INTRODUCTION

Cortistatin (CST) is a novel neuropeptide found in different species including frog, rodents and human (1, 2, 3). The proteolytic processing of pro-cortistatin results in production of multiple mature peptides that include two isoforms CST-14 and CST-29 in rat, and two peptides, CST-17 and CST-29, in human (4, 5). Subsequent studies on cortistatin revealed that this peptide shows high structural homology and functional resemblance with somatostatin (SS) (6, 7).

Initially, CST mRNA was found in the central nervous system, mainly in the cortex and in the hippocampus (5, 8). Further analysis has shown that CST mRNA is present in peripheral tissues including adrenal, thyroid, pancreas, testes, liver, stomach, intestine, kidney and lung, as well as in parathyroid and immune cells (9, 10). SS expression pattern in human overlap CST distribution in some tissues and organs, but SS mRNA distribution is restricted to pancreas, brain, heart, connective and embryonic tissue (11).

Moreover, CST binds with high affinity to all five somatostatin receptors (sst1-sst5) and both peptides, CST and SS, activate receptors with similar efficiency and potency (12). It has been reported that CST but not SS is able to bind to MrgX2, a previously orphan receptor mainly expressed in the dorsal root ganglions. Interestingly, MrgX2 receptor binds also proadrenomedullin and its peptides. However, this receptor has not been...
found in the cerebral cortex, the region with very high expression of CST (13, 14). Recently, it has been also revealed that CST shows ability to displace iodinated, acetylated ghrelin from its binding sites in the pituitary (likely the ghrelin receptor 1A, GHS-R1A) (15).

Numerous studies have revealed that both CST and SS, by affecting somatostatin receptors, exert a wide range of biological activity, including regulation of endocrine and exocrine secretion, neurotransmission, neuropeptide modulation and inhibition of tumor growth (8, 16). On the other hand, when activating non-sst receptors CST does not parallel with SS. Specifically, CST may modulate sleep physiology, locomotor behaviour and hippocampal functions (3, 5). In addition, CST has been reported to possess anti-inflammatory activity (17, 18).

The aim of the present investigation was to evaluate in vivo the effect of peripheral administration of cortistatin on pituitary hormone release in comparison with somatostatin treatment.

MATERIAL AND METHODS

Animals

Adult 3-month old Wistar-Kyoto (240-260g) male rats were used in the experiment. Animals were maintained under standard laboratory conditions on a 12/12 hrs light/dark cycle (lights on at 07.00 AM). Food and water were available ad libitum. All animal procedures were in accordance with the Guiding Principles for the Care and Use of Research Animals and the experiment protocol was approved by the First Warsaw Ethics Committee for Experiments on Animals.

Reagents

Cortistatin-14 (CST-14) trifluoroacetate was delivered from Bachem AG, Switzerland, and somatostatin-14 (SS-14) was purchased from Sigma Chemical Co. (St. Louis, MO, USA)

Peptide injections and sample collection

On the day of experiment, conscious animals, divided into subgroups, were given an intravenous (iv) injection into the tail vein of cortistatin-14 (CST-14), somatostatin-14 (SS-14) and 0.9% NaCl (control group), respectively. Peptides, cortistatin or somatostatin, in a dose of 10 µg per animal were diluted in 0.3 ml of saline. Animals from the control group were treated with 0.3 ml of 0.9% natrium chloratum alone.

Blood samples were collected 60 min after administration of CST-14, SS-14, and 0.9% NaCl (group 1, 2 and 3), or 120 min after the same treatment (group 4, 5 and 6). Serum was separated and frozen in temperature of – 70°C until assays.

Radioimmunoassays

Serum levels of selected pituitary hormones (growth hormone – GH, prolactin – PRL, luteinizing hormone - LH, follicle stimulating hormone - FSH) were measured with RIA methods. Rat serum LH, FSH and PRL concentrations were estimated by using antibodies and hormones preparations generously supplied by Dr A. Parlow and NIDDK, Baltimore, MD, USA. Values were expressed in terms of rat LH (RP-3), FSH (RP-2) and PRL (RP-3), respectively. The detection limit of these assays was 0.1 ng/ml for LH, 1.25 ng/ml for FSH and 0.63 ng/ml for PRL.

