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Relationship Between Occupied Form of Nuclear Estrogen Receptor and Cytosolic Progesterone Receptor or DNA Synthesis in Uteri of Estradiol Implanted Rats

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The effect of 17 β-estradiol (E_2) implantation on the cytosolic progesterone receptor (RcP), DNA and occupied form of nuclear estrogen receptor (o- Rn) content in the uterus of ovariectomized adult rats, is described. Animals were implanted with oil or E_2-oil solution in Silastic capsules. The latter group animals were divided into two subgroups: in subgroup (a), capsules remained *in situ* until decapitation time. In subgroup (b) they were removed 48 h after implantation. The E_2 implantation caused a significant increase in uterine weight, RcP and o-Rn content 48 h later. However, the DNA content increased significantly only after 72 h, but there was no significant difference in the t-Rn concentration in relation to the non-estrogenized animals. In subgroup (a) animals, these values remained unchanged until 96 h. In subgroup (b), the removal of E_2 implants 48 h later caused an almost complete return to the values before the E_2 implantation in terms of uterine weight, RcP and o-Rn content. However the DNA concentration remained higher and the t-Rn level was lower than those values that were obtained for the non- estrogenized rats. These results suggest that the RcP and DNA synthesis induced by E_2 would be connected to the level of o-Rn, although a closer dependency over time seems to exist between the o-Rn and RcP levels than between the o-Rn and DNA concentrations.

Key words: 17 β-estradiol implantation, Occupied nuclear estrogen receptor, Cytosol progesterone receptor, DNA.

It has long been known that estradiol is capable of incrementing the metabolic activities in the rat uterus.

Following the administration of estradiol, a cascade of biological events ensues in the tissue, e.g., hypertrophy, imbibition of water and eventually cell division. At the subcellular level a series of time-dependent events occur (9).

The mechanism of action of estrogens in target tissues is related to the presence of cytoplasmic receptor which bind estradiol as it enters the cell. The binding of the hormone to the receptor forms a complex which is thought to undergo a

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conformational change in its structure facilitating its transfer to the nucleus (8) and retention on specific nuclear acceptor sites in chromatin. Once bound, the nuclear estrogen receptor complex interacts with nuclear chromatin to alter gene expression in target cells (17), which leads to an enhancement of the production of macromolecular components and, if the receptor complex remains in the nucleus for a sufficient period of time (4, 6), true growth of the target tissues is produced.

This paper describes the effect of the implantation of 17 β-estradiol (E_2) oil solution on ovariectomized adult rats in order to establish the relationship between the time course of the concentration of nuclear binding sites (Rn) and the obtention of estrogenic responses, e.g., DNA and cytosolic progesterone receptor synthesis.

Materials and Methods

Animals and tissue preparation. — Adult female Sprague-Dawley rats (200-250 g b.w.) were maintained in 12 h light and 12 h darkness, with water and food available *ad libitum*. Ovariectomy was performed under ether anaesthesia and the animals were used 1 week later. At this time they were implanted subcutaneously with corn oil or estradiol-17β (Sigma) as solution (equivalent to 25 μg E_2/capsule) in Silastic capsules. Rats implanted with E_2 were divided into two subgroups (*a* and *b*): capsules either remained *in situ* during the whole experiment (a), or capsules remained *in situ* for only 48 h (b). All rats were decapitated at chosen times and the two uterine horns were collected, trimmed and weighed. The uteri were placed in 3 ml of ice cold TEDG buffer (10 mM TRIS-HCl, 1.5 mM EDTA- Na_2, 0.5 mM dithiothreitol, 10% glycerol, pH = 7.4) and were homogenized in an all-glass Potter- Eveljheim homogenizer at 0-4° C and 200-500 rpm with 30 s intervals for cooling. The homogenates were centrifuged at 0-4° C and 850 *g* for 15 min and the supernatants 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* uterine pellets were resuspended in TEGD buffer, filtered through cheesecloth, separated into two aliquots for measurement of unoccupied or total nuclear oestrogen receptors and then centrifuged at 850 *g*. One pellet was washed twice in an excess of TEDG buffer, when the unbound nuclear estrogen receptor (f-Rn) was assayed. The pellet resultant from other aliquot was washed twice in an excess of TE buffer (10 mM Tris-HCl, 1.5 mM EDTA-Na_2, pH 7.4) when the total (t-Rn) (bound and unbound) nuclear estrogen receptor was measured. The final pellets were resuspended in buffer TEDG or TE respectively and used as the nuclear myofibrillar fraction for nuclear estradiol binding.

