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RAPID COMMUNICATION

Stimulation by Urocortin of Growth Hormone (GH) Secretion in GH-Producing Human Pituitary Adenoma Cells

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Abstract. Urocortin (Ucn) possesses high homology with CRH and is considered to be a ligand to type-2 CRH receptor. We investigated the effect of Ucn on hormone release from cultured GH-producing human pituitary adenoma cells *in vitro*. GH-producing human pituitary adenoma cells were superfused on a Sephadex G-25 column. Both Ucn (10 nM) and CRH (10 nM) elicited an increase in GH release from the pituitary adenoma cells in one patient with acromegaly. In contrast, GH release from the pituitary adenoma cells was stimulated by Ucn but not by CRH in the other patient with acromegaly. These preliminary findings suggest that type-2 CRH receptors are expressed in some population of GH-producing human pituitary adenoma cells and that Ucn might be involved in GH secretion from tumorous tissues in patients with acromegaly.

Key words: Urocortin, GH, Pituitary adenoma

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UROCORTIN (Ucn), a mammalian member of CRH family, was characterized in the rat [1] and the human [2]. Binding studies revealed that Ucn was more potent than CRH to bind CRH type-2 receptors [1, 2]. Homology of amino acid sequence between Ucn, fish urotensin I and amphibian sauvagine [1], and coincidence of urotensin I-like immunoreactivity and type-2 CRH receptors in the brain [3] suggest that Ucn is an endogenous ligand for type-2 CRH receptor in mammals.

However, physiological role and pathophysiological significance of Ucn remain to be elucidated in human. In this preliminary paper, we report a stimulating effect of Ucn on GH secretion from human pituitary adenoma cells *in vitro*.

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Materials and Methods

Two acromegalic patients were studied (Table 1). Both of them had characteristic clinical features, high plasma levels of GH and IGF-I, and a pituitary mass on brain MRI. Plasma GH levels were not considerably suppressed by oral administration of 75 g glucose in these patients. Either patients did not receive medical treatment before surgery. Pituitary adenoma tissues were obtained at transsphenoidal surgery upon patients' informed consent. Adenoma cells were cultured as previously described [4]. Briefly, the tissues were minced by fine scissors in ice-cold phosphate-buffered saline (PBS) and the cells were dispersed in PBS containing 0.25% trypsin at 37 °C for 20 min by gentle stirring in a use of a spinner flask. The dispersed cells were collected, filtered through 40-μm nylon mesh, washed three times with Dulbecco's modified Eagle's medium supple-

Table 1. Clinical characteristics of patients examined in this study

Patient	Age (year)	Sex	Plasma GH (μg/L)	Plasma IGF-I (ng/ml)	Size of pituitary adenoma on MRI (cm)
1	51	female	1080	2030	3 × 4
2	47	female	107	495	2 × 3

mented with 10% fetal calf serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml). They were cultured in the medium under humidified atmosphere of 5% CO_2-95% air at 37°C. After two to three days, the cells were mechanically harvested. 1.3 to 2 × 10^6 cells were applied onto a small Sephadex G-25 column (diameter, 9 mm; height, 8 mm), and were superfused with Krebs-Ringer bicarbonate buffer, pH 7.4 containing 10 mM glucose and 0.1% bovine serum albumin (KRBG) at a constant flow rate of 0.33 ml/min in a use of a peristaltic pump as previously described [4]. KRBG was equilibrated with 95% O_2-5% CO_2 and was kept at 37°C throughout the experiments. Test substances were dissolved in KRBG at 6.35-fold of the final concentration and were infused into the chamber at a rate of 52 μl/min in a use of an infusion pump. The effluent perfusate was fractionated every five min and was stored at –20°C until assayed for human GH.

Human GH levels in the effluent perfusate were determined by a specific RIA as previously described [5]. Intra- and inter-assay coefficients of variation were less than 5% and 10%, respectively.

Rat Ucn and human CRH were obtained from Yanaihara Institute, Fujinomiya, Shizuoka, Japan and Peptide Institute Inc., Minoh, Osaka, Japan, respectively. TRH and human GH-releasing hormone (GRH) were supplied from Tanabe Parmaceutical Co., Osaka, Japan and Sumitomo Pharmnaceutical Co., Osaka, Japan, respectively. Ucn and CRH were first dissolved in 0.1% acetic acid and were then diluted with KRBG immediately before use.

