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Effects of Estradiol on Corticosterone Secretion in Ovariectomized Rats

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Abstract The effects of estradiol benzoate (EB) on steroidogenesis in rat zona fasciculata-reticularis (ZFR) cells were studied. Female rats were ovariectomized (Ovx) for 2 weeks and then injected subcutaneously with oil or EB for 3 days before decapitation. ZFR cells were isolated and incubated with adrenocorticotropin (ACTH) or prolactin (PRL) for 1 h. Corticosterone concentrations in plasma and cell media, and adenosine 3′,5′-cyclic monophosphate (cAMP) production in ZFR cells were determined by radioimmunoassay. The effects of EB replacement in vivo on the activities of steroidogenic enzymes in ZFR cells were measured by the amounts of intermediate steroidal products separated by thin-layer chromatography. Replacement of EB in vivo resulted in a dose-dependent increase of plasma PRL and corticosterone in Ovx rats. The basal, ACTH-, and PRL-stimulated release of corticosterone by ZFR cells was greater in EB- than in oil-treated animals. Forskolin-induced production of cAMP was greater in the EB-replaced rats than in oil-treated animals, which correlated with the increase of corticosterone production. The 3-isobutyl-l-methylxanthine (IBMX) plus ACTH-, IBMX plus PRL-, and forskolin plus PRL-stimulated productions of cAMP were higher in EB- than in oil-treated rats. The enzyme activities of postpregnenolone were not affected by EB replacement in Ovx rats. These results suggest that the EB-related increase of corticosterone production in Ovx rats is associated with an increase of cAMP generation and the stimulatory effect of PRL on ZFR cells. J. Cell. Biochem. 77:560–568, 2000.

Key words: E2; corticosterone; cAMP; ZFR cells

Estrogen receptors have been shown to be present in the adrenal glands of rodents, and they may have a role in adrenal function [Hirst et al., 1992]. It appears that estrogen can enhance hypothalamic-pituitary-adrenal (HPA) function [Handa et al., 1994]. Postmenopausal women receiving long-term estrogen replacement therapy had higher total cortisol levels than controls (no replacement therapy) [Burleson et al., 1998]. The acute estrogen deficit decreases corticotropin-releasing hormone (CRH)-stimulated secretion of adrenocorticotropin (ACTH) in premenopausal women after ovariectomy [De-Leo et al., 1998]. These studies reflect that the HPA axis is modulated by estrogen in women [Burleson et al., 1998; De-Leo et al., 1998]. Ovariectomy decreases the synthesis and release of ACTH in the pituitary as well as the synthesis of corticosterone in the adrenal [Coyne and Kitay, 1969; Kitay, 1963a]. These effects are reversible by estradiol (E_2) replacement, which subsequently increases both ACTH release and corticosterone secretion [Coyne and Kitay, 1969; Kitay, 1963b]. In the present study, ovariectomized (Ovx) rats were used to observe the in vivo effect of E2 on corticosterone secretion.

Sex differences in the expression of prolactin (PRL) receptors have been observed in a number of tissues, such as the adrenal gland and pituitary [Outhit et al., 1993]. In rats, E_2 treatment increased plasma PRL, corticosterone, and adre-

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nal weights [Gottesfeld and Liehr, 1987]. The hypersecretion of PRL induced by E_2 treatment is highly correlated with the greater levels of plasma corticosterone [Gottesfeld and Liehr, 1987].

It has been demonstrated that the activation of the HPA axis observed during the course of hyperprolactinemia might be explained by a direct stimulatory effect of PRL on both hypothalamic CRH and pituitary ACTH secretion [Weber and Calogero, 1991]. The increased corticosterone secretion during hyperprolactinemia as a possible direct effect of PRL on adrenal steroidogenesis has also been reported [Albertson et al., 1987; Eldridge and Lymangrover, 1984; Glasow et al., 1996].

Numerous studies have examined E_2-related changes of glucocorticoid secretion [Albertson et al., 1987; Coyne and Kitay, 1969; Gottesfeld and Liehr, 1987; Kitay, 1963a,b]. However, the involvement of adenosine 3′,5′-cyclic monophosphate (cAMP) production, the PRL effect, and enzyme activities in steroidogenesis in the regulation of adrenocortical function in Ovx rats by estradiol benzoate (EB) replacement is also unclear. This study was undertaken to evaluate: 1) the effects of E_2 on the secretion of corticosterone both in vivo and in vitro, 2) the possible positive correlations between corticosterone and PRL secretion under the influence of E_2, and 3) the possible correlation between corticosterone and cAMP production or the enzyme activities of steroidogenesis in Ovx rats with different EB-replacement.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing 300–350 g were Ovx 2 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 h). Food and water were given ad libitum. All animal experimentation was conducted in conformance with the policy statement of the Committee of National Yang-Ming University and was conducted humanely.

