Relative and Combined Effects of Estradiol and Prolactin on Corticosterone Secretion in Ovariectomized Rats

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Abstract

***In vivo* and *in vitro* experiments were designed to assess the relationship of the estradiol (E_2) and prolactin (PRL) on glucocorticoid secretion in ovariectomized (Ovx) rats. Female rats were Ovx for two weeks and then subcutaneously injected with oil or estradiol benzoate (EB) for 3 days before experimentation. Venous blood samples were collected from right jugular vein at 0, 30, 60, 90, and 120 min after challenge with adrenocorticotropin (ACTH). Adrenal zona fasciculata-reticularis (ZFR) cells from Ovx rats were isolated and incubated with E_2 or PRL. In the morning and afternoon, EB enhanced the basal and ACTH-stimulated concentrations of plasma corticosterone (CORT) and PRL. Administration of E_2 *in vitro* increased the basal and ACTH-stimulated release of CORT and production of adenosine 3', 5'-cyclic monophosphate (cAMP) in ZFR cells. E_2 enhanced the forskolin-stimulated release of CORT by ZFR cells. However, the 3-isobutyl-l-methylxanthine (IBMX)- or 8-Br-cAMP-stimulated release of CORT was not affected by E_2. E_2 augmented the lower doses of PRL-stimulated release of CORT and cAMP accumulation as compared with the PRL-treated group alone. Incubation of higher doses of PRL increased the production of cAMP. Administration of nifedipine and R(+) BK8644 (classic L-type Ca^{2+} channel blocker) significantly attenuated the PRL-stimulated release of CORT. Taken together, these data indicate that E_2- and PRL-related increase of CORT in Ovx rats is associated with the increase of cAMP accumulation and calcium influx in ZFR cells. In conclusion, E_2 and PRL play a stimulatory role in the co-regulation of CORT secretion.**

Key Words: E_2, PRL, corticosterone, L-type Ca^{2+} channel, cAMP, ZFR cells

Introduction

Hypoestrogenism is believed to impair the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (4, 9). In aged postmenopausal women, reversal of hypoestrogenism resulting from supplemental estrogens may improve the regulation of the HPA axis (4). We found that the hypersecretion of corticosterone (CORT) induced by estradiol (E_2) treatment is in part due to hyperprolactinemia, increased adrenocorticotropin (ACTH)- and prolactin (PRL)-stimulated CORT release in zona fasciculata-reticularis (ZFR) cells of ovariectomized (Ovx) rats (17). These studies reflect that the HPA axis is modulated by estrogen and PRL (4, 17).

The integrity of serotonergic neurotransmission is essential for the expression of the estrogen-induced afternoon PRL surge (14). It has been reported that a significant diurnal change of tuberoinfundibular dopaminergic neuronal activity is coincident with the estrogen-induced afternoon PRL surge in Ovx and E_2-replaced Ovx rats (22). Clinical studies revealed

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that the incidence of hyperprolactinemia in oral contraceptive users is higher than that in control subjects (18). Hypersecretion of PRL induced by E_2 treatment was highly positive correlated with the higher level of plasma CORT (12). These studies demonstrate that the estrogen plays a key role in the regulation of PRL and CORT (12, 14, 18, 22). In the present study, Ovx rats were used to observe the effect of E_2 plus PRL on CORT secretion.

The purpose of this study is to evaluate: [1] the effects of E_2 on the basal and ACTH-stimulated secretion of plasma CORT and PRL both in the morning and in the afternoon, [2] the relationship between E_2 and PRL on the release of CORT and the possible signal-transduction, i.e., adenosine 3', 5'-cyclic monophosphate (cAMP) formation, and [3] the direct effects of E_2 or PRL on the CORT release, adenylate-cAMP pathway or L-type calcium channel.

Materials and Methods

Animals

Female Sprague-Dawley rats weighing 300-350 g were ovariectomized (Ovx) two weeks prior to experimentation. They were housed in a temperature-controlled room (22 ± 1°C) with 14 h of artificial illumination daily (06:00-20:00). Food and water were given ad libitum.