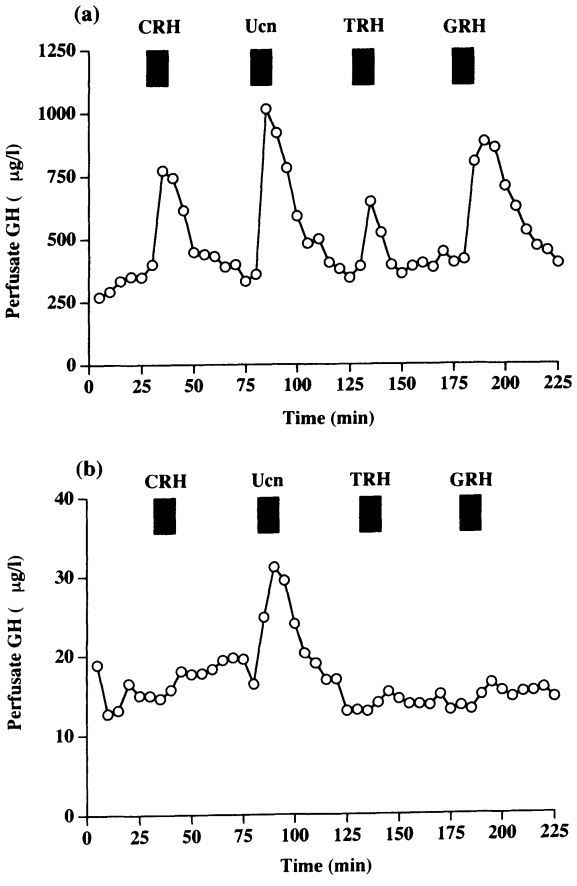


Fig. 1. Effect of human CRH (10 nM), rat urocortin (Ucn) (10 nM), TRH (10 nM) and human GRH (0.1 nM) on GH release from superfused human GH-producing adenoma cells *in vitro*. Human GH concentration in the perfusate of each fraction (5 min) was shown. Each peptide was applied as a 10 min pulse. (a) patient 1. (b) patient 2.

Results

As shown in Fig. 1, Ucn (10 nM) elicited an increase in GH release from GH-producing pituitary tumor cells in both patients 1 and 2. CRF (10 nM) also elicited comparable increase in GH release in patient 1, while the peptide failed to stimulate GH secretion in patient 2. Addition of TRH (10 nM) and GRH (0.1 nM) resulted in an increase in GH release from pituitary tumor cells in patient 1 but not in patient 2.

Discussion

Abnormalities in the GH response to provocative stimuli are a common feature in patients with a GH-producing adenoma. It is known that CRH [6] as well as TRH [7] and GnRH [8] induces paradoxical increase of GH secretion in some patients with acromegaly. These responses could be attributed to an expression of receptors for these peptides associated with dedifferentiation of pituitary adenoma cells [9].

In this experiment, we first found that Ucn increased GH release from GH-producing human pituitary adenoma cells *in vitro*. In one case, CRH and Ucn elicited comparable effects on GH release, while only Ucn but not CRH was effective for stimulating GH release in the other case. Rat Ucn was used in the present study. There are only two amino acid differences between human Ucn and rat Ucn [2]. In addition, the two peptides were almost equally potent to bind to CRH receptors, to accumulate cyclic AMP, and to stimulate ACTH release from rat pituitary [2]. It is plausible, therefore, that the observed effect of rat Ucn might reflect that of human Ucn.

Type-1 CRH receptor is expressed in the pituitary and involved in ACTH secretion. Type-2 CRH receptor was identified in rat brain [3]. It was reported that binding affinities of Ucn and of CRH to type-1 CRH receptor were comparable, while Ucn was a more potent ligand than CRH for CRH type-2 receptors [1, 2]. Taken together, it is suggested that Ucn acted to stimulate GH release from the adenoma cells through type-1 CRH receptor in patient 1 and through type-2 CRH receptor in patient 2, respectively.

In summary, our present findings suggest that type-2 CRH receptor is expressed in some population of GH-producing human pituitary adenoma cells and that Ucn might be involved in GH secretion from tumorous tissues in patients with acromegaly.

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References

1. Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale W (1995) Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 378: 287–292.
2. Donaldson CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE, Vaughan JM, Vale WW (1996) Cloning and characterization of human urocortin. *Endocrinology* 137: 2167–2169.
3. Lobenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T (1995) Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 92: 836–840.
4. Yamauchi K, Murakami Y, Koshimura K, Nishiki M, Tanaka J, Kato Y (1996) Involvement of pituitary adenylate cyclase-activating polypeptide in growth hormone secretion induced by serotoninergic mechanisms in the rat. *Endocrinology* 137: 1693–1697.
5. Murakami Y, Ohshima K, Moshizuki T, Yanaihara N (1993) Effect of human galanin on growth hormone prolactin, and antidiuretic hormone secretion in normal men. *J Clin Endocrinol Metab* 77: 1436–1438.
6. Pieters GFFM, Hermes ARMM, Smals AGH, Kloppenborg PWC (1984) Paradoxical responsiveness of growth hormone to corticotropin-releasing factor in acromegaly. *J Clin Endocrinol Metab* 58: 560–562.
7. Irie M, Tsushima T (1972) Increase of serum growth hormone concentration following tyrotropin-releasing hormone injection in patients with acromegaly or gigantism. *J Clin Endocrinol Metab* 35: 97–100.
8. Rubin AL, Levin SR, Bernstein RI, Tyrrell JB, Noacco C, Forsham PH (1973) Stimulation of growth hormone by luteinizing hormone-releasing hormone in active acromegaly. *J Clin Endocrinol Metab* 37: 160–162.
9. Ishibashi M, Hara T, Tagusagawa Y, Fukushima T, Numata H, Hori T, Yamaji T (1984) Effects of ovine corticotropin-releasing factor and hydrocortisone on growth hormone secretion by pituitary adenoma cells of acromegaly in culture. *Acta Endocrinol* 106: 443–447.