broglio).
[1] First co-authorship.

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and, therefore, both bind all the SST receptor subtypes with similar affinity (Fukusumi et al., 1997; Spier and de Lecea, 2000).

Nevertheless, CST, that is encoded by a different gene than SST (de Lecea et al., 1997), is not simply an alternative SST although it shares some of its biological actions (de Lecea and Castano, 2006). In fact, several reports have recently described that CST also possesses some specific activities in the central nervous system, even in functional antagonism with SST (de Lecea and Castano, 2006). Moreover, even when expressed in the same neurons, CST and SST are regulated by different signals (Calbet et al., 1999; Stumm et al., 2004).

This evidence suggested the existence of previously unknown specific receptors able to bind CST but not SST (Spier and de Lecea, 2000). Consistently with this hypothesis, MrgX2, a previously orphan receptor mainly expressed in dorsal root ganglions, has been reported to bind CST but not SST (Robas et al., 2003). Notably, however, MrgX2 is not expressed in the cerebral cortex, where CST expression is most abundant (Robas et al., 2003). The biological role of CST binding to MrgX2 is therefore, at present, not fully characterized and probably plays a role in the modulatory effect of CST on pain perception (Robas et al., 2003), but is unlikely to explain the functional specificities of CST at the cortical level.

CST-14 and CST-17, but not SST-14, has also been shown able to bind the GH secretagogue receptor type 1a (GHS-R1a), a seven trans-membrane domains G-protein coupled receptor, an endogenous ligand of which is acylated ghrelin (AG) (Van Der Lely et al., 2004; Deghenghi et al., 2001b; Hosoda et al., 2006). GHS-R 1a is expressed in the anterior pituitary gland and in the hypothalamus, mainly in the arcuate nucleus, but also in various extrahypothalamic regions in the central nervous system as well as in multiple peripheral organs (Van Der Lely et al., 2004).

AG is mostly synthesized in the stomach, but it is also expressed in several other central and peripheral tissues (Van Der Lely et al., 2004; Hosoda et al., 2006). Consistently with the widespread expression of ghrelin and of the GHS-R1a, this hormone has been shown to possess several biological actions including stimulatory effects on GH, ACTH/cortisol, prolactin secretion, inhibitory influence on the gonadal axis, a modulatory effect on appetite control, energy balance, gastric motility and acid secretion, cardiovascular function but also influence on the beta cell function, glucose and lipid metabolism (Van Der Lely et al., 2004; Kojima and Kangawa, 2006).

CST-8 (Pro-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Lys-NH2) is a CST-derived synthetic peptide that has been shown able to displace acylated ghrelin from its specific binding sites in human and animal tissues but unable to interfere with somatostatin binding to its receptors (Sibilia et al., 2006). Based on its binding properties, CST-8 has been supposed to represent a tool to explore potential SST-R-independent but GHS-R1a-related biological activities of CST.

In fact, CST-8 has recently been shown to behave as an antagonist of GHS-R1a by counteracting the response of ghrelin on gastric acid secretion (Sibilia et al., 2006) and, in vitro, to modulate GH release from somatotroph cells, possibly influencing GHS-R1a affinity to its ligands by inducing specific dimerizations or conformational transformations of the receptors (Luque et al., 2006).

The endocrine activities of CST-8 in humans have never been investigated so far, and, to address this point, we studied the effects of different doses of CST-8 given as intravenous bolus or as infusion administration on both spontaneous and AG- or hexarelin (HEX)-stimulated GH, PRL, ACTH and cortisol levels in normal young male volunteers.

2. Subjects and methods

Six healthy young male volunteers (age [mean ± SEM]: 28.1 ± 1.0 yr; BMI: 23.0 ± 0.8 kg/m^2 were studied. All the subjects gave their written informed consent to participate in the study which had been approved by an independent Ethical Committee.

All the subjects underwent the following testing sessions in random order and at least 3 days apart:

(a) CST-8 (2.0 μg/kg iv as bolus at 0'; purchased from Neosystem Laboratories, Strasbourg, France);
(b) CST-8 (2.0 μg/kg/h iv as infusion over 120 min);
(c) AG (1.0 μg/kg iv as bolus at 0'; purchased from Phoenix Europe GmbH, Karlsruhe Germany);
(d) HEX (1.0 μg/kg iv as bolus at 0'; purchased from Europeptides, Argenteuil, France);
(e) CST-8 (2.0 μg/kg iv as bolus at 0') + AG (1.0 μg/kg iv as bolus at 0');
(f) CST-8 (2.0 μg/kg/h iv as infusion over 120 min from −30' to +90') + AG (1.0 μg/kg iv as bolus at 0');
(g) CST-8 (2.0 μg/kg iv as bolus at 0') + HEX (1.0 μg/kg iv as bolus at 0');
(h) CST-8 (2.0 μg/kg/h iv as infusion over 120 min from −30' to +90') + HEX (1.0 μg/kg iv as bolus at 0');
(i) isotonic saline (infusion over 120 min).

All the tests have been performed in the morning starting at 08.30–09.00 after overnight fasting, 30' after an indwelling catheter had been placed into an forearm vein kept patent by slow infusion of isotonic saline. Blood samples were taken every 15' in order to assay GH, PRL, ACTH and cortisol levels.

Serum GH levels (ng/ml) were measured in duplicate by immunoradiometric assay (hGH IRMA CT,