J. steroid Biochem. Vol. 23, No. 4, pp. 385–388, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

DEXAMETHASONE SUPPRESSES ESTROGEN ACTION AT THE PITUITARY LEVEL WITHOUT MODULATING ESTROGEN RECEPTOR DYNAMICS

NAOKI TERAKAWA*, IKUYA SHIMIZU, TOSHIHIRO AONO, OSAMU TANIZAWA and KEISHI MATSUMOTO†
Departments of Obstetrics and Gynecology, and †Pathology, Osaka University Medical School, Osaka 553, Japan

(*Received* 18 *December* 1984)

Summary—The administration of glucocorticoid combined with antiestrogen such as clomiphene has been shown to be effective for the induction of ovulation in patients with anovulation. The present study was undertaken to examine the effects of glucocorticoid on estrogen-induced changes in the pituitary gland. A single intraperitoneal (i.p.) administration of 10 μg estradiol-17β (E_2) in ovariectomized and adrenalectomized rats resulted in a significant stimulation of pituitaries with regard to wet tissue weight and progesterone receptor content. An i.p. administration of 1 mg dexamethasone in these animals had no effects on both the values. However, the E_2-induced increases in pituitary weight and progesterone receptor content were significantly inhibited by pretreatment with 1 mg of dexamethasone. The pretreatment with dexamethasone, on the other hand, had no significant effect on the dynamics of pituitary estrogen receptor induced by the injection of E_2, i.e. the degree of nuclear translocation, occupancy and cytoplasmic receptor replenishment. The inhibitory effect of dexamethasone, therefore, does not seem to be mediated through estrogen receptor system in the pituitary. These results suggest that dexamethasone acts directly on the pituitary gland to suppress the action of E_2, and which may be involved in the process of induction of ovulation by glucocorticoid–clomiphene treatment.

INTRODUCTION

Clomiphene citrate (clomiphene) has been used most commonly for the induction of ovulation in patients with anovulatory infertility. Our experience has shown that more than 90% of women with oligomenorrhea can ovulate after clomiphene treatment with a maximum daily dose of 150 mg for 5 days.

It has been suggested that in some patients who are unresponsive to clomiphene, the addition of glucocorticoid might be beneficial. The usefulness of glucocorticoid has been documented, especially in women with polycystic ovaries [1], and the suppression of serum androgen levels by glucocorticoid has been proposed to be a possible cause for restoration of ovulatory cycles [2–4]. However, glucocorticoid alone or in combination with clomiphene is also effective in normoandrogenic patients with anovulatory infertility [5, 6]. These findings suggest that other mechanisms besides the improvement of hyperandrogenism may be involved in induction of ovulation by glucocorticoid.

Evidence from animal studies [7, 8] has indicated that glucocorticoids inhibit estrogen-stimulated changes in female reproductive tissues. Recently, several *in vivo* [9] and *in vitro* studies [10] have suggested that estradiol-17β (E_2) exerts negative as well as positive feedback effects on gonadotropin secretion in mammals, and that the pituitary gland is a possible site of these actions. Our previous findings [11] suggest that clomiphene also acts directly on the pituitary gland as an antiestrogen. In the present study, therefore, the effects of glucocorticoid on E_2-stimulated changes in the pituitary gland were investigated in ovariectomized and adrenalectomized rats. A possible role of glucocorticoid in the induction of ovulation with glucocorticoid–clomiphene treatment is also discussed.

*Correspondence should be sent to: Naoki Terakawa M.D., Department of Obstetrics and Gynecology, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan.

Abbreviations: POPOP, 1,4-*bis*[2-(5-phenyloxazolyl)] benzene; R5020, promegestone(17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione).

EXPERIMENTAL

Animals and treatment

Adult Wistar female rats weighing 200 g were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. Seven days after bilateral ovariectomy and adrenalectomy, either a single dose of 10 μg E_2 in 0.5 ml saline or 1 mg dexamethasone in 0.25 ml sodium phosphate was injected intraperitoneally (i.p.). In another group of ovariectomized and adrenalectomized rats, 10 μg E_2 was administered i.p. 10 min after an injection of 1 mg dexamethasone. The rats were sacrificed by decapitation 1, 4 or 24 h after E_2 injection or 24 h after a dexamethasone injection, and the pituitaries were

immediately removed, weighed and stored at −80°C until use.

