

## Dose-dependent Effects of a Glucocorticoid on Prolactin Production

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**Abstract.** Glucocorticoids are known to stimulate growth hormone (GH) production but to suppress prolactin (PRL) production. However, previous data were obtained with rather high doses of corticosterone. In this study we examined the effects of various doses ( $10^{-12}$ – $10^{-7}$  M) of corticosterone on GH and PRL production in a rat pituitary somatomammotrophic cell line, MtT/SM cells, and found that GH mRNA expression was facilitated by high doses ( $10^{-7}$  and  $10^{-8}$  M). In contrast, a biphasic effect of corticosterone on PRL mRNA expression and secretion was observed, *i.e.*, high doses ( $10^{-7}$  and  $10^{-8}$  M) suppressed and low doses ( $10^{-12}$ – $10^{-10}$  M) facilitated them. In an immunofluorescent staining study, the number of PRL immunopositive cells increased with low doses of corticosterone while it decreased with high doses of it, which corresponded to PRL mRNA expression and hormone secretion, respectively. These effects of corticosterone on PRL production were abolished by a glucocorticoid receptor (GR) antagonist, mifepristone. In addition, co-treatment with low doses of corticosterone ( $10^{-12}$ – $10^{-10}$  M) and  $17\beta$ -estradiol ( $E_2$ , 10 nM) additively increased the number of PRL immunopositive cells. Moreover, a 24 h BrdU incorporation experiment suggested that the increase in the number of PRL immunopositive cells treated with low dose corticosterone was caused by novel synthesis of PRL while, on the other hand, that of those treated with  $E_2$  resulted from PRL cell proliferation. Thus, we concluded that corticosterone biphasically regulates PRL production and the sensitivity of  $E_2$  to different degrees.

**Key words:** Pituitary, Prolactin, Glucocorticoid, Estrogen, Somatomammotrope

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**THE** endocrine cells of the anterior pituitary are classified functionally into five major cell types, *i.e.*, somatotrope, mammotrope, thyrotrope, gonadotrope and corticotrope. It has generally been accepted that these cell types arise from common progenitor cells during development under regulation by lineage-specific tran-

scription factors [1]. Among these lineage-specific transcription factors, pituitary-specific transcription factor 1 (Pit-1) is known to be produced in thyrotrope, somatotrope and mammotrope. Therefore, there might be some similarities among the cells expressing Pit-1. Some studies have confirmed that mammotropes are derived from growth hormone (GH)-producing progenitor cells in rodents [2–5]. In addition, we previously reported that insulin and epidermal growth factor (EGF) caused the transdifferentiation of GH cells into somatomammotrope [6]. These previous reports suggested that PRL cells may arise either from immature

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GH cells or from common progenitor cells. However, the mechanism underlying PRL cell differentiation remains a matter of discussion.

Furthermore, we found that immature GH cells developed from Pit-1 positive cells on stimulation by retinoic acid (RA), and mature GH cells from immature ones on exposure to a glucocorticoid [7]. Several other studies have also shown that glucocorticoids are involved in cell differentiation in rats and chickens, and could promote GH cell differentiation *in vivo* and *in vitro* [8–13]. In addition, glucocorticoid has been shown to suppress PRL cell differentiation and gene expression [14, 15]. In contrast, corticosterone ( $10^{-9}$  M) exposure for more than three days induced PRL production from pituitary cells of chicken embryo [16], which suggests that low doses of corticosterone may have different effects on PRL cell differentiation and PRL production. From this standpoint, we attempted to determine the dose-dependent effects of corticosterone, a principal glucocorticoid, on PRL mRNA expression and PRL production. In this study, we used pituitary clonal cell line MtT/SM, which was derived from an estrogen-induced transplantable rat prolactinoma tumor line [17–19]. MtT/SM cells are known to produce both GH and PRL, which suggests that they are somatomammotrope [18]. Using this cell line, we attempted to determine the dose-dependent effects of corticosterone on rat PRL cells. Here we report a biphasic effect of corticosterone on PRL mRNA expression and production.

## Materials and Methods

### *Cell culture*

MtT/SM cells were established from an estrogen-induced mammotrophic tumor, MtT/F84 [18]. The cells were cultured in phenol-red free DMEM/F12 half medium (Cat. #11039; Life Technologies, Inc., Grand Island, NY, USA) supplemented with charcoal dextran-treated 10% donor horse serum (DHS; Nichimen America, Los Angeles, CA, USA) and 2.5% fetal bovine serum (FBS; Biowhittaker Inc., Walkersville, MD, USA). The cells were incubated in a CO<sub>2</sub> incubator at 37°C under a humidified atmosphere of 5% CO<sub>2</sub> in air.