In Vivo Experiments:

Effects of ACTH on Plasma Corticosterone and Prolactin in Oil- or E_2- Replaced Ovx Rats

The Ovx rats were injected subcutaneously with sesame oil or EB (12.5, 25, 50 μg/ml/kg body weight) once daily for 3 days before experimentation. All rats were anesthetized with ether and catheterized *via* the right jugular vein (15). After 20 h following catheterization, they were intravenously injected with ACTH (5 μg/ml/kg body weight). Blood samples (0.2 ml) were collected from the jugular catheter at 0, 30, 60, 90, and 120 min after the challenge between 0800 and 1400. The lost blood volume was immediately replenished with heparinized saline after each bleeding.

Plasma was separated by centrifugation at 10,000 g for 1 min and stored at -20°C. Plasma (0.1 ml) was mixed with 1 ml diethyl ether (10×vol), shaken for 20 min, centrifuged at 1000 g for 5 min, quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in a buffer solution (0.1 % gelatin in PBS, pH 7.5) before measuring the concentrations of CORT by radioimmunoassay (RIA). The concentration of PRL in plasma was measured by RIA.

In Vitro Experiments:

Preparation of Zona Fasciculata-Reticularis (ZFR) Cells for Cell Culture

Ovx rats were decapitated between 0800-0900 h, the preparation of ZFR cells for culture was performed following a method as previously described (15).

The ZFR cells were incubated with or without hormones or agents dissolved in 1 ml per tube of Krebs-Ringer bicarbonate buffer [3.6 mmol K^+ /l, 11.1 mmol glucose /l] with 0.2% bovine serum albumin (BSA) medium (KRBGA) for 30 min at 37 °C under 95% O_2 and 5% CO_2. To measure the effects of E_2, ovine PRL (oPRL), E_2 plus oPRL on CORT production, ZFR cells of Ovx rats were preincubated for 60 min with KRBGA. After preincubation, the cells were incubated in tubes containing 0.5 ml E_2 (10^{-10} ~ 10^{-7} M, Sigma, USA) or oPRL (10^{-10} ~ 10^{-7} M, Sigma, USA) for 30 min. For studying the relationship of E_2 and oPRL on the adenylate cyclase and accumulation of cAMP, cells were incubated with the medium containing ACTH (10^{-8} M), forskolin (10^{-5} M, an adenylate cyclase activator), 3-isobutyl-1-methylxanthine (IBMX, 5×10^{-4} M, a phospho-diesterase inhibitor), and 8-Br-cAMP (10^{-4} M, a membrane-permeable cAMP) for 30 min. At the end of the incubation period, cells were homogenized in 500 μl of 65% ice-cold ethanol, by polytron (PT-3000, Kinematica Ag, Luzern, Switzerland) and centrifuged at 200 g for 10 min. The supernatants were lyophilized in a vacuum concentrator (SpeedVac, Savant, Instruments, Holbrook, NY, USA) and reconstituted with assay buffer (0.05 M sodium acetate buffer with 0.01 % azide, pH 6.2) before measurement of cAMP concentration by RIA.

To investigate the effects of oPRL (10^{-9} ~ 10^{-7} M) on L-type calcium channel activity, cells were incubated with the classic L-type calcium channel antagonist (nifedipine, 10^{-6} M, or R (+) BK8644, 10^{-6} M) for 30 min. The concentration of CORT in medium was measured by RIA.

RIA of CORT

The concentrations of plasma and medium CORT were determined by RIA as described elsewhere (8, 15) with anti-CORT serum (PSW#4-9), the sensitivity of CORT RIA was 5 pg per assay tube. The intra- and interassay coefficients of variation were 2.9% (n=8) and 4.3% (n=7), respectively.

