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Effects of Oncostatin M on Hormone Release of Rat Pituitary Cells in Primary Culture

It has become increasingly clear that cytokines play an important role in modulating neuroendocrine regulation, especially in the secretion of corticotropin (ACTH) in the pituitary. Oncostatin M (OSM), a cytokine of IL-6 family has been reported to increase ACTH secretion and pro-opiomelanocortin (POMC) transcription in murine corticotroph pituitary tumor cells (AtT20 cells). The present study was undertaken to determine the effects of OSM on hormonal release in primary culture of rat pituitary cells. Growth hormone or prolactin release was not affected by OSM. OSM (1 nM) stimulated ACTH release (35.1% increase versus control, $p<0.001$) in dispersed pituitary cells of rat to a lesser extent than in AtT20 cells. Corticotropin releasing hormone (CRH) (10 nM) also induced a 2.3-fold increase of ACTH secretion ($p<0.001$), but co-treatment of OSM and CRH did not exhibit any synergistic effect on ACTH secretion. We conclude OSM has a stimulatory effect on ACTH secretion in normal rat pituitary cell cultures, and OSM acts mainly on corticotroph, supporting the potential role of OSM to modulate immune-endocrine regulation in the pituitary.

Key Words: *Cytokines, Oncostatin M; Pituitary Gland; Corticotropin*

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INTRODUCTION

Several cytokines are recognized to play an important role in modulating the immune and neuroendocrine systems. Interleukin-6 (IL-6) has been reported to stimulate hypothalamic-pituitary-adrenal axis in vivo (1, 2) and in vitro (3-5). Leukemia inhibitory factor (LIF), a member of IL-6 family, has also been reported to enhance adrenocorticotropin (ACTH) secretion in murine corticotroph tumor cells (AtT20 cells) with the induction of pro-opiomelanocortin (POMC) gene transcription (6). Recently, LIF gene expression was demonstrated in adult rat pituitary gland and LIF was reported to increase ACTH secretion in normal pituitary cultured cells (7).

Oncostatin M (OSM), a glycoprotein of *M*r. approximately 28,000 produced by macrophage and activated T lymphocytes, has diverse biologic effects, including growth inhibition of melanoma and other solid tumors (8, 9). OSM is structurally related to IL-6, LIF and ciliary neurotrophic factor. OSM and LIF have significant similarities in primary amino acid sequence and bind to the same receptor with high affinity as well as mediate an overlapping spectrum of biological activities (10, 11).

Recently, OSM and LIF have been reported to have the same ability to increase ACTH secretion and POMC mRNA level in AtT20 cells (12). However, the effect of OSM on hormone secretion has not been evaluated in normal pituitary cultured cells. Therefore, we studied the direct influence of OSM on hormonal secretion in monolayer primary cultures of rat anterior pituitary cells.

MATERIALS AND METHODS

Cell culture and experimental procedure

Male Wistar-Furth rats (Harlan Co., Indianapolis, IN, U.S.A.) aged 4 to 8 weeks were decapitated and their pituitary glands were removed. The pituitary glands were minced into 1- to 2-mm pieces and were enzymatically dissociated, using 0.35% collagenase and 0.01% hyaluronidase. The dispersed cells were seeded in six-well plates (3.3 cm in diameter) at a density of 5×10^5 cells/well in minimal essential media-D-valine (MEM-D-valine medium) enriched with 10% fetal calf serum (FCS). MEM-D-valine medium was used to restrict fibroblast growth, and the cells were maintained at 37℃ in a humidified atmosphere of 95% air-5% CO_2. After 48 hr of incubation, the culture medium was changed to serum-free defined medium with test materials. Serum-free defined

medium consisted of 1 liter MEM-D-valine, containing BSA (0.2%), T3 (0.6 nM), insulin (1 μg/L), transferrin (10 mg/L), glucagon (10 ng/L), bovine parathyroid hormone (0.2 g/L), FGF (0.2 μg/L), EGF (0.1 μg/L), penicillin (50,000 U/L), streptomycin (50 mg/L) and glutamine (2 mM). Materials for tissue culture such as BSA, penicillin, streptomycin, collagenase, hyaluronidase, transferrin, 3,3',5-tri-iodo-L-thyronine, PTH, insulin, fibroblast growth factor, epidermal growth factor and glucagon were obtained from Sigma (St. Louis, MO, U.S.A.). MEM-D-valine and FCS were purchased from Gibco BRL (Grand Island, NY, U.S.A.).