### *Stimulation with corticosterone and mifepristone*

Cells were seeded onto 48-well culture plates at  $5 \times 10^4$  cells/ml for two days. The cells were exposed to several concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone (Sigma, St Louis, MO, USA) alone or in combination with  $10^{-6}$  M mifepristone (Sigma, St Louis, MO, USA) for three days.

### *Stimulation with corticosterone and 17 $\beta$ -estradiol*

Cells were seeded onto 48-well culture plates at  $5 \times 10^4$  cells/ml, and after two days cultivation, stimulation with several doses of corticosterone was performed for three days with or without 17 $\beta$ -estradiol (E<sub>2</sub>, 10 nM) ( $\beta$ -estradiol-water soluble; Sigma, St Louis, MO, USA).

### *Immunofluorescent staining for GH and PRL*

To detect GH- or PRL-containing cells, we used monkey anti-rat GH serum (a gift from NIH) and rabbit anti-rat PRL serum (HAC-RT26-01RBP85; a gift from Dr. K Wakabayashi). The cells were dispersed enzymatically, allowed to become attached to poly-L-lysine coated culture plates, and then fixed with 10% formalin in phosphate-buffered saline (PBS). The cells were reacted with monkey anti-rat GH serum diluted 1 : 10000 and rabbit anti-rat PRL serum diluted 1 : 2000 for overnight. After rinsing with PBS, the cells were reacted with fluorescence-labeled second antibodies for 2 h. TRITC-labeled goat anti-monkey IgG (EY Laboratories, San Mateo, CA, USA) and Alexa Fluor 488 donkey anti-rabbit IgG (H + L) (Molecular Probes, Inc., Eugene, OR, USA) were used as second antibodies. Samples were mounted with a mounting solution containing 4',6-diamidino-2-phenylindole (DAPI) (VECTASHIELD; Vector Laboratories, Burlingame, CA, USA).

### *Analysis of cell proliferation*

To examine cell growth activity, MtT/SM cells were stimulated with corticosterone and E<sub>2</sub> for 24 h. 5-Bromodeoxyuridine (BrdU; Yamasa Corp., Chiba, Japan) was added to the culture medium simultaneously. After the cultivation, the cells were dispersed enzymatically and incubated on the culture plates, which were treated with poly-L-lysine. The cells were then fixed with 10% formalin in PBS for 30 min. After denatur-

ation of DNA with 4 N HCl, the cells were incubated with monoclonal anti-BrdU antibodies (clone BU-33; Sigma Co.). After rinsing with PBS, the cells were reacted with Alexa Fluor 594 donkey anti-mouse IgG (H + L) (Molecular Probes, Inc., Eugene, OR, USA). Immunofluorescent staining for PRL was performed as described above.

#### *Determination of immunopositive cell numbers*

A sample was observed under a fluorescence microscope (IX-71; Olympus Corporation, Tokyo, Japan) and digital images were obtained with a CCD camera (DP-70; Olympus). Using the digital images, the number of cells labeled with DAPI in a fixed area was determined (total cell number), and then the GH and PRL cell numbers in the same area were determined. More than 500 cells were counted in each treated group ( $n = 3$ ). For BrdU uptake analysis, at least 500 cells in each group ( $n = 4$ ) were counted.

#### *Radioimmunoassay*

The medium concentrations of PRL were measured using a NIDDK kit (NIH, Bethesda, MD, USA) for rat prolactin. The hormone used for iodination was rat prolactin-I-5. The antiserum used was anti-prolactin-S-9. The results are expressed in terms of NIDDK rat prolactin-RP-2. The intra- and interassay coefficients of variation for prolactin were 3.4% and 5.2%, respec-

tively.

#### *Reverse transcriptase-polymerase chain reaction (RT-PCR) and quantitative real time PCR*

Total RNA was isolated using ISOGEN (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol. To digest genomic DNA, total RNA was incubated with DNase (RQ1; Promega, Madison, WI, USA). Complementary DNA was synthesized from 1.5  $\mu$ g total RNA, using Super Script III reverse transcriptase (Invitrogen Corp., Carlsbad, CA, USA). RT-PCR was performed for GH, PRL, mineralocorticoid receptor (MR), GR, estrogen receptor  $\alpha$  (ER $\alpha$ ), and  $\beta$ -actin. PCR was carried out with AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The primers used for PCR amplification, the GenBank accession numbers and the sizes of the products are given in Table 1. Reactions were performed with an i-Cycler (Bio-Rad Laboratories, Hercules, CA, USA). To quantify mRNA expression, we performed quantitative real time PCR. Quantitative real time PCR was performed using SYBR Premix EX Taq (Takara-BIO, Shiga, Japan) according to the manufacturer's protocol. Reactions were then performed using a LightCycler (Roche Diagnostics, Indianapolis, IN, USA). Initial template denaturation was performed for 30 sec at 95°C (denaturation), 20 sec at 60°C (annealing), and 15 sec at 72°C (extension). Forty-five cycles were run and the final cooling step was continued for

