J. steroid Biochem. Vol. 23, No. 1, pp. 77–80, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

TIME COURSE OF ANTERIOR PITUITARY ESTROGEN RECEPTOR AFTER LOW 17β-ESTRADIOL DOSES IN OVARIECTOMIZED RATS

J. GÓMEZ-BENÍTEZ and B. N. DÍAZ-CHICO
Departamento de Fisiología, Colegio Universitario de Las Palmas, Las Palmas de Gran Canaria, Apdo 550 Islas Canarias, Spain

(*Received* 7 *August* 1984)

Summary—The present paper describes the effect of three 17-β-estradiol (E_2) doses (1, 10 and 500 ng E_2/kg) on the cytosolic and nuclear estrogen receptor content of anterior pituitary (Ap) of ovariectomized rats. The estrogen receptors were measured by [^{3}H]E_2 exchange in cytosol and crude nuclear fractions. Two hours after the administration of 10 or 500 ng E_2/kg the Rc showed a depletion to 20–30% of preinjection level. The 1 ng E_2/kg dose did not provoke any Rc depletion. The Rc replenishment was completed 5 h after injection of 10 ng E_2/kg, but it was delayed to 10 h after injection of 500 ng E_2/kg. An increased amount of Rc over the control levels was produced by 1 and 10 ng E_2/kg doses, but not by the 500 ng E_2/kg. The Rn level in Ap increased significantly after all E_2 doses, and their highest levels were similar for 1, 10 and 500 ng E_2/kg. These results suggest that some estrogenic responses like synthesis of the estrogen receptor proteins, can be elicited without previous significant Rc depletion. The relationships between Rc and Rn in Ap suggest an autorregulatory mechanism for the control of the cellular level of unbound estrogen receptors, that can be altered by the exogenous E_2.

INTRODUCTION

There is evidence that estradiol receptors are present in the anterior pituitary (Ap) as cytoplasmic (Rc) and nuclear(Rn) forms, having similar properties to those reported for other target organs [1–10]. It is also known that the binding of [^{3}H]estradiol ([^{3}H]E_2) and the cytosolic receptor (Rc) level in Ap is modified by several conditions such as sex cycle, castration and also after treatment with estrogens or other steroid hormones [10–14]. Following injections of pharmacological doses of 17-β estradiol (E_2) it is possible to deplete the Rc level, causing an increase in the nuclear receptor (Rn) level for several hours [15, 16]. In all estrogen responsive tissues, this process must be previous to the induction of the estrogen receptor protein synthesis and to the Rc replenishment [8, 9, 15]. However we have recently described the induction of Rc synthesis without previous Rc depletion in the uterus of ovariectomized rats injected with 1 or 10 ng E_2/kg [17]. Besides, on the basis of the Rc and Rn relationship obtained in these experiments, we suggested the existence of an autoregulation of the unbound estrogen receptor content in uterine cells [17].

In this work we have attempted to prove whether these results obtained with low estrogen doses in the uterus of ovariectomized rats are reproducible in the anterior pituitary.

EXPERIMENTAL

Animals and tissue fractionation

Adult female Sprague–Dawley rats (200–250 g b.wt) from our colony were maintained in 12 h light and 12 h darkness, with water and food *ad libitum*. Ovariectomy was performed under either anaesthesia, and 2 or 3 weeks later, the rats were injected intraperitoneally with 0.1 ml/100 g b.wt of 17-β estradiol (Merck) dissolved in saline solution containing 5% ethanol. The animals were decapitated 0.5–42 h after injection. Only the anterior lobe of the pituitary gland was used.

The tissue pieces were washed in ice-cold TE buffer (10 mM Tris–HCl, 1.5 mM EDTA, pH 7.4) and two Ap were pooled and homogenized in all-glass Potter–Eveljhem homogenizer at 0–4°C and 200–500 rpm with 30 s intervals for cooling.

The homogenate was centrifuged at 850 *g* for 15 min and the supernatant centrifuged again in a MSE Centriscan 75 ultracentrifuge at 4°C for 90 min at 105,000 *g*, to obtain the cytosol. The 850 *g* pellet was washed 3 times in an excess of TE buffer, and used as crude nuclear preparation for [^{3}H]E_2 binding.