Effects of EB Replacement on the Concentrations of Plasma E_2, PRL, and Corticosterone in Ovx Rats

The Ovx rats were injected subcutaneously with sesame oil or EB (12.5, 25, or 50 μg/ml/kg body wt) once daily for 3 days before experimentation. Rats were then decapitated between 09:00–10:00 h, trunk blood was collected, and plasma samples were withdrawn, and then separated and stored at −20°C. The concentrations of E_2, PRL, and corticosterone in plasma were measured by radioimmunoassay (RIA).

Plasma (0.1 ml) was mixed with 1 ml diethyl ether (10×volume), shaken for 20 min, centrifuged at 1,000*g* for 5 min, and then 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 E_2 and corticosterone by RIA.

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

An adrenocortical preparation enriched with ZFR cells for culture was performed following a method as previously described [Lo et al., 1998a]. Rat adrenal glands were rapidly excised and stored in an ice-cold 0.9% NaCl solution. The adipose tissues were removed. The encapsulating bands were separated into capsule (mainly zona glomerulosa) and inner-zone (mainly ZFR) fractions with forceps. The fractions of inner zone from rat adrenals were incubated with collagenase (2 mg/ml, Sigma Chemical Co., St. Louis, MO) at 37°C in a shaking water bath, 100–110 strokes/min, for 60 min. The collagenase was dissolved in 2–4 ml of Krebs-Ringer bicarbonate buffer (3.6 mmol K^+/1, 11.1 mmol glucose/l) with 0.2% bovine serum albumin (BSA) medium (KRBGA), pH 7.4. ZFR cells were dispersed by repeated pipetting and filtered through a nylon mesh. After centrifugation at 200*g* for 10 min, the cells were washed in KRBGA medium and centrifuged again. Erythrocytes were eliminated from ZFR cells by washing with 4.5 ml distilled water for a few seconds. The ZFR cells were then mixed with 0.5 ml of 10× Hanks balanced salt solution (pH 7.4). After centrifugation at 200*g* for 10 min, the supernatant was discarded, and the pellet was resuspended in 3 ml of KRBGA solution. An aliquot (20 μl) was used for cell counting in a hemocytometer after staining with 0.05% nigrasin stain. Cells in culture medium were further diluted to a concentration of 5×10^4 cells/ml and divided into the test tubes.

The ZFR cells were incubated with or without hormones dissolved in 1 ml per tube of KRBGA medium for 60 min at 37°C under 95% O_2 and 5% CO_2. To measure the effects of ACTH and PRL on corticosterone production with different EB-replaced Ovx rats, ZFR cells were preincubated for 60 min with KRBGA medium. After preincubation, the cells were incubated in tubes containing 0.5 ml ACTH (10^{-10} and 10^{-9} M, Sigma) or ovine PRL (oPRL, 10^{-8} and 10^{-7} M, Sigma). For studying the in vitro effect of different EB replacement (12.5, 25, or 50 μg/ml/kg body weight) on the adenylyl cyclase and accumulation of cAMP, cells were incubated for 60 min with the medium containing forskolin (10^{-6} M) or 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM). After priming with forskolin or IBMX, the cells were incubated for 60 min in tubes containing 0.5 ml forskolin or IBMX in the presence or absence of hormones, such as ACTH (10^{-10} and 10^{-9}M) or oPRL (10^{-8} and 10^{-7} M). At the end of the incubation period, cells were homogenized in 500 μl of 65% ice-cold ethanol, by polytron (PT-3000, Kinematica Ag, Lucerne, Switzerland), and centrifuged at 200*g* for 10 min. The supernatants were lyophilized in a vacuum concentrator (SpeedVac, Savant, Instruments, Holbrook, NY) and reconstituted with assay buffer (0.05 M sodium acetate buffer with 0.01% azide, pH 6.2) before measuring the concentration of cAMP by RIA.

To measure the effects of EB replacement on the 3β-hydroxysteroid dehydrogenase (3β-HSD), 21β-hydroxylase, and 11β-hydroxylase activities, ZFR cells were incubated for 60 min with KRBGA medium. After preincubation, the cells were incubated in tubes containing 0.5 ml pregnenolone (10^{-8} M, Sigma).