Assay of cytosolic progesterone receptor. — The method described by VU HAI and MILGROM (15) was used with minor modifications. Briefly, 100 μl uterine cytosol aliquots, were incubated for 18 h at 4° C with 20 nM ^{3}H-R5020 (17 α-methyl-^{3}H Promegestone, sp.act.77.1 Ci/mmol, New England Nuclear) either alone for total binding or with 4 μM cold R5020 (New England Nuclear) for non-specific binding. A suspension of dextran-coated charcoal (0.05 and 0.5% respectively) was added to remove unbound steroids. After a 15 min incubation the samples were centrifuged at 4° C for 15 min at 3,000 *g* and an aliquot mixed with scintillation cocktail (0.5% PPO and 0.025% POPOP in toluene) and counted for radioactivity in a Packard Spectrometer (mod 3390) at 37% efficiency. Determinations on each uterus were performed in quadruplicate.

Assay of nuclear oestrogen receptor. — The amount of unbound nuclear binding

sites was measured according to the method of ZAVA *et al.* (18). Briefly, 250 μl aliquots of the TEDG resuspended nuclear-myofibrillar fractions were incubated over-night at 0-4° C with 10 nM ^{3}H-E_2 ([2, 4, 6, 7- ^{3}H]-estradiol 17 β, sp. act. 90 Ci/mmol, New England Nuclear) with or without 2 μM DES (diethylestilbestrol, Sigma). The excess of unbound steroids was removed by means of 3 washes in 1 ml of TEDG buffer.

The total nuclear binding sites were measured by nuclear E_2 exchange assay (3). Briefly 250 μl aliquots of the TE resuspended nuclear-myofibrillar fractions were incubated with ^{3}H-$E_2$10 nM with or without 2 μM DES for 1 h at 30° C with shaking. After this period 1 ml of cold TE buffer was added to the incubation tubes and centrifuged at 0-4° C for 15 min. Pellets were washed twice in an excess of TE buffer.

The final pellets were extracted with scintillation cocktail and counted for radioactivity.

The occupied form of nuclear estrogen receptor (o-Rn) was obtained by subtracting the mean concentration of f-Rn from the mean concentration of t-Rn from each treatment group.

Determinations on each uterus were performed in quadruplicate.

Assay of protein and DNA. — Protein and DNA were measured by the LOWRY *et al.* (13) and BURTON (5) procedures, respectively.

Statistics. — Comparison between groups was made using the Student's t-test.

Results

The implantation of 17 β-estradiol in subgroup (a) animals caused a significant increase in uterine weight after 48 h (table I), which remained unchanged (p = 0.858) 48 h later. In this subgroup the DNA value increased significantly over 72 h and remained unchanged (p = 0.341) 24 h later. In rats of subgroup (b) the uterine weight decreased significantly over 96 h. However, the DNA value had not dropped with respect to the level obtained 48 h before the removal of the capsules. The RcP concentration in OVX rats, increased significantly 48 h after E_2 implantation, remaining unchanged (p = 0.868) 48 h later in subgroup (a) rats. In subgroup (b), the RcP value fell 24 h after the removal of the implants and it showed no significant differences in relation to the non-estrogenized animals at 96 h (table II).

Table I. *Effects of the implantation of 17β-estradiol (E_2) into one-week ovariectomized (OVX) adult rats on uterine weight and DNA content, with respect to length of time E_2 implants remains* in situ.
Number of quadruplicate determinations in parenthesis. Statistical differences were calculated by means of Student's t-test (*** p < 0.001; ** p < 0.01; * p < 0.05; n.s.: non significant differences, with respect to the group indicated by an asterisk).

Decapitation Time (h)		Treatment	Uterine weight (mg)	DNA (mg/uterus)
0	(9)	OVX	169 ± 8	0.326 ± 0.049
96	(6)	OVX, Oil 0-96 h	158.6 ± 7.6	0.338 ± 0.035
48	(12)	(OVX, E_2 0-48 h)*	301 ± 19	0.3468 ± 0.025
72	(11)	OVX, E_2 0-72 h	313 ± 15$^{n.s.}$	0.6388 ± 0.0429***
96	(10)	OVX, E_2 0-96 h	305 ± 10$^{n.s.}$	0.5829 ± 0.0368***
72	(11)	OVX, E_2 0-48 h	238.6 ± 7**	0.42108 ± 0.0348*
96	(8)	OVX, E_2 0-48 h	190 ± 8***	0.5035 ± 0.0276***

Table II. *Effects of the implantation of 17β-estradiol (E_2) into one-week ovariectomized (OVX) adult rats on uterine cytosolic progesterone receptor (RcP), total nuclear estrogen receptor (t-Rn) and occupied form of nuclear estrogen receptor (o-Rn) content, with respect to length of time E_2 implants remain* in situ. Number of quadruplicate determinations in parenthesis. Statistical differences were calculated by means of Student's t-test (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s.: non significant difference, with respect to the group indicated by an asterisk).