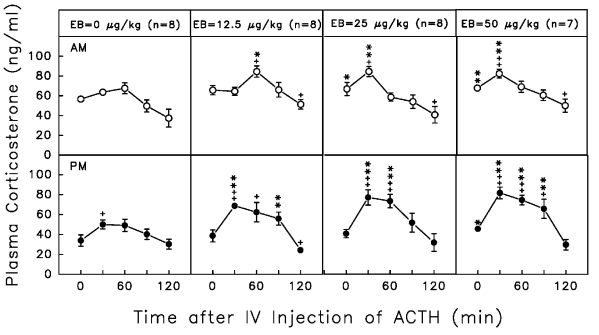

Fig. 1. Effects of a single intravenous (IV) injection of adrenocorticotropin (ACTH; 5 μg/ml/kg body wt) in both the morning (8AM, top) and the afternoon (2PM, bottom) on the plasma corticosterone (CORT) concentrations in oil or EB-replacement ovariectomized (Ovx) rats. Female rats were Ovx for two weeks. Ovx rats were subcutaneously injected with sesame oil or EB (12.5, 25, 50 μg/ml/kg body wt) once daily for 3 days. Blood samples were collected through a jugular catheter at times indicated. *, **, $P < 0.05$, $P < 0.01$ as compared with oil-treated rats at corresponding time, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the corresponding basal value, respectively.

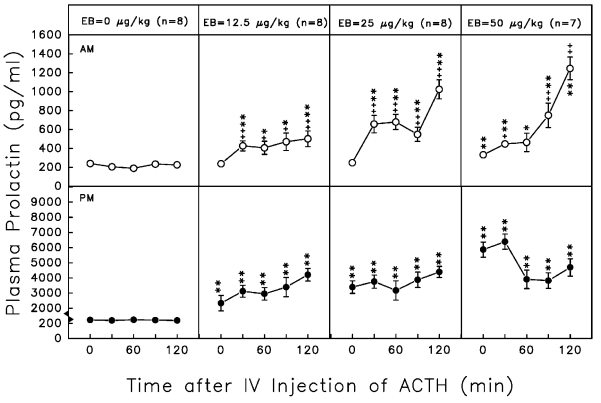

Fig. 2. Effects of a single IV injection of ACTH (5 μg/ ml/kg body wt) in both the morning (8AM, top) and the afternoon (2PM, bottom) on the plasma PRL concentrations in oil or EB (12.5, 25, 50 μg/ml/kg body wt) replaced Ovx rats. Blood samples were collected through a jugular catheter at times indicated. *, **, $P < 0.05$, $P < 0.01$ as compared with the oil-treated rats at corresponding time, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with basal level within each treatment value, respectively.

RIA of PRL

The concentration of plasma PRL was measured by RIA as previously described (21). The rat PRL-I-5 used for iodination and the PRL-RP-3 served as a standard preparation were provided by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the U. S. Department of Agriculture, USA. The sensitivity of rat PRL RIA was 3 pg per assay tube. The intra- and interassay coefficients of variation were 4.1% (n=5) and 5.2% (n=7), respectively.

RIA of cAMP

The concentration of adrenal cAMP was determined by RIA as described elsewhere (15, 16) with anti-cAMP serum No. CV-27 pool. The sensitivity of cAMP RIA was 2 fmol per assay tube. The intra- and interassay coefficients of variation were 4.7 % (n=7) and 5.6 % (n=9), respectively.

Statistical Analysis

All data are expressed as the mean ± SEM. In the *in vitro* studies, the treatment means were tested for homogeneity using an ANOVA, and the difference between specific means was tested for significance using Duncan's multiple-range test (19). In the *in vivo* experiments, Student's *t*-test was employed. A difference between two means was considered statistically significant at a *P* level less than 0.05 or 0.01.

Results

Effects of Intravenous (IV) Injection with ACTH on Plasma CORT in Ovx Rats

Besides oil-injected Ovx rats, a single IV injection of ACTH increased (1.2~2.1-fold) plasma concentrations of CORT at 30, 60, or 90 min compared with the basal level in the same group, both in the morning and in the afternoon ($P<0.05$ or $P<0.01$, Fig. 1, top and bottom).