Subcellular fractionations

All procedures were performed at 4°C. Four pituitaries were collected and homogenized in 2 ml of TEM (10 mM Tris, 1.5 mM EDTA and 2 mM mercaptoethanol, pH 7.4) buffer with a Teflon-glass homogenizer. The homogenate was first centrifuged at 800 *g* for 10 min. The supernatant was then centrifuged at 105,000 *g* for 60 min, and clear supernatant (cytosol) obtained was then incubated with 0.2 ml of 2.5% charcoal–0.025% dextran suspension for 15 min to remove unbound steroids. After centrifugation of the cytosol–charcoal mixture, the supernatant was used for the cytosol exchange assay. The pellet from the initial 800 *g* centrifugation was washed 3 times with 2 ml of TEM buffer, resuspended in 2 ml of TEM buffer and used without further purification for the nuclear exchange assay.

Exchange assay

The methods used were essentially the same as reported previously [11]. For the cytosol exchange assay, duplicate 0.3 ml aliquots of cytosol preparation were incubated with 5 nM [^{3}H]E_2 in the presence or absence of a 100-fold excess of unlabeled diethylstilbestrol (DES) in a final volume of 0.35 ml at 4°C for 2 h and then at 25°C for 2 h. After incubation, 35 μl of the charcoal–dextran solution was added and the mixtures were incubated at 0°C for 15 min. The charcoal was removed by centrifugation and 0.2 ml aliquots of the supernatant were counted. For the nuclear exchange assay, duplicate 0.45 ml aliquots of the crude cell nuclear pellet suspension were incubated with 5 nM [^{3}H]E_2 in the presence or absence of a 100-fold excess of unlabeled DES in a final volume of 0.5 ml at 4°C for 2 h followed by incubation at 35°C for 1 h. After incubation, the pellet was washed three times with 2 ml of TEM buffer, and the final pellets were counted.

The concentrations of estrogen receptor (ER) were expressed as fmol per 10 mg pituitary.

Assay of progesterone receptor

For cytoplasmic progesterone receptor assay, TEMG (10 mM Tris, 1.5 mM EDTA, 2 mM mercaptoethanol and 20% v/v glycerol, pH 7.4) buffer was used. The procedures to obtain cytosol fraction were the same as described in ER assay. Duplicate 0.3 ml aliquots of the cytosol were incubated with 10 nM [^{3}H]R5020 in a final volume of 0.35 ml at 4°C for 4 h. After incubation, 35 μl of the charcoal-dextran solution was added and the mixtures were incubated at 0°C for 10 min. The charcoal was removed by centrifugation and 0.2 ml aliquots of the supernatant were counted.

The concentrations of progesterone receptor were also expressed as fmol per 10 mg pituitary.

The radioactivity of tritium was assayed in 5 ml scintillation fluid (100 mg POPOP, 3 g 2,5-diphenyloxazole in 666 ml toluene, 333 ml Triton X-100) at an efficiency of 45% in an Isocap 300 liquid scintillation counter.

Chemicals and reagents

[2,4,6,7-^{3}H]E_2 (110 Ci/mmol), [17α-methyl-^{3}H] R5020 (77 Ci/mmol) and unlabeled R5020 were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Unlabeled steroids and acid washed charcoal were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. Dextran T70 was a product of Pharmacia Fine Chemicals, Uppsala, Sweden. Dexamethasone sodium phosphate was provided by Organon Oss, the Netherlands. All other chemicals used were of analytical grade.

Statistics

All values were expressed as mean ± SE. Student's unpaired *t*-test was used for the statistical analysis.

RESULTS

As shown in Fig. 1, the weight of the pituitary gland in the ovariectomized and adrenalectomized rats increased significantly from 10.1 ± 0.2 mg to 11.2 ± 0.2 mg 24 h after a single i.p. injection of 10 μg E_2. On the other hand, a single dose of 1 mg dexamethasone did not have any effect on the pituitary