Decapitation Time (h)		Treatment	fmol RcP/mg prot.	fmol t-Rn/mg DNA	fmol 0-Rn/mg DNA
0	(9)	OVX	87 ± 12	1.134 ± 83	25
96	(6)	OVX, Oil 0-96 h	68 ± 8	1.224 ± 85	12
48	(12)	*(OVX, E_2 0-48 h)	441 ± 15	1.000 ± 168	685
96	(10)	OVX, E_2 0-96 h	447 ± 38$^{n.s.}$	660 ± 43$^{n.s.}$	521
72	(11)	OVX, E_2 0-48 h	240 ± 19***	908 ± 117$^{n.s.}$	837
96	(8)	OVX, E_2 0-48 h	72 ± 7***	548 ± 77*	10

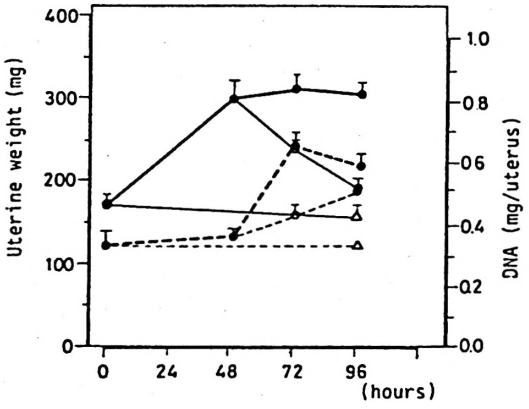

Fig. 1. *Time course of weight (full line) and DNA (broken line) in the uterus of: one-week ovariectomized rats (⊙); one-week ovariectomized rats after E_2 implantation.*
Implants remain *in situ* until decapitation time (●); one-week ovariectomized rats after E_2 implantation and removal after 48 h (○); and one-week ovariectomized rats after oil implants (△). Each point indicates mean ± SE of at least six quadruplicate assays.

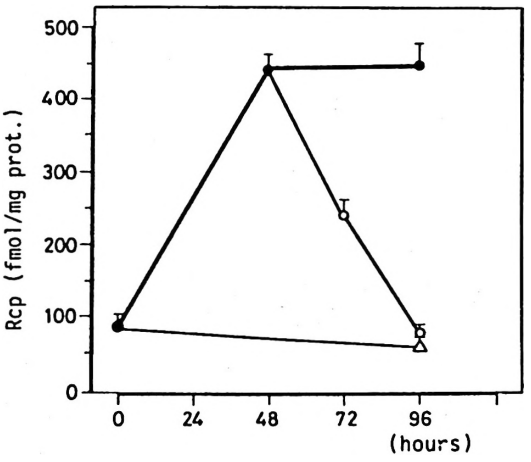

Fig. 2. *Time course of the concentration of the uterine cytosolic progesterone receptor (RcP).*
Symbols have the same meaning as in fig. 1. Each point indicates mean ± SE of at least six quadruplicate assays.

The t-Rn level remained unchanged ($p = 0.465$) but the o-Rn level increased significantly 48 h after E_2 implantation. In subgroup (b), the t-Rn concentration showed no significant difference ($p = 0.655$) with respect to the value obtained 24 h before removing the capsules. However, the o-Rn level still remained high. Both values decreased significantly 48 h after the removal of E_2 implants. (table II).

There was no significant difference between the values obtained for OVX rats and for those implanted with oil.

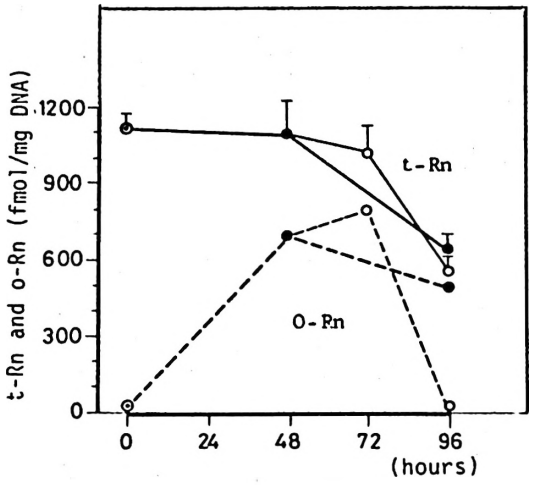

Fig. 3. *Time course of the uterine concentration of total nuclear estrogen receptor (t-Rn) (full line) and occupied nuclear estrogen receptor (o-Rn) (broken line).*

Symbols (⊙) (●) and (○) have the same meaning as in fig. 1. Each point for the (t-Rn) indicates mean ± SE of at least six quadruplicate assays. Each point for the (o-Rn) is derived by subtracting the mean concentration of (f-Rn) from the mean concentration of (t-Rn) from each treatment group.

