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Estradiol and corticosterone stimulate the proliferation of a GH cell line, MtT/S
Proliferation of growth hormone cells

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ABSTRACT

Objectives: Estrogens are known as a potent growth-stimulator of the anterior pituitary cells such as prolactin cells and somatomammotroph cell lines, while glucocorticoids often inhibit cellular proliferation in the pituitary gland as well as in the extra-pituitary tissues. In this study, the involvement of these steroid hormones in the regulation of proliferation was examined in the MtT/S cells, secreting growth hormone (GH).
Design: Effects of estrogens and glucocorticoids were examined in MtT/S cells grown in the medium containing dextran-coated charcoal treated serum. The relative cell density after culture was estimated by the Cell Titer-Glo Luminescent Cell Viability Assay System, and the proliferation rate was determined by the BrdU incorporation method. The mRNA levels were determined by real-time PCR.
Results: Estradiol and the specific agonist for both estrogen receptor (ER) α and ERβ stimulated MtT/S growth at a dose dependent manner. The membrane impermeable estrogen, 17β-estradiol-bovine serum albumin conjugate also stimulated the MtT/S proliferation. The effects of all estrogens were inhibited by an estrogen receptor antagonist, ICI182780. Corticosterone stimulated the proliferation of MtT/S cells at doses lower than 10 nM without stimulating GH gene transcription, whereas it did not change the proliferation rate at 1 μM. The effects of corticosterone were inhibited by glucocorticoid receptor inhibitor, RU486, but not by the mineralocorticoid receptor antagonist, spironolactone. Both estrogens and glucocorticoids were found to stimulate the proliferation of MtT/S, increasing the mRNA expression of cyclins D1, D3, and E.
Conclusions: The results suggest that estrogens and glucocorticoids may be involved in the mechanisms responsible for the proliferation of GH cells in the course of pituitary development, to maintain the population of GH cells in the adult pituitary gland, and also in the promotion of GH cell tumors.

1. Introduction

Growth hormone (GH) cells develop in the anterior pituitary gland during late gestation in rodents [1,2] and increase in number to constitute approximately 30% of anterior pituitary cells in the mature animal [3]. Several lines of evidences suggest that a hypothalamic hormone, GH-releasing hormone (GHRH), is a major factor for the expansion of GH cell population in the course of pituitary development [4–7], because the excess or depletion of GHRH or its signaling molecules results in the increase or decrease in the GH cell population. However, it is considered that the proliferation of anterior pituitary cells is regulated by the multiple factors including not only the hypothalamic releasing hormones, but also growth factors and the hormones secreted by the peripheral endocrine glands [8–10], and it is likely that factors other than GHRH are also involved in the regulation of the GH cell population. Although the involvement of estrogen [11] and epidermal growth factor [12] in the proliferation of GH cells has been reported in the monolayer culture of rat pituitary and a cell line of the immature GH cells, respectively, the knowledge of the factors that regulate the proliferation of GH cells are not understood well. On the other hand, the mechanisms for the proliferation of prolactin (PRL) cells and somatomammotrophs, both of which are closely related to GH cells with respect to the cell lineage, have been examined more extensively than GH cells. Estrogen has been shown to have a potent stimulatory effect on the growth of PRL cells and somatomammotrophs [13–15]. Recently, CXCL12 [16] and ghrelin [17] have been reported to stimulate the proliferation of GH3, a somatomammotroph cell line, through the activation of Erk1/2. Furthermore, bone morphogenetic protein-4 [18], insulin-like growth factor-I [3] and TGFβ [19] stimulated, and somatostatin [20] inhibited the GH3 proliferation.

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In this study, the effects of hormonal and growth factors on the proliferation of a GH cell line MtT/S [21] was examined. The MtT/S cells are mono-hormonal GH cells derived from the pituitary tumor induced by estrogen in the rat. The results indicate that estrogens and glucocorticoids stimulate proliferation of MtT/S cells by inducing G1 cyclins at the doses lower than those effective in their genomic effects. The results also suggest a possible involvement of membrane bound estrogen receptor in the estrogen-induced MtT/S proliferation.

2. Materials and methods

2.1. Chemicals

Culture medium (DMEM/F12 with or without phenol red) and the sera were obtained from Invitrogen (Groningen, The Netherlands). Corticosterone and 17β-estradiol were obtained from Sigma-Aldrich (St Louis, MO). The specific agonists for estrogen receptor (ER) α (PPT), and for ERβ (DPN), the ER antagonist (ICI182780), the glucocorticoid receptor antagonist (RU486) and the mineralocorticoid receptor antagonist (spironolactone) were from TOCRIS Bioscience (Bristol, UK). Bovine serum albumin conjugated 17β-estradiol hemisuccinate (E2BSA, Steraloids, Newport, RI), and corticosterone hemisuccinate-BSA conjugate (CSBSA, Steraloids) were dissolved in distilled water and extracted with dextran-coated charcoal to eliminate possible contamination of unconjugated free steroids, followed by extensive dialysis against Dulbecco's phosphate buffered saline (PBS, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) for 24 h.