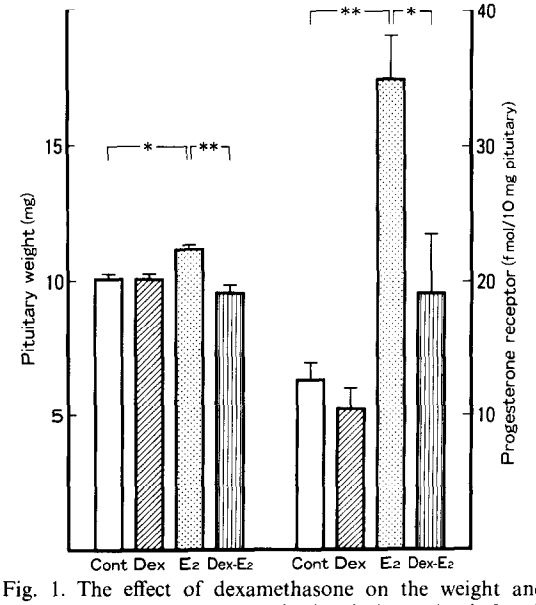

Fig. 1. The effect of dexamethasone on the weight and progesterone receptor content in the pituitary gland. Ovariectomized and adrenalectomized rats were injected i.p. with saline (control) or a single dose of 1 mg dexamethasone or 10 μg E_2. Another group of the rats was injected i.p. with 1 mg dexamethasone 10 min before injection of 10 μg E_2. The rats were sacrificed 24 h after saline, dexamethasone or E_2 injection. Each value represents the mean ± SE (sixteen determinations for pituitary weight and four determinations for progesterone receptor content). Differences between the values of saline and E_2, or between the values of E_2 and dexamethasone combined with E_2 (P): * < 0.01, ** < 0.001.

weight in these animals. The pretreatment with 1 mg dexamethasone completely inhibited the increase in pituitary weight induced by E_2.

The pituitary content of progesterone receptor increased significantly from 12.8 ± 1.2 fmol to 34.9 ± 3.4 fmol/10 mg pituitary 24 h after a single injection of E_2 in both ovariectomized and adrenalectomized rats (Fig. 1). A single dose of dexamathasone did not cause any significant change of the progesterone receptor content. The dexamethasone pretreatment also suppressed the increase in the progesterone receptor content induced by E_2, suggesting that dexamethasone antagonizes E_2-stimulated responses in the pituitary gland.

It was found that the level of cytoplasmic ER in the ovariectomized and adrenalectomized rats (71.4 ± 6.0 fmol/10 mg pituitary) was not altered significantly by a single dose of 1 mg dexamethasone (67.7 ± 9.3 fmol/10 mg pituitary at 24 h). In order to know whether dexamethasone antagonizes E_2 action in the pituitary through ER system, the dynamics of pituitary ER was studied. As shown in Fig. 2, the administration of 10 μg E_2 in dexamethasone-pretreated animals caused a rapid increase in nuclear receptor with a concomitant decrease in cytoplasmic receptor, and the translocated receptor in the crude cell nuclear pellet (51.3 ± 9.4 fmol/10 mg pituitary at 1 h) remained almost unchanged for 3 h (41.0 ± 6.6 fmol/10 mg pituitary at 4 h). Replenishment of cytoplasmic receptor could be detected 24 h after E_2 injection. The dynamics of pituitary ER after E_2 administration in the dexamethasone-pretreated rats were similar to those in the dexamethasone-nontreated rats. Changes in levels of nuclear ER after E_2 administration in the absence or the presence of dexamethasone in the ovariectomized and adrenalectomized rats are summarized in Fig. 3. There were no significant differences in the levels of nuclear ER 1, 4 and 24 h after a single dose of E_2 between the absence or the presence of dexamethasone, suggesting that ER system does not seem to be involved in inhibition by dexamethasone of E_2 action in the pituitary.

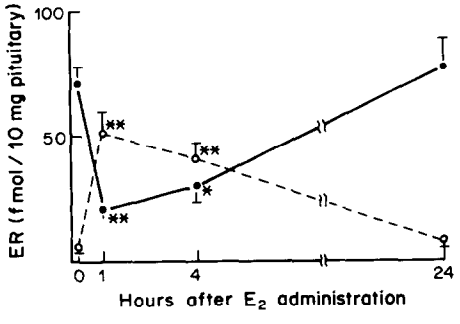

Fig. 2. The effect of dexamethasone on ER distribution in the pituitary of ovariectomized and adrenalectomized rats. The rats were injected i.p. with 10 μg E_2 10 min after injection of 1 mg dexamethasone. The rats were sacrificed at the times indicated. Cytoplasmic (●——●) and nuclear (○- - -○) ER were measured by the exchange assay. Each point represents the mean ± SE of four determinations. Differences from 0 h (P): * < 0.01, ** < 0.001.