Cytosolic estrogen receptor assay

The method described by Mester *et al.* [18] and Mester and Baulieu [19] was adapted for the measurement of cytosol binding of 17-β-estradiol ([^{3}H]E_2) in Ap. Cytosol aliquots of 250 μl were incubated in triplicate with 50 μl of [^{3}H]E_2 ([2,4,6,7-^{3}H]estradiol-17β, sp. act. 100 Ci/mmol, The Radiochemical Centre) for 3.33 nM final concentration, either alone for total binding or with 1 nM DES (diethylstilbestrol, Sigma) for non specific binding overnight at 0–4°C. A suspension of dextran-coated charcoal (0.005 and 0.5% respectively) was added and after 15 min incubation, centrifuged. Aliquots of supernatant were mixed with scintillation

cocktail (0.5% PPO and 0.025% POPOP in toluene), shaken vigorously and counted for radioactivity in a Packard 3390 spectrometer with 37% efficiency. For maximal binding capacity and K_d determination, assays were performed as previously described, but with [^{3}H]E_2 concentration in the range of 0.1–5 nM, and the results were subjected to Scatchard analysis.

Nuclear estrogen receptor assay

The number of Ap nuclear estrogen binding sites was measured by nuclear exchange assay as described by Anderson *et al.*[4]. Aliquots of 250 μl of nuclear suspensions, were incubated overnight at 0–4°C with 50 μl of [^{3}H]E_2 for a final concentration of 3.33 nM, either alone or with 1 nM DES. The excess of [^{3}H]E_2 was removed by 3 washes in 2 ml of TE buffer. The last pellet was extracted with the scintillation cocktail, and counted for radioactivity.

Assay of proteins and DNA

Proteins and DNA were measured following the Lowry *et al.*[20] and Burton[21] procedures, respectively.

Statistics

Comparisons between groups were performed by Student's t-test.

RESULTS

Saturation curves for both Rc and Rn showed complete saturation of receptors above 2.5 nM of [^{3}H]estradiol. The range of K_d values for Rc was from 0.2 to 0.7 nM and between 0.5–1.1 nM for Rn. (data not shown) and they were in agreement with those reported previously [8]. No significant differences in K_d values were observed between controls and E_2 treated groups for both Rc and Rn.

The time course of the cytosolic and nuclear receptors of ovariectomized rats after 1, 10 and 500 ng E_2/kg of body weight is shown in Figs 1 and 2 respectively. Table 1 shows the obtained results as fmol/mg w.wt tissue.

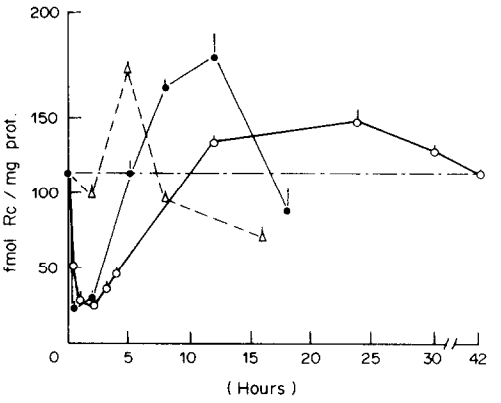

Fig. 1. Time course of anterior pituitary cytosolic receptor, expressed as fmol/mg protein, of ovariectomized rats injected with 1 (△), 10 (●), or 500 (○) nanograms of E_2/kg b.wt. Each point is the mean (+ or − SEM) of at least 4 triplicate determinations of 2 or 3 pooled anterior pituitaries.

Two hours after the injection of 500 or 10 ng E_2/kg, a significant depletion of Rc from 113 to 25 and 30 fmol/mg of cytosolic protein (P) respectively, occurred in Ap ($P < 0.01$ in both doses). At no time after injection did the dose of 1 ng E_2/kg provoke Rc depletion.

The Rc replenishment was completed 5 h after injection of 10 ng E_2/kg and at 10 h after 500 ng E_2/kg dose. The excess of Rc accumulated over the control after E_2 injection (the so called overshoot) was significantly higher in the doses of 1 and 10 ng E_2/kg than in the controls, with levels of 189 and 182 fmol/mg P respectively ($P < 0.01$). The overshoot in the dose of 500 ng E_2/kg did not show significant differences versus the controls. The overshoot rate was positively correlated with the assayed dose. After the overshoot, the Rc level declined to control levels.