RIA of E_2

The concentration of plasma E_2 was determined by RIA as previously described, using antiestradiol serum W-1 [Lu et al., 1998]. The sensitivity of E_2 RIA was 1 pg per assay tube. The intra- and interassay coefficients of variation were 4.8% (n = 3) and 6.5% (n = 4), respectively.

RIA of PRL

The concentration of plasma PRL was determined by RIA, as previously described [Wang et al., 1989]. Rat PRL-I-5 used for iodination and 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 (all 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 = 5), respectively.

RIA of Corticosterone

The concentrations of plasma and media corticosterone were determined by RIA as described elsewhere [Chen et al., 1997; Lo et al., 1998a], with anti-corticosterone serum (PSW #4-9, by Dr. P.S. Wang, NYMU, Taipei, Taiwan, ROC); the sensitivity of corticosterone RIA was 5 pg per assay tube. The intra- and interassay coefficients of variation were 4.2% (n = 5) and 7.2% (n = 5), respectively.

RIA of cAMP

The concentration of adrenal cAMP was determined by RIA as described elsewhere [Lo et al., 1998a; Lu et al., 1996]. With the anti-cAMP serum no. CV-27 pool, the sensitivity of cAMP was 2 fmol per assay tube. The intra- and interassay coefficients of variation were 5.6% (n = 5) and 8.5% (n = 5), respectively.

Activities of 3β-HSD, 21β-Hydroxylase, and 11β-Hydroxylase

The enzyme activities of postpregnenolone in rat ZFR cells were determined by thin-layer chromatography (TLC), as described elsewhere [Lo et al., 1998b]. ZFR cells (50,000 cells per tube) were preincubated for 60 min at 37°C in 95% O_2 and 5% CO_2 in 1 ml KRBGA medium. After centrifugation at 200*g* for 10 min, the supernatant was discarded, and the cells were incubated for 60 min in tubes in 0.2 ml KRBGA containing [^{3}H] pregnenolone (8,000–10,000 cpm, 4.5–5.0 pmol, NEN-DuPont, Boston, MA). After incubation, the medium containing radioactive products was removed from cultures by centrifugation at 200*g* for 10 min. The media were extracted with diethyl ether (5×volume), shaken for 30 min, centrifuged at 200*g* for 3 min, and then quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in 100% eth-

anol. Aliquots of 50 μl of each sample and 5 μl of unlabeled carrier steroids (1 mg/ml) were spotted on silica gel G sheets containing fluorescent indicators (Macherey-Nagel, Germany) and chromatographed in carbon tetrachloride-acetone (4:1, v/v) solution. The sheets were then dried, and steroid-containing spots were located under UV light. The migration rates (R_f values) were as follows: progesterone = 0.95, deoxycorticosterone (DOC) = 0.7, and corticosterone = 0.3. Next, the spots were cut off and transferred into vials containing 1 ml of liquid scintillation fluid (Ready Safe, Beckman Instruments, Inc., Fullerton, CA) before counting the radioactivity in an automatic beta counter (Wallac 1409, Pharmacia, Turku, Finland). The recovery of [^{3}H] corticosterone after ether extraction and TLC was 62%.

In the experiment of the incubation of ZFR cells with [^{3}H] pregnenolone, the activities of 3β-HSD, 21β-hydroxylase, and 11β-hydroxylase were expressed as the radioactivities of [^{3}H] progesterone, [^{3}H] DOC, and [^{3}H] corticosterone, respectively.

Statistical Analysis

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 [Steel and Torrie, 1960]. In the in vivo experiments, Student's *t*-test was employed. A difference between two means was considered statistically significant when P was less than 0.05.

RESULTS

Effects of EB on Plasma Estradiol, PRL, and Corticosterone in Ovx Rats

Plasma estradiol increased 4.7- and 14.8-fold, respectively, in 25 and 50 μg/kg EB-replaced rats as compared with oil-injected animals ($P < 0.01$, Fig. 1, top). However, no significant change was observed for plasma estradiol in Ovx rats injected with 12.5 μg/kg EB as compared to oil-injected animals.

Compared to control animals, the basal levels of plasma PRL were significantly increased by 10-, 15-, and 26-fold ($P < 0.05$ or $P < 0.01$, Fig. 1, center) in Ovx rats replaced with 12.5, 25, and 50 μg/kg EB, respectively.

The levels of plasma corticosterone significantly increased by 53%, 75%, and 103% in Ovx rats replaced with 12.5, 25, and 50 μg/kg EB ($P < 0.05$ or $P < 0.01$, Fig. 1, bottom), respectively. In cyclic rats , the mean level of plasma corticosterone was 53.6 ± 3.9 ng/ml (n = 8).