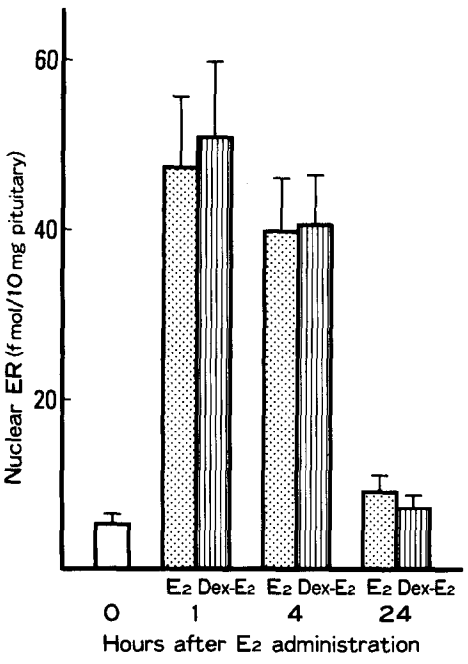

Fig. 3. The content of nuclear ER following a single injection of E_2 in the absence or the presence of dexamethasone. The ovariectomized and adrenalectomized rats were injected i.p. with a single dose of 10 μg E_2 or injected i.p. with 10 μg E_2 10 min after injection of 1 mg dexamethasone. The rats were sacrificed at the times indicated. Each point represents the mean ± SE of 4 determinations.

DISCUSSION

The present results demonstrate that dexamethasone antagonizes E_2 action in the pituitary gland of ovariectomized and adrenalectomized rats. To our knowledge, the present study demonstrates for the first time that dexamethasone shows an inhibitory effect on E_2-induced increases in the weight and progesterone receptor content in the pituitary gland, though dexamethasone alone does not exhibit any significant effects on these parameters.

It is generally accepted that estrogen-induced phenomena result from the long term retention of ER in the nucleus of target cells and that the degree of agonist activity depends on the concentration of nuclear ER [12]. Since 1 mg of dexamethasone did not affect ER dynamics induced by 10 μg E_2 in the pituitary gland, the inhibitory effect of dexamethasone does not seem to be mediated through ER system. These findings are inconsistent with those reported in the uteri of adrenalectomized immature female rats [8], in which dexamethasone significantly impaired nuclear translocation of ER. It has been shown that dexamethasone does not exhibit any apparent interference with the binding of estrogen to its receptor [8, 13]. At present, the mechanism of this

dexamethasone effect on E_2-induced changes in the pituitary is not known.

Many investigators reported that combined administration of glucocorticoid and clomiphene could induce ovulation in patients with anovulation, who were unresponsive to clomiphene alone. The ovulation rates obtained with this combined therapy among non-responders to clomiphene were 80–87% and the pregnancy rates were 47–48% [6, 14]. It has been postulated that a decrease in plasma androgen levels by glucocorticoid may enhance ovulation by clomiphene, since hyperandrogenism has been suggested to be responsible for anovulation and menstrual disturbance [2-4]. Indeed glucocorticoid has been shown to inhibit androgen production from the ovary as well as from the adrenal gland, and restore ovulation and conception in hyperandrogenic patients [1, 5]. However, glucocorticoid alone or in combination with clomiphene is also effective for induction of ovulation in normoandrogenic anovulatory women [5, 6]. Our experience has also shown that combined administration of glucocorticoid and clomiphene is useful to induce ovulation, especially in patients with polycystic ovaries. However, we previously reported [15] that androgen levels in patients with polycystic ovaries were apparently lower in Japan than in western countries. From our experience, patients with low endogenous estrogen levels rarely respond to the combined regimen with glucocorticoid and clomiphene. Although the suppression of androgen by glucocorticoid may be a possible factor for restoration of ovulatory cycles in hyperandrogenic women, these findings suggest that other mechanisms seem to be involved in these processes.