Discussion

The implantation of 17 β-estradiol caused the weight to almost double in 96 h. This increase is in agreement with the limits previously described for the fluctuations in the uterine weight during the rat estrous cycle (14, 16). The removal of E_2 implants caused an atrophy in the uterus. Thus, 48 h later, an almost complete return to the values before E_2 implantation was obtained. The level of DNA showed an increase 72 h after the implantation of E_2. This indicates that the synthesis of DNA is delayed in relation to the uterine hypertrophy. This situation has been previously described (10). The removal of the E_2 implants also showed an increase in the level of DNA both 24 and 48 h later. It suggests that the synthesis of DNA seems to depend on the nuclear receptor content more than on the plasmatic E_2 level. The former will be discussed later.

The relatively high uterine RcP value obtained in OVX animals indicates that this level could represent a RcP population whose synthesis does not seem to depend on the ovaric estradiol. It is likely that this level is the result of a constitutive property of the target tissue cells to synthesize RcP, and that in the presence of estradiol this property would be activated. Thus, the E_2 implantation caused a noticeable increase in the concentration of RcP. It would represent the estrogen-dependent RcP population (D-Rp), which has been defined in this manner by several authors (1, 2, 7, 11). Because the RcP value was significantly higher in subgroup (b) than in the control rats 24 h after the removal of E_2 implants and also because a short half-time (20-30 min) for serum estradiol after the removal of subcutaneous estradiol implants has been described (12), this value suggests that the plasmatic E_2 that entered into the target tissues is not the only factor to be considered for the induction of RcP synthesis. Moreover, the significant drop obtained in the RcP value 48 h after the removal of E_2 implants suggests that the (D-Rp) population could have a short semi-desintegration time, perhaps due to a processing activity which would regulate the RcP content together with its synthesis.

The results obtained for the nuclear receptors demonstrate that the concentration of t-Rn showed no significant difference between the values obtained in both subgroups. However, the o-Rn behaved differently. The lowest levels of o-Rn obtained before E_2 implantation and 48 h after the removal of these E_2 implants indicate that all the receptors which were measured at these two times were predominantly unoccupied nuclear receptors. Likewise, the o-Rn level obtained 24 h after the removal of the E_2 implants indicates the persistence of these complexes

in the nucleus for a relatively long time. This result could explain why the RcP and DNA contents were significantly higher than in the non-estrogenized rats, even 24 h after the removal of the E_2 implants. It suggests that the estradiol operativity causing certain responses in the target tissues would be related to the o-Rn level. Thus, while the nuclear content of the receptors was predominantly o-Rn, these responses would be obtained.

Figures 1, 2 and 3 show a greater similarity between the RcP and o-Rn levels than between the o-Rn and DNA. This suggests that just as the DNA synthesis is the slowest response obtained after the administration of E_2 (8), the decrease in the production of DNA that occurs in the absence of o-Rn could also be a slow response.

Resumen

Se describe el efecto de la implantación de 17 β-estradiol (E_2) sobre el contenido, en el útero de ratas adultas ovariectomizadas, del receptor citosólico progesterona (RcP), del DNA y del receptor nuclear estrogénico en su forma ocupada (o-Rn). Los animales implantados con una solución oleosa de E_2 son separados en dos subgrupos: en uno (a), las cápsulas se mantienen hasta el momento de la decapitación, en el otro (b), se retiran 48 h después de su implantación. La implantación de E_2 causa incrementos significativos en el peso uterino, RcP y o-Rn 48 h más tarde. El contenido en DNA incrementa 72 h después y la concentración en receptores nucleares estrogénicos totales (t-Rn) no presenta diferencias significativas respecto a animales no estrogenizados. En los animales del subgrupo (a) estos valores permanecen estables hasta las 96 h. En el subgrupo (b), la eliminación de los implantes de E_2 origina una disminución en la concentración de RcP, o-Rn y en el peso, alcanzando valores similares a los obtenidos antes de la implantación. Sin embargo, el DNA permanece más alto y la concentración de t-Rn es inferior en relación a los valores obtenidos en animales no estrogenizados. Estos resultados sugieren que la síntesis de RcP y DNA que es inducida por el E_2 estaría relacionada con el nivel de o-Rn, aunque parece existir una relación mayor entre la evolución temporal del o-Rn y del RcP que entre el o-Rn y el DNA.

Palabras clave: Implantación de 17 β-estradiol, Receptores estrogénicos nucleares ocupados, Receptores citosólicos de progesterona, DNA.

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