**Table 1.**

gene		sequence	product size (bp)
GenBank accession No.			
GH U62779	FWD	5'-CAAGAGGCTGGTGCTTTACC-3'	123
	BWD	5'-AATGTAGGCACGCTCGAACT-3'	
PRL AF022935	FWD	5'-AGACAAGGAACAAGCCCAGA-3'	220
	BWD	5'-TTCAGGATAGGCCTGGCTAA-3'	
GR M14053	FWD	5'-TTCCTGCAGCATTACCACAG-3'	163
	BWD	5'-ACTGCTGCAATCACTTGACG-3'	
MR M36074	FWD	5'-TGTCTCTCCTCCACGACTAGC-3'	111
	BWD	5'-TGTGTGACCTTGAGCCTCTG-3'	
ER $\alpha$ NM012689	FWD	5'-CCACTGACCATGACCATGAC-3'	150
	BWD	5'-GCTGTTGTCCACGTACACCT-3'	
$\beta$ -actin NM031144	FWD	5'-TGGCACCACACTTTCTACAATGAG-3'	105
	BWD	5'-GGGTCATCTTTTCACGGTTGG-3'	

30 sec at 40°C. Quantitative measurement of each mRNA was achieved by establishing a linear amplification curve with serial dilutions of each plasmid containing the amplicon sequence. The amplicon size and specificity were confirmed by melting curve analysis and 2% agarose gel electrophoresis. The indicated values are normalized as to  $\beta$ -actin mRNA.

### Statistical analysis

The data are expressed as means  $\pm$  S.E. Statistical analysis was performed by non-repeated measurement analysis of variance (ANOVA) followed by Bonferroni correction.  $P < 0.05$  was considered to be statistically significant.