At the end of 24-hr incubation with recombinant OSM (R&D systems Inc., Minneapolis, MN, U.S.A.) or CRH (American Peptide Company, Santa Clara, CA, U.S.A.), the media were taken from each wells and stored at -20℃ until measurement of ACTH, growth hormone (GH) and prolactin (PRL) levels. All test incubations were performed in triplicate or quadruplicate, and six independent experiments were performed.

Hormone determinations & statistical analysis

ACTH was determined in duplicate, using a RIA kit purchased from Nichols Institute (San Juan Capistrano, CA, U.S.A.). The concentration of rat GH and PRL was also measured in duplicate by RIA, using reagents kindly provided by the National Hormone and Pituitary Program, NIDDK (Bethesda, MD, U.S.A.). Sensitivity of ACTH, GH and PRL assays were 5.5 pg/mL, 1.56 ng/mL, and 4.0 ng/mL, respectively. Hormonal concentrations from individual wells treated with OSM or CRH were compared to those from the control wells treated with an equal volume of medium alone, and the results of the OSM or CRH treatment were expressed as the difference from control culture whose secretion was expressed as 100%.

All data are presented as mean±SD. The difference between group means was analyzed by unpaired t-test.

RESULTS

Dispersed anterior pituitary cells were cultured in monolayer and then incubated in serum-free medium with OSM (1 nM), or CRH (10 nM) for 24 hr. The optimal concentrations of OSM and CRH showing maximal effect on ACTH secretion in AtT20 cells have been reported to be 1 nM and 10 nM, respectively (6), and so the same concentrations were applied in the present study.

Effect of OSM on ACTH secretion in the primary cultured rat pituitary cells

OSM significantly increased ACTH secretion by 35.1 ±26.0% compared to the control wells ($p<0.01$). CRH used as a positive control also caused about a 2.3-fold increase in ACTH secretion ($p<0.01$), but the addition of OSM to CRH did not show a synergistic effect (248.1% of control) (Fig. 1).

Effect of OSM on GH and PRL secretion in the primary cultured rat pituitary cells

OSM did not show any statistically significant change

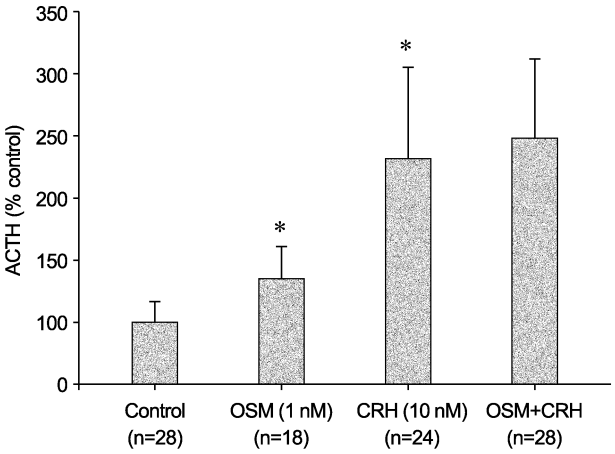

Fig. 1. ACTH release by rat AP cell monolayers treated with OSM or CRH. *$p<0.05$ compared to control. Values are means of 6 independent experiments; each experimental condition was performed in three to four wells. N means the numbers of total wells treated with OSM or CRH. OSM, oncostatin M; CRH, corticotropin releasing hormone; AP cell, anterior pituitary cell