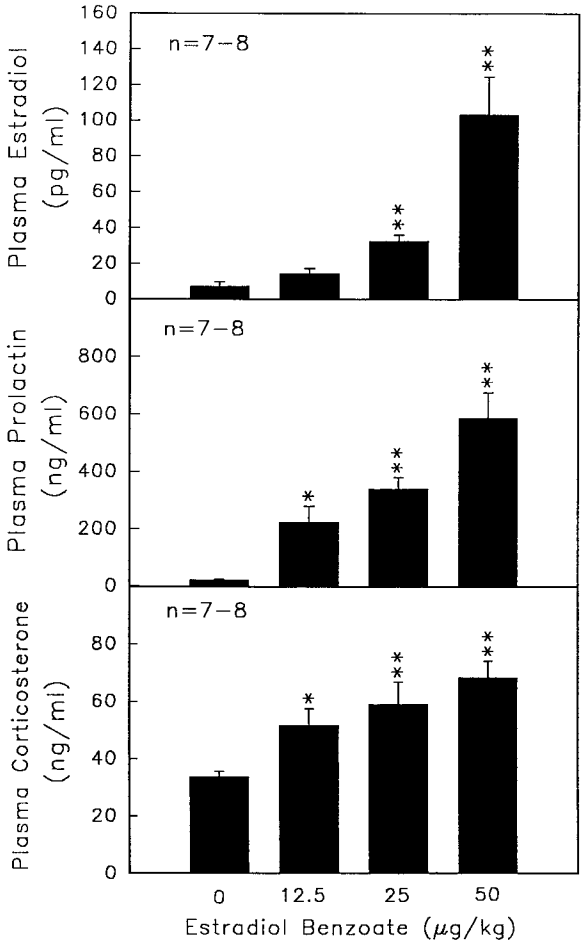

Fig. 1. Effects of estradiol benzoate (EB) replacement on the plasma E_2 (**top**), PRL (**center**), and corticosterone (**bottom**) concentrations in ovariectomized (Ovx) rats. Female rats were Ovx for 2 weeks. Ovx rats were injected subcutaneously with sesame oil or EB (12.5, 25, or 50 μg/kg) once daily for 3 days. $^*P < 0.05$, $^{**}P < 0.01$ as compared with oil-treated rats. Each value represents the mean ± SEM.

Effects of EB on Basal and ACTH-Induced Corticosterone Release in Response to Vehicle or IBMX In Vitro

Compared to animals receiving oil, Ovx rats treated with 12.5, 25, or 50 μg/kg EB showed an increased corticosterone release in ZFR cells ($P < 0.05$ or $P < 0.01$) following incubation with either vehicle, IBMX, ACTH (10^{-10} or 10^{-9} M), or IBMX plus ACTH (Fig. 2, top and bottom).

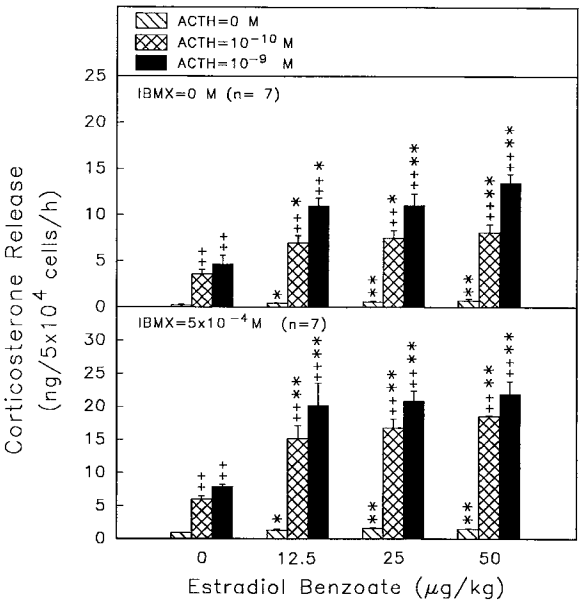

Fig. 2. Effects of EB replacement in vivo on the ACTH (**top**) or IBMX plus ACTH (**bottom**)-stimulated release of corticosterone from ZFR cells of Ovx rat in vitro. $*P < 0.05$, $**P < 0.01$ as compared with oil-treated rats. $^{++}P < 0.01$ as compared with ACTH = 0 M. Each value represents the mean ± SEM.