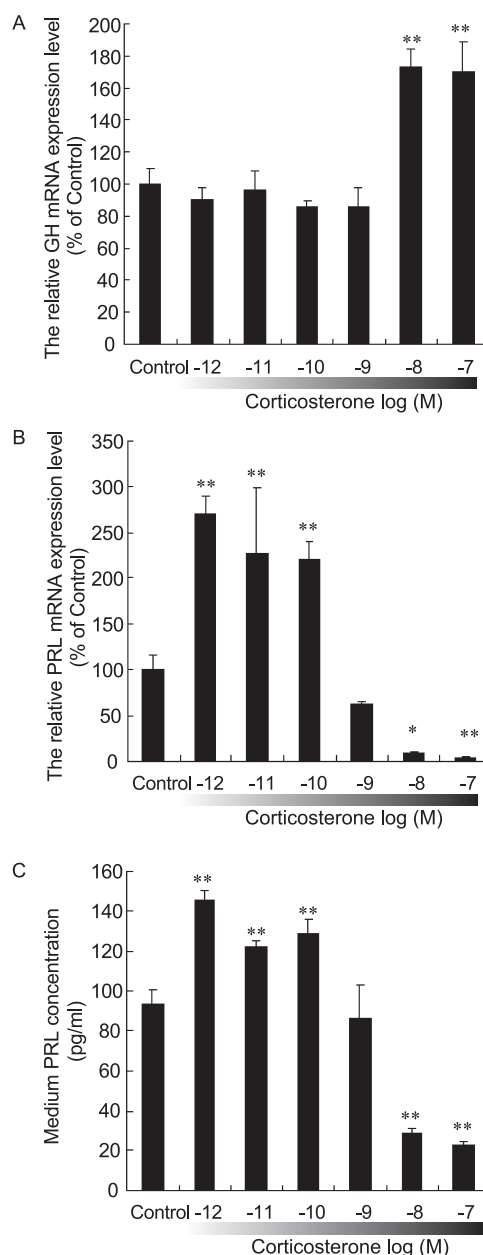
## Results

### Quantitative analysis of GH and PRL mRNA expression, and PRL secretion on MtT/SM cells

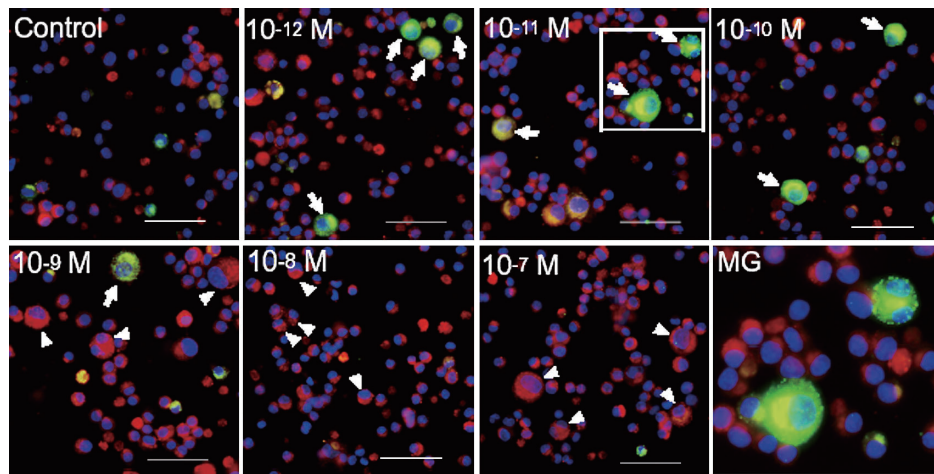
To quantify the changes in GH and PRL mRNA expression caused by corticosterone, MtT/SM cells were treated with various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. As shown in Fig. 1A, high doses ( $10^{-7}$  and  $10^{-8}$  M) of corticosterone significantly increased GH mRNA expression whereas low doses ( $10^{-12}$ – $10^{-9}$  M) had no effect. In contrast, PRL mRNA expression was significantly decreased with high doses ( $10^{-7}$  and  $10^{-8}$  M), but increased with low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone (Fig. 1B). The medium PRL level for MtT/SM cells treated with corticosterone was determined by radioimmunoassay. As shown in Fig. 1C, high doses ( $10^{-7}$  and  $10^{-8}$  M) suppressed the medium PRL level, while low doses ( $10^{-12}$ – $10^{-10}$  M) increased it.

### Double immunofluorescent staining for GH and PRL on MtT/SM cells treated with several doses of corticosterone

Typical appearances of MtT/SM cells treated with several concentrations of corticosterone and non-treated cells are represented in Fig. 2. The cells were visualized with both rabbit anti-rat PRL antibodies (*green*) and monkey anti-rat GH antibodies (*red*). *Blue* indicates the nuclear staining with DAPI (see Materials and Methods). As shown in Fig. 2, cells mainly producing



**Fig. 1.** Quantitative analysis of GH and PRL mRNA expression, and PRL secretion on MtT/SM cells. MtT/SM cells were treated with the vehicle (Control) or various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. Quantitative real time PCR was performed for GH (A) and PRL (B). Values were normalized as to the  $\beta$ -actin mRNA expression level in each sample. The results shown are means  $\pm$  S.E. (C) The medium PRL levels for MtT/SM cells treated with corticosterone were determined by radioimmunoassay. Values are expressed as means  $\pm$  S.E. Significant differences compared with control values are denoted by asterisks (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ ).



**Fig. 2.** Typical appearance of MtT/SM cells with or without treatment with corticosterone. Double immunofluorescent staining for GH (red) and PRL (green) was performed for MtT/SM cells treated with or without corticosterone. Blue indicates nuclear staining with DAPI. Bars, 50  $\mu$ m. Arrows indicate cells mainly producing PRL with mature granules. Arrowheads indicate cells mainly producing GH with mature granules. MG; magnified image of Cort  $10^{-11}$  M (indicated the boxed area).

GH with dotted immunoreactivity were predominantly found in the high dose corticosterone-treated groups, while cells mainly producing PRL with dotted immunoreactivity were mostly found in the low dose corticosterone-treated ones. Typical appearance of dotted immunoreactivity was shown in Fig. 2 MG.

*Expression level of mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) mRNA on MtT/SM cells treated with several doses of corticosterone*

It is well known that corticosterone binds both MR and GR. To quantify the changes in MR and GR mRNA expression caused by corticosterone, MtT/SM cells were treated with various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. We then performed quantitative real time PCR. As shown in Fig. 3, high doses ( $10^{-7}$  and  $10^{-8}$  M) of corticosterone induced both MR and GR mRNA expression significantly. However, low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone only induced GR mRNA expression significantly.