2.2. Cell culture

The MtT/S cell is a gift from Dr. Kinji Inoue, Saitama University, Saitama, Japan. The MtT/S cell line is a GH cell line established from a mammotropic pituitary tumor in the rat, secreting only growth hormone with a negligible level of prolactin expression [21]. The cells were grown in poly-L-lysine coated dishes in the control medium (CM, DMEM/F12 containing 10% horse serum, 2.5% fetal bovine serum and penicillin and streptomycin (all from Invitrogen). Prior to the experiments, the cells were seeded onto a 24-well plate (for RNA extraction) or a 48-well plate (for the determination of cell number) coated with poly-L-lysine. The medium for the experiments (DCC-medium) was the same composition as CM, but made up with the serum which had been extracted with dextran-coated charcoal, and DMEM/F12 which did not contain phenol red. The cells were seeded at different densities as indicated and the hormones or growth factors were added to the culture after 6 h-incubation. The cells were incubated for 4 days unless otherwise stated. The each experiment was repeated at least 3 times and the representative data were shown.

2.3. Determination of cell number

In the experiment of Fig. 1A, the cells were dispersed by trypsin digestion and counted by a hemocytometer. In other experiments, the cells were lysed by cell culture lysis buffer (Promega, Madison, WI), and the relative cell number was determined by the Cell Titer Glo reagent for cell viability assay according to the protocol. The luminescence was determined by a luminometer (Turner Biosystems, Sunnyvale, CA).

2.4. 5′-Bromodeoxyuridine (BrdU) incorporation

The cells were seeded on poly-L-lysine coated-coverslips (9 mm in diameter) placed in a 24 well plate and incubated for 6 h in a DCC-medium, followed by overnight incubation in the same medium containing test steroids. The BrdU was added to the culture (10 μM, final concentration), and the cells were fixed after 2 h incubation in 1:3 mixture of formalin and saturated aqueous solution of picric acid at 4 °C for 24 h. Then the coverslips were washed with PBS, and sequentially treated with 1% Triton X100/PBS for 15 min, and 1 N HCl in distilled water for 30 min. Then the cover slips were immersed in 5% normal goat serum (NGS, Invitrogen) in PBS for 2 h, and incubated with 1:200 diluted mouse monoclonal antibody against BrdU (BD Biosciences, San Jose, CA) in 5% NGS at 4 °C for 24 h. The BrdU positive cells were visualized by the standard ABC procedure. The number of BrdU positive cells and total cell number were determined on a light micrograph taken at 200×. The mean of the percentages of positive cells calculated in 5 different randomly selected fields was considered as a representative value of a cover slip, and 5 cover slips were used for one group.

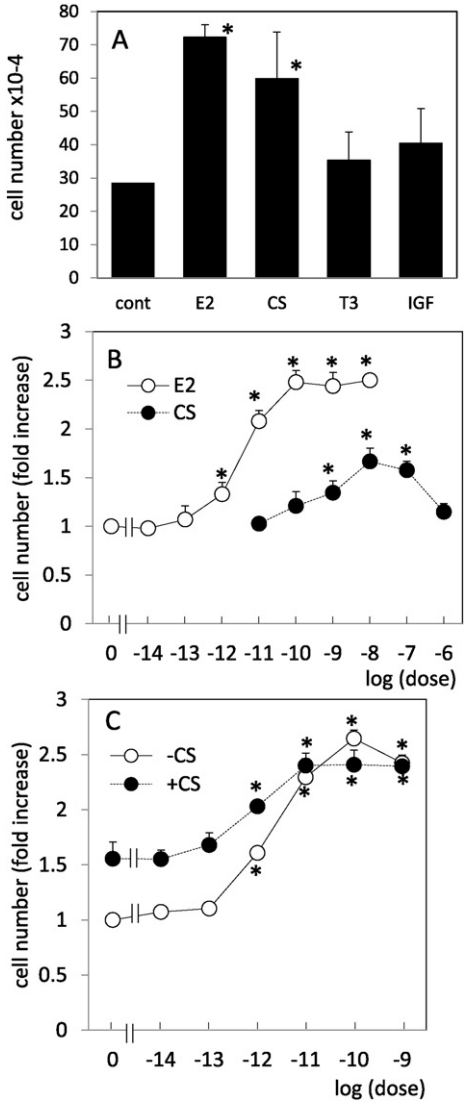

Fig. 1. Corticosterone and estradiol stimulate the proliferation of MtT/S cells in a dose dependent manner. (A) MtT/S cells (2×10^4 cells/well) were seeded onto a 24 well plate, and incubated for 4 days with estradiol (E2, 10 nM), corticosterone (CS, 100 nM), triiodothyronine (T3, 1 nM) or IGFI (IGF, 1 μM). The control cells (cont) were incubated with 0.01% EtOH, the solvent of steroid hormones. In this and the following experiments, the plates were coated with poly-L-lysine, and the cells were incubated in a DCC-medium for 4 days. After the incubation, the cells were harvested by trypsin digestion, and the cell number was determined using a hemocytometer. (B) MtT/S cells were seeded onto a 48 well plate at a concentration of 1×10^4 cells/well, and incubated in a DCC-medium containing different concentrations of E2 or CS for 4 days. After the incubation, the cells were lysed and the relative cell density was determined by the Cell Titer Gro reagent. (C) The effects of different doses of E2 on MtT/S proliferation were determined as described in (B) with or without 100 nM of CS. The values were means ± SEM ($n = 4$). $^*p < 0.05$ *vs* control (cont), or free of the test steroid.