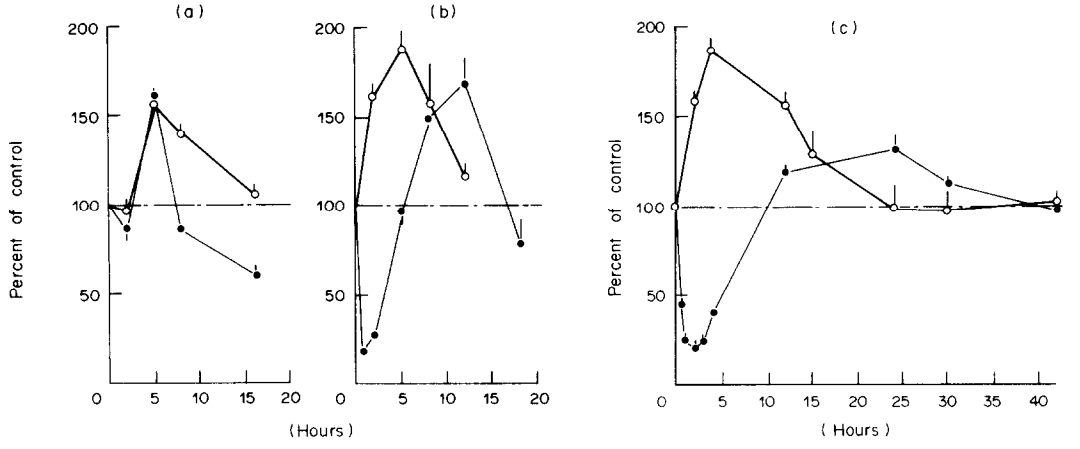

Fig. 2. Time course of anterior pituitary cytosolic (●) and nuclear (○) receptors after injection of 1 (a), 10 (b) or 500 (c) ng E_2/kg b.wt. The results are expressed as percent of control values: cytosolic receptor, 113.4 ± 5.9 fmol/mg protein (30 observations); Nuclear receptor, 413.8 ± 24.5 fmol/mg DNA (27 observations). Each point is the mean (+ or − SEM) of at least 4 triplicate determinations of 2 or 3 pooled anterior pituitaries.

Table 1. Effect of 500, 10 or 1 ng of 17-β-estradiol/kg on the cytosolic and nuclear estrogen receptor content of anterior pituitary, expressed as fmol/mg w.wt tissue in adult ovariectomized rats, in relation to the time after treatment.

Time (h)	fmol Rc/mg tissue	fmol Rn/mg tissue
Control		
0	(30) 5.46 ± 0.2	(27) 1.63 ± 0.07
500 ngE_2/kg		
2	(10) 1.43 ± 0.32a	(12) 3.38 ± 0.33a
12	(12) 5.86 ± 0.20	(15) 2.00 ± 0.77c
24	(18) 7.62 ± 0.50c	(10) 2.21 ± 0.35c
30	(6) 5.67 ± 0.29	(6) 2.18 ± 0.40c
42	(15) 5.29 ± 0.26	(12) 1.86 ± 0.04c
10 ngE_2/kg		
2	(6) 1.64 ± 0.06a	(12) 1.67 ± 0.30
5	(6) 5.33 ± 0.45	(6) 2.81 ± 0.15a
8	(6) 8.58 ± 0.52a	(6) 1.98 ± 0.36
12	(15) 9.60 ± 1.30a	(6) 1.72 ± 0.08
1 ngE_2/kg		
2	(6) 5.14 ± 0.43	(6) 2.29 ± 0.07b
5	(9) 9.40 ± 0.26a	(6) 3.54 ± 0.79b
8	(6) 5.02 ± 0.08	(6) 1.85 ± 0.14
16	(6) 3.64 ± 0.32b	(6) 1.77 ± 0.07

Number of determinations between brackets. Statistical differences with control group were calculated by means of the Student t-test (a = $P < 0.001$; b = $P < 0.01$; c = $P < 0.05$; none = no significant differences versus controls).

The effect of the application of the same treatments on the pituitary Rn levels is shown in Fig. 2. Two to four hours after E_2 injection there were significant Rn increases in relation to the control. The highest Rn level of the 3 assayed doses occurred 4 or 5 h after the injection. The highest values reached were 777, 782 and 652 fmol/mg DNA for 500, 10 and 1 ng E_2/kg respectively without significant differences between them. After these peaks, there was a decrease in the Rn towards the control levels that was faster in the case of the lower doses, than in that of the 500 ng E_2/kg one.