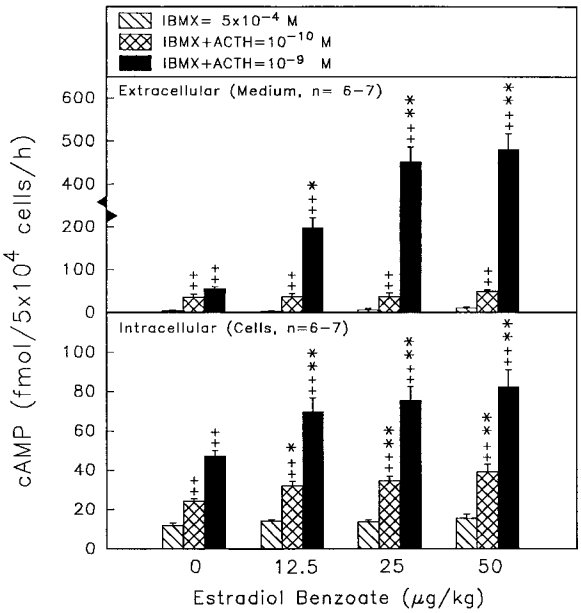

Fig. 3. Effects of EB replacement in vivo on the basal and ACTH (10^{-10} or 10^{-9} M)-stimulated levels of extracellular (**top**) and intracellular (**bottom**) cAMP after incubation of rat ZFR cells with 0.5 mM IBMX. $*P < 0.05$, $**P < 0.01$ as compared with oil-treated rats. $^{++}P < 0.01$ as compared with the group treated with IBMX alone. Each value represents the mean ± SEM.

Administration of ACTH or IBMX plus ACTH (10^{-10} or 10^{-9} M) markedly increased ($P < 0.01$) the corticosterone release by ZFR cells in all animals compared to those treated with vehicle or IBMX (Fig. 2, top and bottom).

Effects of EB on cAMP Production in Response to IBMX or IBMX Plus ACTH In Vitro

The levels of extracellular (i.e., medium) and intracellular (i.e., cell) cAMP after incubation of ZFR cells from oil or EB-replaced Ovx rat with 0.5 mM IBMX or IBMX plus ACTH are shown in Figure 3.

There were no difference between extra- and intracellular cAMP production in all groups in response to IBMX (Fig. 3, top and bottom). EB replacement (12.5, 25, and 50 μg/kg) enhanced the stimulatory effect of ACTH (10^{-10} or 10^{-9} M) on the levels of extra- and intracellular cAMP ($P < 0.05$ or $P < 0.01$) in comparison with oil-replaced rats (Fig. 3, top and bottom).

Incubation of IBMX plus ACTH resulted in greater extra- and intracellular cAMP production in ZFR cells as compared to the IBMX-treated groups (Fig. 3, top and bottom).

Effects of EB on Basal and oPRL-Induced Corticosterone Release in Response to Forskolin or IBMX In Vitro

Corticosterone release by rat ZFR cells following incubation of forskolin or IBMX with or without oPRL (10^{-8} or 10^{-7} M) in vitro are shown in Figure 4.

Treatment of EB (12.5, 25, and 50 μg/kg) in vivo in Ovx rats significantly increased the forskolin-, IBMX-, and/or oPRL (10^{-8} or 10^{-7} M)-stimulated release of corticosterone as compared to oil-replaced Ovx rats (Fig. 4, top and bottom).

Administration of oPRL (10^{-8} or 10^{-7} M) resulted in a higher ($P < 0.01$) release of corticosterone in response to forskolin and IBMX as compared to the groups treated with forskolin or IBMX alone (Fig. 4, top and bottom).

Effects of EB on cAMP Production in Response to Forskolin, IBMX, and/or oPRL In Vitro

Administration of EB in vivo in Ovx rats increased the forskolin-stimulated production of intracellular cAMP in ZFR cells by 57–63% (Fig. 5, top). However, only in Ovx rats treated