Although the effect of dexamethasone treatment on plasma gonadotropins was not examined in the present animal study, it has been reported that serum gonadotropin levels are increased during the combined administration of prednisolone and clomiphene [16]. Our previous study [11] has suggested that clomiphene acts as an antiestrogen on the pituitary gland and may stimulate the release of gonadotropin by disruption of negative feedback effect of ovarian estrogen, though the role of hypothalamus cannot be ruled out. The present results, combined with our previous findings suggest that both glucocorticoid and clomiphene may exert their antiestrogenic effects on the pituitary gland cooperatively, and may increase the gonadotropin secretion, stimulate the maturation of follicles and then induce ovulation in infertile women. However, the present findings do not rule out the suppressive effect of glucocorticoid on the production of androgens from the adrenal and the ovary in hyperandrogenic patients.

REFERENCES

1. Perloff W. H., Smith K. D. and Steinberger E.: Effect of prednisone on female infertility. *Int. J. Fert.* **10** (1965) 31–40.
2. Chang R. J. and Abraham G. E.: Effect of dexamethasone and clomiphene citrate on peripheral steroid levels and ovarian function in a hirsute amenorrheic patient. *Fert. Steril.* **27** (1976) 640–646.
3. Toaff R., Toaff M. E., Gould S. and Chayen R.: Role of androgenic hyperactivity in anovulation. *Fert. Steril.* **29** (1978) 407–413.
4. Lobo R. A., Paul W., March C. M., Granger L. and Kletzky O. A.: Clomiphene and dexamethasone in women unresponsive to clomiphene alone. *Obstet. Gynec.* **60** (1982) 497–501.
5. Steinberger E., Smith K. D., Tcholakian R. K. and Rodrigues-Rigau L. J.: Testosterone levels in female partners of infertile couples. Relationship between androgen levels in the woman, the male factor, and the incidence of pregnancy. *Am. J. Obstet. Gynec.* **133** (1979) 133–138.
6. Higashiyama S., Yasuda J., Ohtsubo K. and Okada H.: Ovulation induction with prednisolone–clomiphene therapy in clomiphene failure. *Jap. J. Fert. Steril.* **26,** (1981) 1–6.
7. Pietras R. J. and Szego C. M.: Steroid hormone-responsive, isolated endometrial cells. *Endocrinology* **96** (1975) 946–954.
8. Campbell P. S.: The mechanism of the inhibition of uterotrophic responses by acute dexamethasone pretreatment. *Endocrinology* **103** (1978) 716–723.
9. Nakai Y., Plant T. M., Hess K. L., Keogh E. J. and Knobil E.: On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. *Endocrinology* **102** (1978) 1008–1014.
10. Frawley L. S. and Neill J. D.: Biphasic effects of estrogen on gonadotropin-releasing hormone-induced luteinizing hormone release in monolayer cultures of rat and monkey pituitary cells. *Endocrinology* **114** (1984) 659–663.
11. Tsutsumi H., Terakawa N., Aono T., Kurachi K. and Matsumoto K.: Effects of clomiphene citrate on the pituitary gland in chronically estrogenized rat. *J. steroid Biochem.* **19** (1983) 1805–1809.
12. Anderson J. N., Peck E. J. Jr and Clark J. H.: Estrogen-induced uterine responses and growth: relationship to receptor estrogen binding by uterine nuclei. *Endocrinology* **96** (1975) 160–167.
13. Higgins S. J., Rousseau G. G., Baxter J. D. and Tomkins G. M.: Nature of nuclear acceptor sites for glucocorticoid– and estrogen–receptor complexes. *J. biol. Chem.* **248** (1973) 5873–5879.
14. Diamant Y. Z. and Evron S.: Induction of ovulation by combined clomiphene citrate and dexamethasone treatment in clomiphene citrate nonresponders. *Eur. J. obstet. Gynec. Reprod. Biol.* **11** (1981) 335–340.
15. Aono T., Miyazaki M., Miyake A., Kinugasa T., Kurachi K. and Matsumoto K.: Responses of serum gonadotrophins to LH-releasing hormone and oestrogens in Japanese women with polycystic ovaries. *Acta. endocr., Copenh.* **85** (1977) 840–849.
16. Higashiyama S., Kobayashi H., Ohno Y., Yamamoto T. and Okada H.: Ovulation induction with prednisolone–clomiphene therapy in clomiphene failure and its mechanism of action. *Acta. Obstet. Gynec. Jap.* **30** (1978) 1424–1432.