*Effect of a glucocorticoid receptor antagonist on corticosterone-induced PRL production*

To determine the specific effect of corticosterone on the GR, we used the GR antagonist mifepristone (RU486). Cells were exposed to several doses ( $10^{-12}$ – $10^{-7}$  M) of corticosterone alone or in combination with  $10^{-6}$  M mifepristone for three days. We performed no

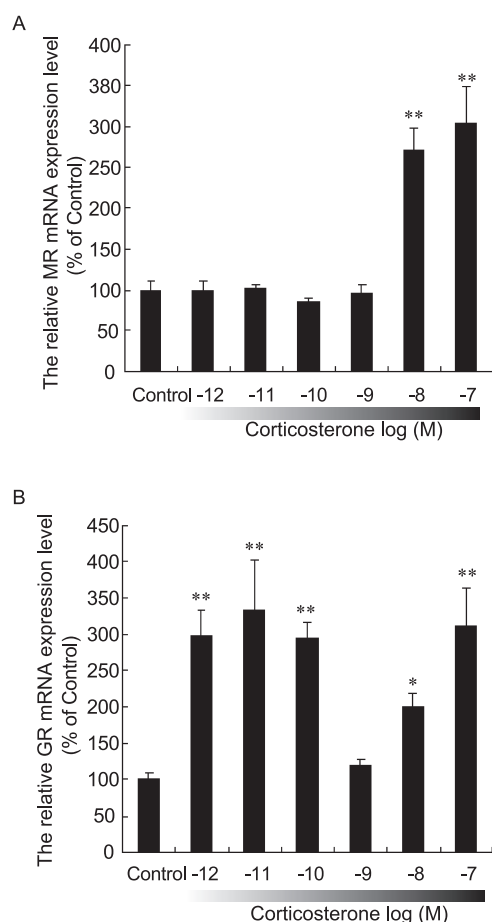
medium change for three days. These cells were stained with anti-rat PRL antibodies and the immunopositive cell number was determined. As shown in Fig. 4, mifepristone significantly inhibited the change in the number of PRL immunopositive cells caused by corticosterone.

*Expression level of estrogen receptor  $\alpha$  (ER $\alpha$ ) mRNA on MtT/SM cells treated with several doses of corticosterone*

Since it is well known that  $17\beta$ -estradiol ( $E_2$ ) can affect PRL production and cell differentiation, we examined whether or not corticosterone could affect the estrogen receptor  $\alpha$  (ER $\alpha$ ) mRNA expression level. MtT/SM cells were treated with various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. We then performed quantitative real time PCR. A high dose ( $10^{-7}$  M) of corticosterone significantly suppressed the ER $\alpha$  mRNA expression, while a low dose ( $10^{-12}$  M) significantly increased it (Fig. 5). With  $10^{-8}$ – $10^{-11}$  M stimulation, ER $\alpha$  mRNA expression tended to negatively correspond to the corticosterone concentration in a dose-dependent manner.

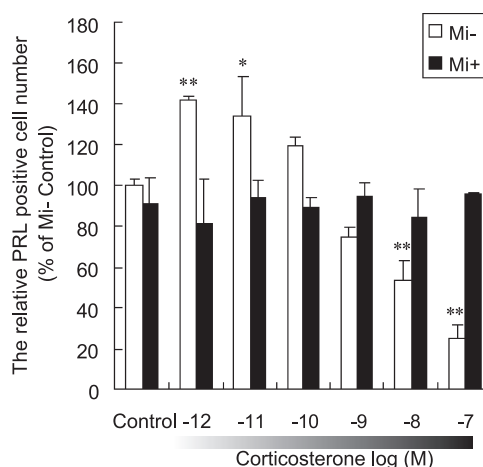
*Additive effects of corticosterone and  $17\beta$ -estradiol on PRL production and PRL cell appearance*

Since low dose of corticosterone increased both ER $\alpha$  mRNA expression and PRL production, respec-



**Fig. 3.** Quantitative analysis of MR and GR mRNA expression on MtT/SM cells treated with corticosterone. MtT/SM cells were treated with the vehicle (Control) or various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. Quantitative real time PCR was performed for MR (A) and GR (B). Values were normalized as to the  $\beta$ -actin mRNA expression level in each sample. The results shown are means  $\pm$  S.E. Significant differences compared with control values are denoted by asterisks (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ ).

tively, we examined the combined effect of  $E_2$  and corticosterone on PRL production and PRL cell appearance. We performed co-stimulation with  $E_2$  and corticosterone for three days. As shown in Fig. 6,  $E_2$  alone increased the number of PRL immunopositive cells, moreover, co-treatment with  $E_2$  and low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone also additively increased the number of PRL immunopositive cells.