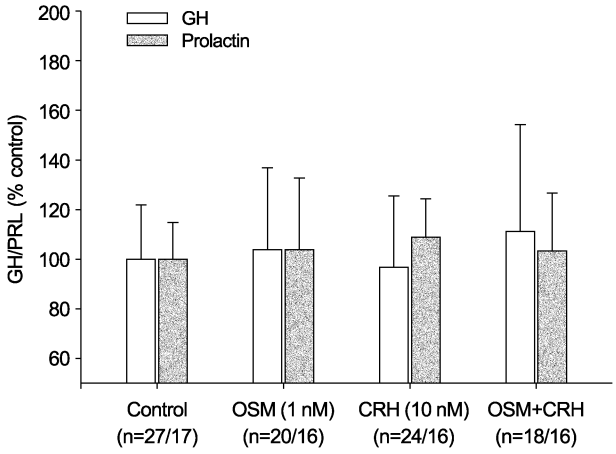

Fig. 2. GH and PRL release by rat AP cell monolayers treated with OSM or CRH. Values are means of 6 independent experiments; each experimental condition was performed in three to four wells. N means the numbers of total wells treated with OSM or CRH. OSM, oncostatin M; CRH, corticotropin releasing hormone; AP cell, anterior pituitary cell

in GH or PRL secretion. GH level was 103.8% and PRL level was 103.8% relative to those of the control well (100%) when treated with OSM. CRH did not significantly increase GH or PRL secretion either, 96.8% and 108.9%, respectively (Fig. 2).

DISCUSSION

Inflammatory cytokines such as interleukin-1, tumor necrosis factor α and IL-6 have been reported to activate the hypothalamic-pituitary-adrenal (HPA) axis (13-15). The regulation by IL-6 on anterior pituitary hormone secretion has been previously described by several groups. In vivo, administration of recombinant IL-6 into the third ventricle of freely moving, conscious male rats induced increase of plasma ACTH (3), and injection of IL-6 in patients with cancer caused marked and prolonged elevations of plasma ACTH and cortisol (2). IL-6 also stimulated the release and synthesis of ACTH in the AtT20 cells in vitro (5).

OSM, a cytokine tested in the present study, is a member of a recently defined family of functionally and structually related cytokines, including IL-6, IL-11, LIF and ciliary nerve neurotrophic factor. They share a common gp130 receptor subunit, and OSM acts through a specific receptor subunit forming a heterodimeric complex with gp130 (16, 17). Akita et al. (12) reported enhanced ACTH secretion to 160-180% of basal ACTH level with the treatment of LIF or OSM, inducing the gene transcription of POMC in AtT 20 cells. The combination of CRH with either LIF or OSM in AtT 20 cells resulted in a synergistic enhancement of the ACTH response. Ray (6) and Stefana (18) also reported a similar response of ACTH secretion to OSM (up to 6-fold increase) and enhancement of POMC transcription (1.6 fold). However, all these data evaluating OSM action on ACTH secretion came from murine corticotropic pituitary tumor cell line (AtT-20 cell). Recently, we found LIF also stimulated ACTH release (143.7% of control) in the anterior pituitary cells of rat (7), and we now provide another evidence supporting OSM action on ACTH secretion in primary cultured pituitary cells.

In the present study, OSM significantly increased ACTH secretion compared to the control in primary pituitary cultures, but to a lesser extent than in the case of murine corticotrophic tumor cell line. CRH also induced ACTH secretion to a 2.3 fold of control, but showed no synergistic effect on ACTH secretion with OSM. These results suggest there may be some differences in ACTH response to OSM and CRH between primary culture of pituitary cells and corticotropic tumor cell lines, and it is conceivable that these differences may result from the heterogenous population of cells or cell-to-cell interaction in primary culture (19).

The significance of enhanced ACTH release in response to OSM and LIF has been established. These cytokines mediate the endocrine response to endotoxic shock or other stresses. Wang (20) reported LIF mRNA and LIF receptor mRNA were induced in vivo in response to lipopolysaccharide endotoxin administration. This observation indicates that these cytokines play a role in mediating immune-neuroendocrine interface within the pituitary. In addition, the OSM treatment in our study showed flat response to GH and PRL levels. A similar result was also recently reported for LIF (7). These results support evidence that OSM selectively acts on corticotroph in the pituitary.

In conclusion, OSM selectively acts on corticotroph and enhances ACTH secretion in dispersed anterior pituitary cells. These findings raise the possibiltiy that OSM acts as an inflammatory cytokine which has the potential to regulate pituitary function.

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