In the morning and afternoon, EB enhanced the basal and ACTH-stimulated release of plasma CORT ($P<0.05$ or $P<0.01$, Fig. 1, top and bottom).

Effects of IV Injection with ACTH on Plasma PRL in Ovx Rats

Either morning or afternoon, there were EB-dependent increases in basal secretion of plasma PRL ($P<0.05$ or $P<0.01$, Fig. 2, top and bottom).

In the morning but not in the afternoon, EB (12.5, 25 and 50 μg/kg)-treated rats showed the significant elevation in plasma PRL from 30 to 120 min following ACTH challenge in the same group ($P<0.05$ or $P<0.01$, Fig. 2, top).

Effects of E_2 on the Basal and ACTH-Induced CORT Release and cAMP Production in ZFR Cells of Ovx Rats

In vitro administration of E_2 ($10^{-10} \sim 10^{-7}$ M) for

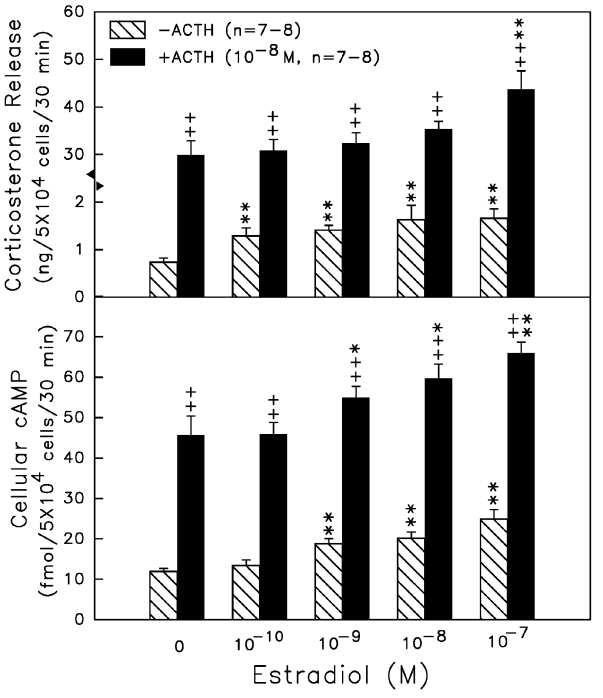

Fig. 3. Effects of estradiol (10^{-10} ~ 10^{-7} M) on the basal and ACTH (10^{-8} M)-stimulated release of corticosterone (top) and cAMP production (bottom) in ZFR cells of Ovx rats. *, **, $P < 0.05$, $P < 0.01$ as compared with the group treated with estradiol = 0 M, respectively. ++, $P < 0.01$ as compared with the group treated with ACTH = 0 M.