DISCUSSION

The cytosolic estrogen receptor depletion described by other authors as the first receptor event after E_2 administration [2, 9] was observed in Ap following the dose of 10 or 500 ng E_2/kg.

The obtained Rc results might be slightly underestimated because of a possible incompleteness of exchange under the incubations conditions used. However these results are in the range of those obtained by Cidlowski and Muldoon, who used larger E_2 doses [10, 11].

The lack of significant Rc depletion after injection of 1 ng E_2/kg could be due either to the low plasma E_2 levels reached by this dose, or to another more complex process that will be discussed later. The process of Rc replenishment after estrogenic stimulus, and also the overshoot of Rc over its basal level, depends on protein synthesis which is induced by the estrogen receptor complex acting at nuclear level [6, 8]. From this point of view, the excess of Rc over the control level observed in Ap cytosolic receptor after the injection of 1 ng E_2·kg, suggest a response of the cellular protein synthesis system to this very low E_2 dose.

This finding reveals that the Rc depletion is not a neccessary condition for eliciting some cellular response to estrogen, and is in agreement with previous results we obtained in uterus [17].

In the rats injected with 10 ng E_2/kg the Rc replenishment is faster and the overshoot greater than in those injected with 500 ngE_2/kg. The Rc concentration in the estrogen responsive tissues can be considered as the result of an equilibrium between Rc synthesis and its own breakdown by binding with plasma E_2. From this reasoning the time course of Rc after injection of 10 or 500 ng E_2/kg can be interpreted as the manifestation of a progressive disturbance of the Rc synthesis/breakdown ratio when the E_2 dose is increased. The fast increase of Rc between 2 and 5 h after 1 ng E_2/kg injection, suggest that this induction of Rc synthesis may also be present in Ap of rats injected with higher doses than this. If this process has not been seen in the rats injected with 500 ng E_2/kg, it could be because the Rc binding with plasma E_2 overlaps this synthetic process. The lack of Rc depletion after injection of 1 ng E_2/kg, suggest that the basal Rc synthesis could be sufficient to compensate the Rc breakdown caused by the weak plasma levels reached by this dose.

The absence of a balance between pituitary Rc decrease and Rn increase with the used doses is in agreement with the results described previously in the uterus [17, 19]. This could either lead to a rapid processing of the receptor in the nuclei or to an incomplete exchange of the Rc–E_2 complexes attached to the chromatin. The possibility of contamination of crude nuclear pellets with Rc must also be considered. This contamination has been stated as 10% of Rn concentration in uterine crude nuclei prepared as above [19], and in pituitary should be in the same range.

The nuclear receptor increase after 500 ng E_2/kg shows an accumulation pattern similar to the one described by Anderson *et al.* [5] who administered higher doses than the ones used in this study. The similarity between the highest Rn values after the 3 assayed doses, and the simultaneous Rc and Rn increases after 1 ng E_2/kg, are in agreement with the results we obtained previously in uterus [17]. They suggest an entry of unbound receptors into the nucleus and their fixation to its structures.

The possibility of a translocation of unbound Rc into the nucleus of pig uterus in absence of estrogens has also been suggested previously [22]. Besides, an equilibrium of free receptors distributed between the cytosol and the nucleus of the immature rat uterus has been proposed by Sheridan *et al.* [23]. The results described in this study and in previous work [17] support the conclusions of the authors [22, 23].

On this basis it can be suggested that the entrance of unbound receptors into the nucleus might be a general property of estrogen receptors that could be

present in all estrogen responsive tissues. On the other hand, the simultaneous loss of Rc and Rn in Ap suggest the appearance of a receptor processing which causes its inactivation or degradation. This could be part of an autorregulatory mechanism operating on the unbound receptor, the characteristics of which require more experiments before being completely understood.

Acknowledgements—Thanks to Mr J. Rivero Báez for his technical assistance, to Mr A. Lleó Martel for his help in animal handling, and to Mrs S. Huntley for her help in the English correction of the manuscript.