Rat serum GH was measured using the kit from Linco (USA). The limit of detection was 0.5 ng/ml. For each hormone all serum samples were analyzed in one assay. The intra-assay coefficients of variation were less than 7%. To avoid inter-assay variability all samples among one hormone estimation were tested with the same RIA kit.

Statistical analysis

All results are expressed as means ±SD. Statistical evaluation of differences between groups was performed using the Kruskal-Wallis rank test followed the Mann-Whitney U test. Differences were considered statistically significant when p<0.05.

RESULTS

Peripheral injection of CST-14 resulted in significant decrease in circulating levels of growth hormone compared to saline injected control rats (p<0.05). However, this effect was seen only 60 min after peptide administration as after 120 min GH concentration resumed to baseline values. (Fig. 1)

As expected, SS-14 treatment also significantly influenced serum GH levels and decrease in GH concentration was observed at 60 min in comparison with control group (p<0.05), but shortly thereafter, in another 60 min, this inhibitory action was diminished. (Fig. 1)

Increase in serum prolactin concentration was noticeable only at 60 min after administration of cortistatin (p<0.01). (Fig. 2) Somatostatin treatment failed to produce any significant changes in
circulating levels of prolactin throughout the 120 min period of observation. (Fig. 2)

Serum gonadotropins levels, both LH and FSH, did not differ significantly between CST-14 treated group and saline injected controls at any time examined. Administration of SS-14 markedly decreased LH levels at 60 min as compared to control group (p<0.05) and lowered FSH secretion after 120 min (p<0.05). (Fig. 3 and Fig. 4, respectively)

**DISCUSSION**

In the present study we have focused on the influence of cortistatin on selected pituitary hormones secretion in animal *in vivo* model. It has been shown previously that both rat and human form of cortistatin, CST-14 and CST-17, may exert similar activity to somatostatin (19). This phenomenon could be explained by the close resemblance of these peptides in chemical structure and binding affinity to somastostatin receptors. However, CST does not mimic SS activity thoroughly (20). Therefore, recent studies have raised the question whether CST acts *via* different to SST receptors and the findings indicate that CST exert it effect also through MrgX2 and GHRS-1A receptors.

Several *in vitro* and *in vivo* experiments have shown that CST is involved in modulation of GH release from pituitary. However, data remain equivocal. The study by Rubinfeld *et al.* showed that CST-14 in a dose of 10 nMol inhibits basal GH secretion from human fetal pituitary cells (21). It is worth to notice that suppression was greater than induced with equal dose of SS-14 under the same experimental procedure (21). In addition, in the above study it was also found that CST-14 and CST-17 are able to inhibit basal GH secretion in GH- and GH/PRL-adenomas.

Moreover, CST was reported to possess a dual activity as it could act as an inhibiting or stimulating factor of GH release from isolated porcine somatotropes. In detail, treatment with high doses of CST, comparable to somatostatin, resulted in regression of GH release stimulated by GH-RH (growth hormone releasing hormone) while low, subnanomolar dose of CST produced an increase in GH secretion (22). Authors of this particular investigation concluded that the same receptors and signaling pathways are likely to be involved in both CST and SS effects. Furthermore, our previous *in vitro* study with use of rat pituitary cells revealed that
cortistatin, contrary to somatostatin, exerts an stimulatory, dose- and time-dependent effect on GH release (23).

Data from the studies conducted on healthy humans showed that either CST-14 or CST-17 administration inhibited GH secretion and decreased insulin concentration without changing glucose levels. These results were related to those of SS and found to be overlapping (19, 24, 25). Consequently, the GH response to GH-RH and ghrelin or its synthetic analogs in the presence of CST and SS was also investigated. Accordingly, there was no significant difference in inhibitory potency when compared CST to SS (19, 24, 25).

Besides, consistently with results obtained from the healthy humans, a study comparing the effects of CST and somatostatin on endocrine response in patients with acromegaly and prolactinoma showed that both peptides shared the same inhibitory effect on GH secretion as it was found in healthy controls (26). Moreover, decrease in insulin secretion under either CST or SST infusion was recorded in acromegalic subjects, a well as in patients with prolactinoma and controls. However, no changes in glucose levels were found in all groups (26).