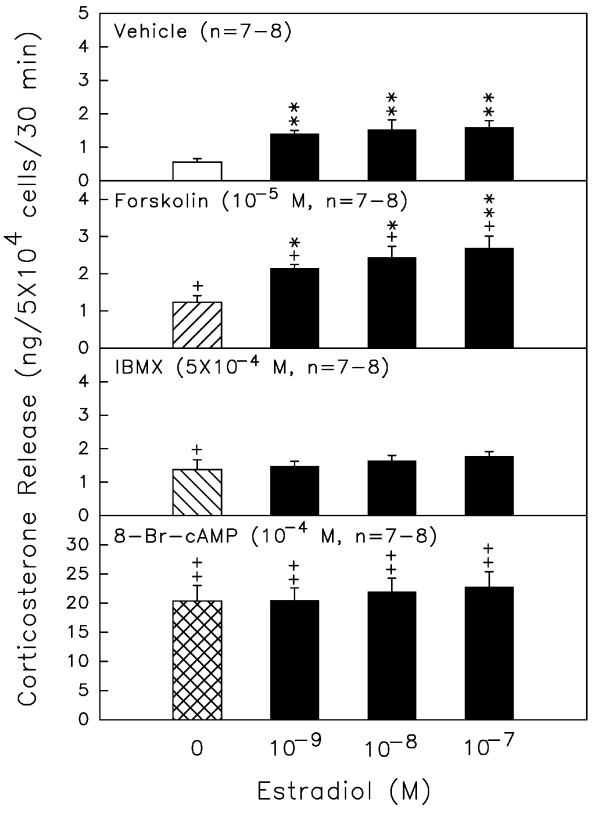

Fig. 4. Effects of estradiol (10^{-9} ~ 10^{-7} M) on the basal, forskolin (10^{-5} M)-, IBMX (5×10^{-4} M)-, and 8-Br-cAMP (10^{-4} M)-stimulated release of CORT in ZFR cells of Ovx rats. *, **, $P < 0.05$, $P < 0.01$ as compared with the group treated with estradiol = 0 M, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the corresponding vehicle group, respectively.

30 min resulted in an increase by 1.8~2.3- and 1.5-fold in basal and ACTH-stimulated release of CORT (P<0.01, Fig. 3, top) and 1.6~2.1- and 1.2~1.4-fold in basal and ACTH-stimulated production of intracellular cAMP (P<0.05 or P<0.01, Fig. 3, bottom).

Effects of E_2 on the Basal, Forskolin-, IBMX- and 8-Br-cAMP-Induced CORT Release in ZFR Cells of Ovx Rats

Incubation of forskolin (10^{-5} M) plus E_2 (10^{-9}~10^{-7} M) for 30 min resulted in a greater CORT release in ZFR cells as compared with the forskolin-treated groups (P<0.05 or P<0.01, Fig. 4).

The IBMX (5×10^{-4} M)- or 8-Br-cAMP (10^{-4} M)-induced CORT release in ZFR cells was not altered by E_2 as compared with the IBMX- or 8-Br-cAMP-treated alone (Fig. 4).

Effects of oPRL on the Basal and E_2-Induced CORT Release and cAMP Production in ZFR Cells of Ovx Rats

In the presence or absence of E_2 (10^{-7} M), oPRL (10^{-10} ~ 10^{-7} M) resulted in a dose-dependent increase in CORT release (P<0.01, Fig. 5 top). However, only higher dose of oPRL (10^{-7} M) or oPRL (10^{-7} M) plus E_2 (10^{-7} M) treatment significantly increased the production of intracellular cAMP as compared to the group of vehicle or E_2 alone (P<0.05 or P<0.01, Fig. 5, bottom).

Administration of E_2 (10^{-7} M) for 30 min enhanced the lower doses of oPRL (10^{-10} and 10^{-9} M)-stimulated release of CORT as compared with the groups treated with oPRL alone (10^{-10} and 10^{-9} M) (Fig. 5, top). E_2 combined with oPRL (10^{-10} and 10^{-9} M) produced a greater accumulation of intracellular cAMP in ZFR cells than the group of oPRL (Fig. 5, bottom).

Effects of Nifedipine or R (+) BK8644 on oPRL-Induced CORT Release in ZFR Cells of Ovx Rats

Administration of nifedipine or R (+) BK8644 for 30 min significantly decreased the release of CORT by 45.2% or 49.6% (P<0.05) compared to the vehicle group (Fig.6, top, middle and bottom). After coincubation of oPRL (10^{-8} or 10^{-7} M) with nifedipine or R (+) BK8644, there was a significant decrease of (P<0.05 or P<0.01) the oPRL-stimulated release of CORT (Fig. 6, top, middle and bottom).