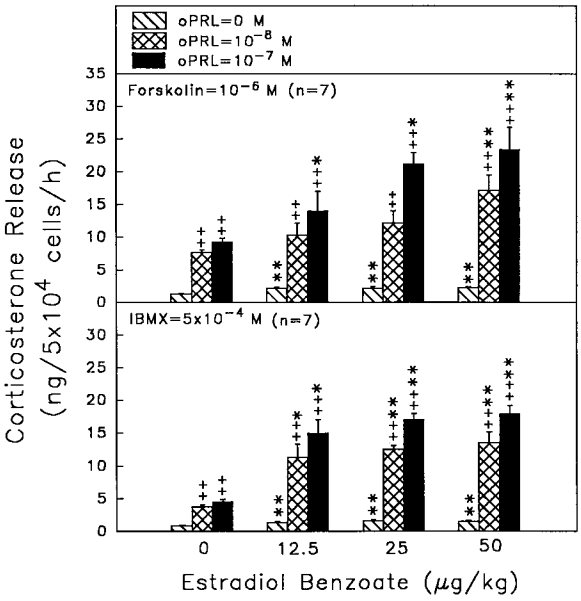

Fig. 4. Effects of EB replacement in vivo on the basal and oPRL (10^{-8} or 10^{-7} M)-stimulated release of corticosterone after incubation of rat ZFR cells with forskolin (10^{-6} M, **top**) or IBMX (5×10^{-4} M, **bottom**). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with oil-treated rats. $^{++}P < 0.01$ as compared with oPRL = 0 M. Each value represents the mean ± SEM.

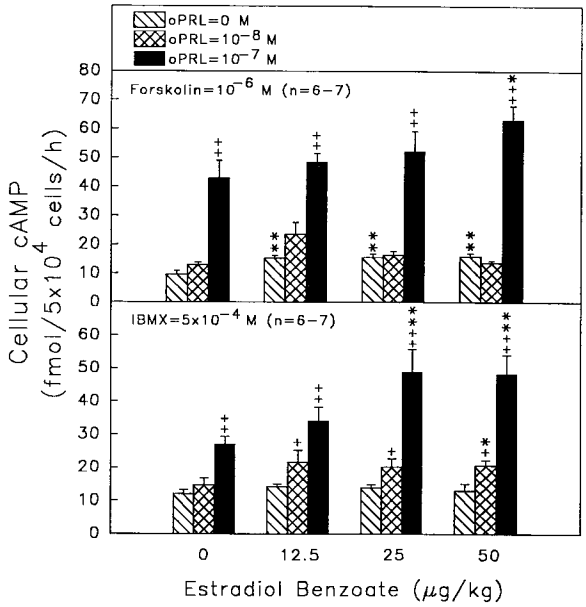

Fig. 5. Effects of EB replacement in vivo on the basal and oPRL (10^{-8} or 10^{-7} M)-stimulated production of intracelluar cAMP after incubation of rat ZFR cells with forskolin (10^{-6} M, **top**) or IBMX (5×10^{-4} M, bottom). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with oil-treated rats. $^{+}P < 0.05$, $^{++}P < 0.01$ as compared with oPRL = 0 M. Each value represents the mean ± SEM.

with 50 μg/kg EB was there a significant enhancement of the cellular cAMP by forskolin plus oPRL (10^{-7} M) as compared to the oil-treated group (Fig. 5, top).

The IBMX-induced cAMP production in ZFR cells was not altered by EB replacement in the Ovx rats (Fig. 5, bottom). After coincubation of oPRL (10^{-8} or 10^{-7} M) with IBMX, there was a significant increase ($P < 0.05$ or $P < 0.01$) in the intracellular cAMP production in Ovx rats receiving 25 or 50 μg/kg EB as compared to oil-injected Ovx rats (Fig. 5, bottom).

Administration of oPRL plus forskolin or oPRL plus IBMX (10^{-8} or 10^{-7} M) significantly increased ($P < 0.05$ or $P < 0.01$) the intracellular cAMP levels in response to forskolin or IBMX alone (Fig. 5, top and bottom).

Effects of EB on the In Vitro Release of Corticosterone in Response to Pregnenolone

Administration of EB in vivo in Ovx rats did not affect the production of corticosterone in vitro in response to pregnenolone (10^{-8} M) (Fig. 6).

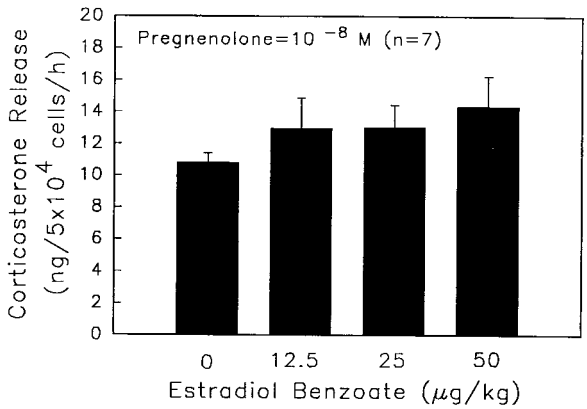

Fig. 6. Effect of EB replacement in vivo on the pregnenolone (10^{-8} M)-stimulated release of corticosterone from rat ZFR cells for 60 min. Each value represents the mean ± SEM.