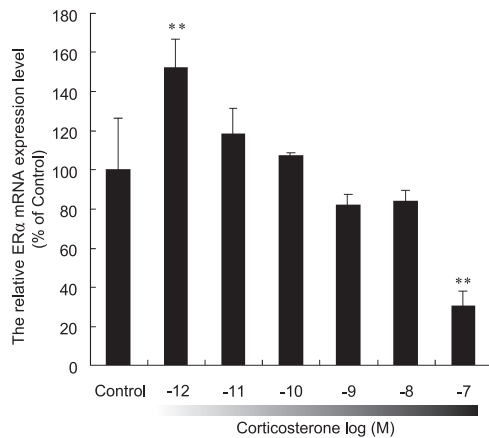


**Fig. 4.** Effect of a glucocorticoid receptor antagonist on corticosterone-induced PRL production. To determine the specificity of the effect of corticosterone on PRL cell differentiation, we used the glucocorticoid receptor (GR) antagonist mifepristone (Mi). MtT/SM cells were exposed to several concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone alone (Mi-, open bars) or in combination with  $10^{-6}$  M mifepristone (Mi+, closed bars) or the vehicle (Control), for three days. After cultivation, the number of PRL immunopositive cells was determined. The values indicated are percentages as to the 'Mi-Cont' group. The results shown are means  $\pm$  S.E. Significant differences compared with control values are denoted by asterisks (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ ).

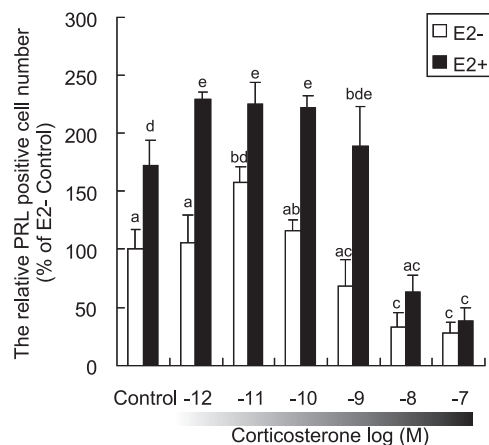
#### *Effects of $E_2$ and corticosterone on PRL cell proliferation*

We also examined whether the increased PRL cell number is due to PRL cell proliferation or novel PRL production by means of a bromodeoxyuridine (BrdU) uptake study. MtT/SM cells were treated with the vehicle (Control), corticosterone (CORT,  $10^{-12}$  M) alone,  $17\beta$ -estradiol ( $E_2$ ; 10 nM) alone, or corticosterone ( $10^{-12}$  M) and  $E_2$  (10 nM) together (CE) for 24 h. BrdU (10  $\mu$ M) was simultaneously added to the culture medium at the start of the treatment. The cells were then prepared for double immunofluorescent staining for PRL and BrdU. Immunopositive cells after performing double immunofluorescent staining are shown in Fig. 7. The number of PRL immunopositive cells was significantly increased in each treated group (CORT, corticosterone  $10^{-12}$  M:  $120.2 \pm 4.4\%$  of Control;  $E_2$ ,  $E_2$  10 nM:  $120.1 \pm 1.4\%$  of Control; CE, corticosterone  $10^{-12}$  M plus  $E_2$  10 nM:  $138.6 \pm 3.6\%$  of Control).

Interestingly, between the control and  $E_2$  treated groups there was no significant difference in the



**Fig. 5.** Quantitative analysis of ER $\alpha$  mRNA expression on MtT/SM cells treated with corticosterone. MtT/SM cells were treated with the vehicle (Control) or various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone for three days. Quantitative real time PCR was performed for ER $\alpha$ . Values were normalized as to the  $\beta$ -actin mRNA expression level in each sample. The results shown are means  $\pm$  S.E. Significant differences compared with control values are denoted by asterisks (\*\*,  $P < 0.01$ ).



**Fig. 6.** Effects of corticosterone and 17 $\beta$ -estradiol on the appearance of PRL cells. MtT/SM cells were treated with the vehicle (Control) or various concentrations ( $10^{-12}$ – $10^{-7}$  M) of corticosterone with E $_2$  (10 nM, E2+, closed bars) or without E $_2$  (E2-, open bars) for three days. After cultivation, the number of PRL immunopositive cells was determined. The values indicated are percentages as to the 'E2- Cont' group. The results shown are means  $\pm$  S.E. Values with the same letters (a–e) are not significantly different ( $P < 0.05$ ).

number of BrdU– PRL+ cells for total PRL immunopositive cells ( $n = 4$ , Control:  $63.0 \pm 4.7\%$ ; E $_2$ :  $65.5 \pm 0.9\%$ ), while the number of BrdU+ PRL+ cells for total PRL immunopositive cells was significantly different ( $P < 0.05$ ;  $n = 4$ , Control:  $37.0 \pm 0.8\%$ ; E $_2$ :  $54.6 \pm 1.0\%$ ). There was a significant difference for BrdU– PRL+ cells, with respect to the effect of corticosterone stimulation ( $P < 0.05$ ;  $n = 4$ , Control:  $63.0 \pm 4.7\%$ ; CORT:  $83.8 \pm 5.4\%$ ). Moreover, the treatment with corticosterone and E $_2$  together had an additive effect on the number of PRL immunopositive cells that resulted from both novel synthesis (corticosterone) and cell proliferation (E $_2$ ), respectively.