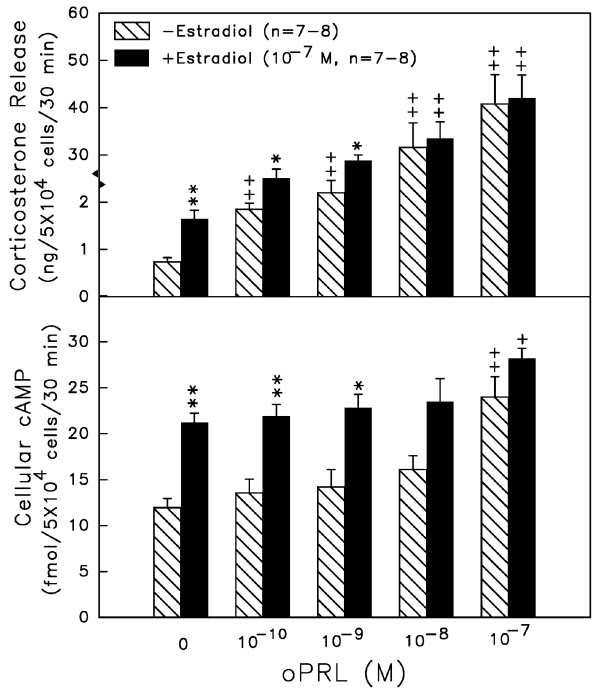

Fig. 5. Effects of oPRL (10^{-10} ~ 10^{-7} M) on the basal and estradiol (10^{-7} M)-stimulated release of CORT (top) and cAMP production (bottom) in ZFR cells of Ovx rats. *, **, $P < 0.05$, $P < 0.01$ as compared with the group treated with estradiol = 0 M, respectively. +, ++, $P < 0.05$ or $P < 0.01$ as compared with the group treated with oPRL= 0 M, respectively.

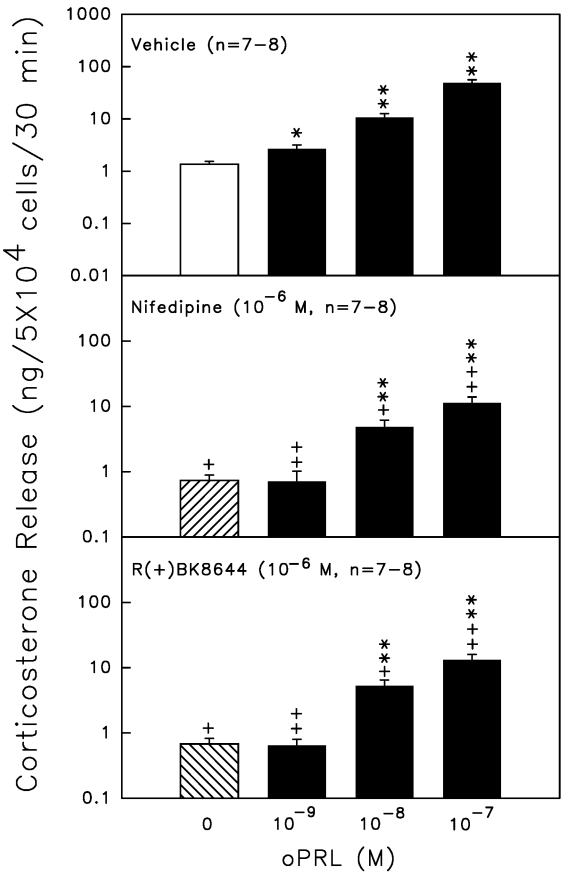

Fig. 6. Effects of oPRL (10^{-9} ~ 10^{-7} M) on the basal (top), nifedipine (10^{-6} M, middle)- and R (+) BK8644 (10^{-6} M, bottom)-induced release of CORT in ZFR cells of Ovx rats. *, **, $P < 0.05$, $P < 0.01$ as compared with the group treated with oPRL= 0 M, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the corresponding vehicle group, respectively. Each value represents the mean ± SEM. Please note log scale on y-axis.

Discussion

Acute estrogen deficit induces changes in the HPA axis characterized by reducing stimulated secretion of ACTH but normal stimulated production of cortisol (10). After long-term estrogen replacement therapy, postmenopausal women had higher total cortisol levels than controls (2, 10). Our previous studies have revealed that after replacement of EB for 3 days in Ovx rats, plasma CORT concentration and basal release of CORT from ZFR cells elevated as compared with oil-treated rats (17). In the present study, we found that estrogen treatment increased the plasma CORT secretion (in the morning and in the afternoon) and the basal release of CORT from ZFR cells of Ovx rats (Figs.1 and 3). These studies reflect that estrogen plays a stimulatory role in glucocorticoid secretion both *in vivo* and *in vitro*.