Effects of EB on the Activities of 3β-HSD, 21β-Hydroxylase, and 11β-Hydroxylase

The activities of 3β-HSD (conversion of [^{3}H] pregnenolone to [^{3}H] progesterone), 21β-hydroxylase (conversion of [^{3}H] pregnenolone to [^{3}H] progesterone, and [^{3}H] DOC), and 11β-hydroxylase (conversion of [^{3}H] pregnenolone to [^{3}H] progesterone, [^{3}H] DOC, and [^{3}H] cortico-

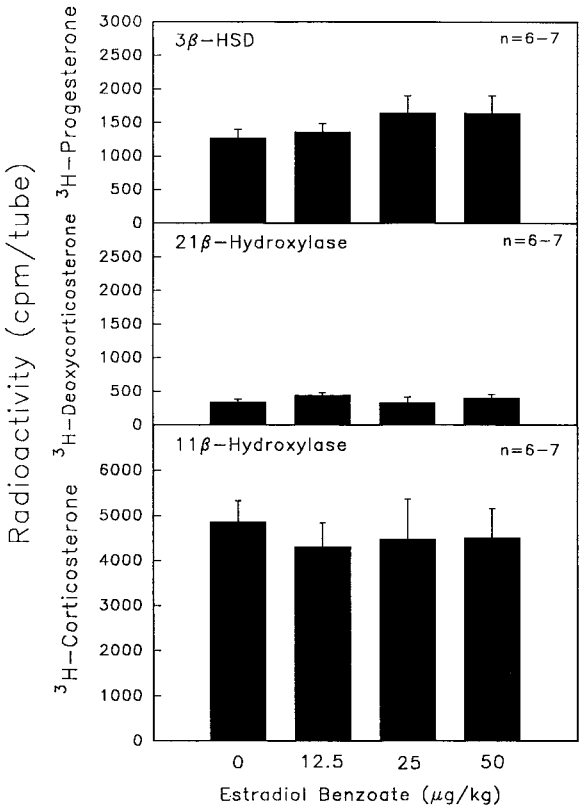

Fig. 7. Effects of EB replacement in vivo on the activities of 3β-HSD (**top**), 21β-hydroxylase (**center**), and 11β-hydroxylase (**bottom**) in ZFR cells of Ovx rats. Cells were incubated with 200 μl [^{3}H]-pregnenolone (5 pmol). Radioactive products in the medium were extracted with ether and analyzed by TLC. Each value represents the mean ± SEM.

sterone) were not affected by EB replacement in Ovx rats (Fig. 7, top, center, and bottom).

DISCUSSION

It has been shown that in rats, estrogen receptors exist as two subtypes, α and β [Kuiper et al., 1997]. The rat tissue distribution and/or relative level of estrogen α and β expression seems to be quite different, i.e., moderate to high expression in adrenal for estrogen receptor α [Kuiper et al., 1997]. In the adult rhesus monkey, immunocytochemistry revealed that staining for estrogen receptor was restricted to the cell nucleus and was most dense in the zona fasciculata [Hirst et al., 1992]. These studies reflect that estrogen may influence the function of adrenal cortex [Hirst et al., 1992; Kuiper et al., 1997]. Kitay [1963a,b] showed that the effects of E_2 in Ovx rats are reversible, which subsequently increases ACTH and corticosterone secretion. Our in vivo and in vitro studies have shown that after replacement of EB for 3 days in Ovx rats, both plasma corticosterone concentration and basal release of corticosterone from ZFR cells had increased as compared with oil-treated rats (Figs. 1, 2).

It has been well-established that the effects of estrogen on plasma and pituitary PRL could be a direct action of estrogen on pituitary lactotrophs [Terry et al., 1985] or due to indirect effects of estrogen on hypothalamic PRL regulatory systems, i.e., inhibition of dopamine release [Casanueva et al., 1982] or stimulation of serotonin [Nakagawa et al., 1980]. We confirmed that in vivo treatment with EB in Ovx rats resulted in a dose-dependent increase the secretion of plasma PRL compared to oil-treated Ovx rats. This is consistent with the observation that acute and chronic effects of E_2 on male rats yield a significant increase in plasma PRL and pituitary PRL [Terry et al., 1985]. However, these results indicate that there is positive correlation between plasma PRL and corticosterone under the influence of E_2.