## Discussion

Glucocorticoids, steroid hormones, were previously demonstrated to have an inducing effect on GH mRNA expression and GH production both *in vitro* and *in vivo* [10, 11, 14]. On the contrary, glucocorticoids have also been shown to have a suppressing effect on PRL production [14, 15]. However, these previous studies were conducted with rather high doses ( $10^{-7}$  and  $10^{-8}$  M) of glucocorticoids, while only Fu and Porter (2004) used a relatively low dose ( $10^{-9}$  M) of corticosterone, and found an inducing effect on PRL cell differentiation and PRL gene expression in primary cultured chicken pituitary cells [16]. All these previous reports suggest that glucocorticoids might have a bidirectional effect on PRL cell differentiation and/or hormone production. The present study clearly shows that low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone significantly increase PRL mRNA expression, PRL hormone secretion and production through the GR. High doses ( $10^{-7}$  and  $10^{-8}$  M) of corticosterone, however, suppressed PRL mRNA expression and PRL hormone secretion in our study, as reported previously [14, 15]. Notably our data is the first to indicate the bidirectional effects of a glucocorticoid (*i.e.*, used for corticosterone) on PRL mRNA expression and PRL hormone production *in vitro*.

Immunofluorescent staining confirmed an increased number of PRL immunopositive cells among large PRL cells which contain dotted immunoreactivity in the MtT/SM cell line. Those dotted immunoreactivities might correspond to secretory granules. When MtT/SM cells were stimulated with high doses ( $10^{-7}$  and  $10^{-8}$  M) of corticosterone, large mature cells, with a diameter of about 30–40  $\mu$ m (normal diameter, about

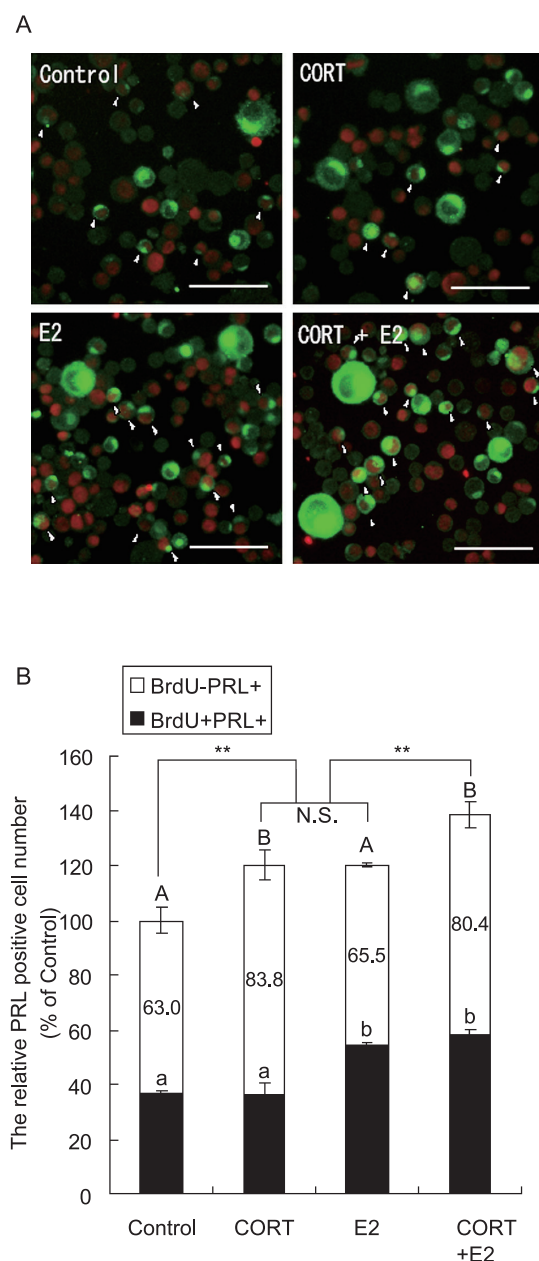


10  $\mu$ m), were mainly stained with anti-GH antibodies. On the other hand, with low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone, large mature cells were mainly stained with anti-PRL antibodies. Therefore, corticosterone seems to induce hormone synthesis by somatomammotrope bidirectionally.