The dual action, stimulatory or inhibitory, of cortistatin on GH release is difficult to explain, but it is worth to notice that cortistatin may act via the activation of other to sst specific receptors MrgX2 and/or GHS-R. It has been reported in literature that CST-17 and CST-14 but not SS bind GH secretagogue receptor (GHS-R) (15). Ghrelin as a natural ligand stimulates GH and PRL secretion, influences on insulin secretion and hyperglycemic effect as well as plays an important role in the control of food intake (27, 28).

Broglio et al. have demonstrated that both SS and CST similarly inhibited ghrelin secretion in humans (29). On the other hand, the authors showed persistent inhibition of ghrelin release after the withdrawal of SS or CST despite normalization of GH and insulin and this result may suggest that inhibitory effect of CST or SS on the GH and insulin release is not mediated by ghrelin (29).

We confirmed the findings of other authors that in vivo CST inhibits GH secretion in rats and this effects is in parallel in those exert by somatostatin (31). We demonstrated that intravenous administration of cortistatin, CST-14, similarly to SS-14 resulted in inhibition of GH release. However, this response was abolished after 120 min.

Previous studies have indicated that cortistatin may influence prolactin secretion. It has been reported that inhibition of PRL release under CST-17 was seen in cultured pituitary adenomas both
prolactinomas and mixed GH/PRL-adenomas (21). Moreover, it has also been established that somatostatin receptor subtype 5 (sst5) is the main receptor mediating this inhibitory effect as the lack of sst5 was found in CST-resistant adenomas (21). Data from study conducted by Grottolli et al. showed that both CST and SS suppress prolactin secretion in patients with prolactinoma and, at least partially, with acromegaly (26). However, no effect of cortistatin administration on prolactin concentration was seen in normal healthy subjects (26). Furthermore, Broglio et al. demonstrated that, in humans, the prolactin response to ghrelin infusion was not changed by CST or SS administration (24). Recently, Prodam et al. found that CST-8, a synthetic CST-analogue, did not modify both spontaneous and ghrelin- or hexarelin-stimulated GH, PRL, ACTH and cortisol secretion in humans in vivo (31).

The results of present study revealed that CST-14 treatment resulted in significant but short-lived increase in prolactin secretion. This finding in connection with discrepancies in effect of CST on PRL secretion proves the statement that extensive research is necessary to explain the correlation between those two peptides.

Data concerning the influence of CST on gonadotropes are lacking. As mentioned above, there is an evidence indicating that CST is able to bind different receptors including sst specific receptors, MrgX2 and GHS-R. Therefore, it could be predicted that CST, by activating sst receptors, would behave as an inhibitor of gonadotropin secretion. Moreover, modulatory effect of CST on gonadotropes via GHS-R also should be put into consideration. Ghrelin, the endogenous ligand of GHS-R1A, shows pleiotropic biological effects not only regulating energy homeostasis but also influencing reproductive functions. Recent in vivo studies have shown that ghrelin is able to inhibit LH and FSH secretion. However, data from in vitro experiments reveal that ghrelin decreases LH responsiveness to GnRH but enhances LH and FSH basal secretion at the pituitary levels (32, 33). Extrapolating results from studies on ghrelin, it might be supposed that peripheral injection of cortistatin will result in decrease in gonadotropins concentration. In the present study, we observed that there was no significant effect of CST-14 administration on both LH and FSH concentration in the rat, although after 1 hour the tendency to lower values of both gonadotropins was seen. However, SS administration influenced gonadotropin secretion. It is plausible that cortistatin affects gonadotropes by activating different types of receptors but consequently, more trials are needed to confirm this hypothesis.

**CONCLUSIONS**

Cortistatin plays a regulatory role in pituitary secretion. Moreover, some differences have been found when compared cortistatin to somatostatin. Thus, when analyzing the mechanism of cortistatin activity it is worth to consider the effect of binding with all receptors of somatostatin (SSTR 1-5), specific receptor for CST (MrgX2) and GH secretagouge receptor of ghrelin (GHS-R). Accordingly, it would be essential to include the influence of immune system on endocrine targets as it has been found recently that cortistatin possesses anti-inflammatory properties.

**REFERENCES**


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