It has been documented that many substances such as hormones (3, 5, 15), drugs (16), and Chinese herbal medicine (6) also influence the basal and ACTH-stimulated plasma CORT concentration. Previous reports indicated that E_2 treatment increased the synthesis and release of ACTH and CORT (3, 17). The present study demonstrated that administration with E_2 *in vitro* enhanced the basal and the ACTH-stimulated on CORT release and intracellular cAMP production in ZFR cells of Ovx rats. Incubation of forskolin (an adenylate cyclase activator) plus E_2 resulted in a greater CORT release in ZFR cells as compared with the forskolin-treated group. However, the IBMX (a phosphodiesterase inhibitor)- or 8-Br-cAMP (a membrane-permeable cAMP)-induced CORT release in ZFR cells was not altered by E_2. These results indicate that the increase of adenylate cyclase activity may be involved in the stimulation of physiological doses of E_2 on CORT secretion. These results suggest *in vitro* administration of E_2 increases the adrenocortical function in part via an activation of adenylate cyclase-cAMP pathway in ZFR cells of Ovx rats.

Estrogens are the well-known regulators of the expression of anterior pituitary PRL (11, 12). Acute and chronic effects of E_2 on male rats produced a significant increase in plasma PRL and pituitary PRL (2). We confirmed that *in vivo* treatment with EB

resulted in a dose-dependent increase of the basal and ACTH-induced secretion of plasma PRL compared to oil-treated Ovx rats. However, the results of afternoon showed no changes in plasma PRL levels following ACTH stimulation compared to the basal levels of the same group. One possible explanation might be due to the estrogen-induced afternoon PRL surge. Therefore, the higher basal level is difficultly stimulated by ACTH challenge in EB-replaced groups. This result indicates that there is a positive correlation between plasma PRL and CORT under the influence of E_2.

Our previous study had demonstrated that PRL directly increased the production of CORT in rat ZFR cells *via* the activations of cAMP cascades and 3β-hydroxysteroid dehydrogenase (3β-HSD) (5). The EB-related increase of CORT production in Ovx rats is associated with an increase of cAMP generation and the stimulatory effect of PRL in ZFR cells (17). Our present *in vitro* study confirms that administration of E_2 for 30 min enhanced the productions of CORT and intracellular cAMP in the presence of lower doses of oPRL. This study confirms that E_2 and PRL play a stimulatory role in the co-regulation of CORT secretion.

In bovine or rat adrenocortical cells, calcium influx is critical in maintaining a secretory response of aldosterone or glucocorticoid (1, 7, 13). It has been demonstrated that the increase of aldosterone secretion by oPRL is in part due to the activation of both L- and T-type Ca^{2+} channels (13). We found that the PRL-stimulated secretion of CORT was partially reduced by the L-type Ca^{2+} channel blockers, i.e., nifedipine and R (+) BK8644. It implies that the extracellular Ca^{2+} influx might be involved in the secondary messenger-signaling pathway of oPRL on CORT secretion. However, our previous study indicated that the nifedipine did not inhibit the basal or oPRL-stimulated CORT secretion (5). Three factors might attribute to the difference between past (5) and present finding: [1] the concentration difference of nifedipine (the previous 10^{-7} M vs. the present 10^{-6} M), [2] the duration difference for cell incubation (the previous 60 min vs. the present 30 min), and [3] the gender difference (the previous male rats vs. the present Ovx rats). However, we need to perform another new experiment to confirm that E_2 enhanced CORT secretion is associated with the activation of L- or T-type Ca^{2+} channels by PRL. Taken together, these data showed that a direct stimulatory effect of PRL on CORT secretion in adrenal ZFR cells was in part due to the activation of L-type Ca^{2+} channel.

In summary, the increase of CORT secretion by E_2 is due in part to an elevated adenylate cyclase-cAMP activity and an enhanced stimulatory effect of PRL in ZFR cells of Ovx rat.

Acknowledgments

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