It is well known that the action of corticosterone is mediated through two distinct intracellular transcription factors, MR and GR. These receptors, which are activated by corticosterone, regulate transcription positively and negatively through either direct binding to

DNA or protein-protein interactions [20]. In order to determine the mode of action of corticosterone, we assessed the changes in the expression of both MR and GR mRNA quantitatively in MtT/SM cells, and found that high doses ( $10^{-7}$  and  $10^{-8}$  M) of corticosterone increased both MR and GR mRNA expression significantly. However, low doses ( $10^{-12}$ – $10^{-10}$  M) of corticosterone induced GR mRNA expression significantly but not MR mRNA. To determine whether or not the change in PRL production caused by corticosterone stimulation occurred through the GR, we treated MtT/SM cells with mifepristone, a GR antagonist. The effect of corticosterone on PRL production was completely abolished by mifepristone treatment. This finding indicates that both the inducible and suppressive effects of corticosterone on PRL production occurred through the GR. Generally, it was accepted that corticosterone had a high affinity for MR more than GR, therefore it is conjectured that low doses of corticosterone mainly bind to MR. However, our present study showed that the effect of low doses of corticosterone on PRL production was abolished by GR antagonist, which supports the view that GR may mediate the effects of corticosterone on PRL production. However, further studies are needed to explain this phenomenon.

As it is also known that estrogen affects PRL production and cell differentiation, we examined whether or not corticosterone has any effect on ER $\alpha$ . A high dose ( $10^{-7}$  M) of corticosterone significantly suppressed the ER $\alpha$  mRNA expression, while a low dose



**Fig. 7.** Effects of  $E_2$  and corticosterone on PRL cell proliferation. MtT/SM cells were treated with the vehicle (Control), corticosterone (CORT,  $10^{-12}$  M) alone,  $17\beta$ -estradiol ( $E_2$ ; 10 nM) alone, or corticosterone ( $10^{-12}$  M) and  $E_2$  (10 nM) together (CORT +  $E_2$ ) for 24 h. BrdU (10  $\mu$ M) was added to the culture medium simultaneously. (A) Typical results of double immunofluorescent staining for PRL (green) and BrdU (red). The arrowheads indicate the cells which were double positive for PRL and BrdU. After double immunofluorescent staining, the number of immunopositive cells was determined (B). The values indicated are percentages as to the 'Control' group. The open bars indicate the percentages of BrdU-PRL+ cells and the closed bars indicate the percentages of BrdU+PRL+ cells. The results shown are means  $\pm$  S.E. Values with the same letters (A and B, a and b) are not significantly different ( $P < 0.05$ ). Significant differences compared with control values are denoted by asterisks (\*\*,  $P < 0.01$ ). N.S. = not significant.



( $10^{-12}$  M) of corticosterone significantly increased it. With  $10^{-8}$ – $10^{-11}$  M stimulation, ER $\alpha$  mRNA expression tended to negatively correspond to the corticosterone concentration in a dose-dependent manner. This finding suggests that a low dose of corticosterone can also promote the ER $\alpha$  mRNA expression and thereby support the transdifferentiation of GH-producing cells into PRL-producing cells.

The relationship between  $E_2$  and PRL secretion has been well studied. In mouse, it has been reported that disruption of the ER $\alpha$  gene reduced the PRL cell number and PRL mRNA expression [21]. It is well known that long-term  $E_2$  treatment induces mammatrophic pituitary tumors [22, 23]. Moreover, diethylstilbestrol could induce PRL cell differentiation in the fetal mouse pituitary [24], and promote PRL cell proliferation in the adult pituitary [25, 26]. In this study, we assessed the effect of co-treatment with  $E_2$  and corticosterone on MtT/SM cells, and found that co-treatment with  $E_2$  and corticosterone ( $10^{-12}$ – $10^{-9}$  M) had an additive effect on the induction of PRL-immunopositive cells.

In order to determine how  $E_2$  and corticosterone increase the PRL level, we performed a BrdU uptake study. As revealed in the present study,  $E_2$  and corticosterone increased the PRL level in two distinct ways. Whereas corticosterone increased the number of PRL-positive cells without cell proliferation, increasing the number of somatomammotrope derived from GH cells,  $E_2$  increased the number of PRL-positive cells through

cell proliferation. These data suggest that both  $E_2$  and corticosterone additively affect novel PRL synthesis and PRL cell proliferation. However, because Ozawa *et al.* (1999) have reported that mature PRL cells in the rat pituitary do not express GR [27], this inducible effect of low doses of corticosterone on PRL production might occur on GR-expressing immature somatomammotrope and stimulate their differentiation into mature PRL cells. Indeed, MtT/SM cells expressed both MR and GR mRNA, and the expression of these receptors changed with several doses of corticosterone.

Finally, in this communication, we report for the first time that low doses of corticosterone enhance PRL production by somatomammotrope without cell proliferation. Because of the finding of the stimulatory action of corticosterone toward PRL production *in vitro*, we need to further verify that low doses of corticosterone promotes the ER $\alpha$  mRNA expression involved in the transdifferentiation from somatomammotrope to PRL cells, and thereby mature PRL cells. Therefore, there is need for additional study in this regard.

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