REFERENCES

1. Jensen E. V. and Jacobson H. I.: Basic guides to the mechanism of estrogen actions. *Recent Prog. Horm. Res.* **18** (1962) 387–414.
2. Gorski J., Toft D., Shyamala G., Smith D. and Notides A.: Hormone receptors: Studies on the interaction of estrogen with the uterus. *Recent Prog. Horm. Res.* **29** (1968) 45–79.
3. Jensen E. V.: A two step mechanism for the interaction of estradiol with rat uterus. *Proc. natn. Acad. Sci., U.S.A.* **59** (1968) 632–638.
4. Kato J., Atsumi Y. and Muramatsu M.: Nuclear estradiol receptor in rat anterior hypophisis. *J. Biochem.* **67,** (1970) 871–872.
5. Anderson N. J., Peck E. J. Jr and Clark J. H.: Nuclear estrogen receptor complex: Accumulation, retention and localization in the hypothalamus and pituitary. *Endocrinology* **93** (1973) 711–717.
6. McEwen B. S.: Gonadal steroid receptors in neuroendocrine tissues In *Receptors and Hormone Actions* (Edited by B. W. O'Malley and L. Birnbauer). Academic Press, New York (1978) pp. 353–400.
7. Jensen E. V. and DeSombre E. R.: Steroid sex hormone receptors and actions. In *Endocrinology* (Edited by Leslie J. de Groot). Grune and Stratton, New York (1979) pp. 2055–2061.
8. Muldoon T. G.: Role of receptors in the mechanism of steroid hormone action in the brain. In *Endocrine Functions of the Brain* (Edited by M. Motta). Raven Press, New York (1890) pp. 51–93.
9. Jensen E. V., Greene G. L., Closs L. E., DeSombre E. R. and Nadh M.: Receptor reconsidered: a 20-year perspective. *Recent Prog. Horm. Res.* **38** (1982) 1–40.
10. Cidlowski J. A. and Muldoon T. G.: Estrogenic regulation of cytoplasmic receptors populations in estrogen-responsive tissues of rat. *Endocrinology.* **95** (1974) 1621–1629.
11. Cidlowski J. A. and Muldoon T. G.: The dynamics of intracellular estrogen receptor regulation as influenced by 17-β estradiol. *Biol. Reprod.* **18** (1978) 234–246.
12. Morris I. D.: Changes in brain, pituitary and uterine cytoplasmic estrogen receptors induced by estradiol-17β in the ovariectomized rat. *J. Endocr.* **71** (1976) 343–349.
13. Lisk R. D. and Reuter L. A.: Progesterone treatment increases pituitary estradiol retention in the ovariectomized normal female rat but not in the male nor in the androgen or estrogen sterilized female rat. *Acta endocr., Copenh.* **84** (1977) 268–280.
14. Thrower S. and Lim L.: The nuclear oestrogen receptor in the female rat. *Biochem. J.* **198** (1981) 385–389.
15. Gorski J. and Gannong F.: Current models of steroid hormone action: a critique. *A. Rev. Physiol.* **38** (1976) 425–450.
16. Menon K. M. J. and Gunaga K. P.: Cytoplasmic and nuclear receptor-estradiol complex in hypothalamus and pituitary: Relationship with pituitary sensitivity to GnRH and gonadotropin secretion in the rat. *Neuroendocrinology* **22** (1976) 8–17.
17. Diaz-Chico B. N., Sosa-Gonzalez A. and Gómez-Benítez J.: Relations between cytosolic and nuclear estrogen receptors in castrated rat uterus following low 17-β estradiol doses. *J. steroid Biochem.* **20** (1984) 1135–1139.
18. Mester J., Robertson D. M., Feherty P. and Kellie A. E.: Determination of high-affinity estrogen receptors sites in uterine supernatant preparations. *Biochem. J.* **120** (1970) 831–836.
19. Mester J. and Baulieu E. E.: Dynamics of estrogen receptors distribution after estradiol administration. *Biochem J.* **146** (1975) 617–623.
20. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin-phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
21. Burton K.: Study of conditions and mechanism of diphenilamine reaction for colorimetric estimation of deoxiribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
22. Jungblut P. W., Hughes A., Garren J., Kallweit E., Mescher I., Paol F., Sierralta W., Szendro P. I. and Wagner P. K.: Mechanism involving in the regulation of steroid receptor levels. *J. steroid Biochem.* **11** (1979) 273–278.
23. Sheridan P. J., Buchanan J. M., Anselmo V. C. and Martin P. M.: Equilibrium: The intracellular distribution of steroid receptor. *Nature* **282** (1979) 579–582.