In rats, corticosterone is the major adrenal steroid secreted and is released in direct response to ACTH secreted from the anterior pituitary gland [Koroscil and Gallant, 1980]. Our in vitro study indicates that the ACTH-stimulated release of corticosterone in EB-replaced rats was higher than in oil-replaced Ovx rats. These results are in agreement with previous reports that E_2 treatment increases synthesis and release of ACTH and corticosterone [Coyne and Kitay, 1969; Kitay, 1963a,b]. However, Nowak et al. [1995] found no change in ACTH-stimulated corticosterone secretion after E_2 therapy of Ovx animals. The disagreement between past and present findings might be due to the different methods for cell culture preparation or experimental procedures. Adrenal cells from a patient with ACTH-independent Cushing's syndrome cultured in different concentrations of E_2 secrete cortisol in a dose-dependent manner in the absence of ACTH [Caticha et al., 1993]. These results suggest that either in vivo or in vitro administration of E_2 increases adrenocortical function.

In the adrenal glands, initial events in ACTH action include binding of the hormone to the cell surface receptor, followed by an activation of the adenylyl cyclase-cAMP-protein kinase system [Koroscil and Gallant, 1980]. We

found that administration with E_2 in vivo enhances the stimulatory effect of ACTH on extra- and intracellular cAMP production in Ovx rats. The reason might be due to increased number of ACTH binding sites or binding affinity in Ovx rat ZFR cells after EB replacement. Whether administration of EB in vivo increases the ACTH-stimulated corticosterone release by mediating the increased number of ACTH binding sites or the binding affinity in ZFR cells is unknown.

The PRL receptor has been reported in rat, guinea pig, and human adrenals [Glasow et al., 1996; Nagano and Kelly, 1994; Outhit et al., 1993; Sautin et al., 1989]. In humans, PRL receptors exist in all three zones of the adrenal cortex [Glasow et al., 1996]. It has been shown that PRL has a direct effect on adrenal steroidogenesis, thereby regulating adrenal function [Albertson et al., 1987; Glasow et al., 1996]. Recently, we demonstrated that PRL increases the production of corticosterone by acting directly on rat ZFR cells via cAMP cascades and 3β HSD activity [Chang et al., 1999]. Our present in vitro study confirms that PRL stimulated the release of corticosterone by ZFR cells and demonstrates a close correlation between EB-related increase of the levels of plasma PRL and that of plasma corticosterone in rats.

It has been known that PRL inhibits hCG-stimulated steroidogenesis and cAMP accumulation in preovulatory granulosa cells by enhancement of phosphodiesterase activity [Gitay-Goren et al., 1989]. The present study was carried out to examine if the production of cAMP was involved in the action of PRL on the release of corticosterone in EB-replaced Ovx rat ZFR cells. We found that the forskolin (an adenylyl cyclase activator)-stimulated cAMP production in EB-replaced Ovx rats was still higher than in oil-replaced animals; the activity of phosphodiesterase should be decreased in rat ZFR cells by E_2. Since both forskolin + PRL and IBMX + PRL-stimulated production of cAMP was greater in EB-replaced Ovx rats than in oil-replaced animals, the increased accumulation of cAMP induced by PRL was correlated with the hypersecretion of corticosterone in EB-treated Ovx rats. Our data suggest that the direct and stimulatory effects of PRL on the corticosterone releases were in part via an increase of cAMP production in ZFR cells.

In rat adrenals, biosynthesis of corticosterone involves the participation of cholesterol side-chain cleavage (rate-limiting step), 3β-HSD, 21β-hydroxylase, and 11β-hydroxylase activities [Lieberman et al., 1984]. Furthermore, it has been known that E_2 directly affects rat adrenocortical secretion, mainly by acting on the rate-limiting step of steroidogenesis [Nowak et al., 1995]. In the present study, we evaluated the possible correlation between corticosterone secretion and the postpregnenolone steroid enzyme activity on EB-increased steroidogenesis in Ovx rats by use of the substrate of pregnenolone and [^{3}H] pregnenolone. Our results indicate that the activities of 3β-HSD, 21β-hydroxylase, and 11β-hydroxylase were not affected by EB replacement in Ovx rats.

In summary, these findings suggest that the hypersecretion of corticosterone induced by E_2 treatment in Ovx rats is in part due to: 1) increase of basal, ACTH-, and PRL-stimulated release of corticosterone in ZFR cells; 2) hyperprolactinemia; and 3) an increase in the production of cAMP in ZFR cells. However, the enzyme activities of postpregnenolone might be unaffected by EB-replacement in Ovx rats.

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