NUCLEAR TRANSLOCATION OF THE RAT PITUITARY CYTOSOL 17β-ESTRADIOL RECEPTOR

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1. Introduction

The association of 17β-estradiol with an 8 S binding protein in the cytoplasm is a generally acknowledged primary step in the cellular action of the steroid hormone. The complex crosses the nuclear membrane either before or after conformational change of the protein to a 5 S species [1]. The presence of an estrogen receptor in the pituitary is well documented [2–5] and the ontogeny of the pituitary cytosol 17β-estradiol receptor was described recently [6]. In addition, the actions of estrogen on the hypothalamus–pituitary axis have been studied extensively. Little is known, however, about the molecular mechanisms of LH–RH stimulated gonadotropin release modulated by 17β-estradiol at the pituitary level.

The present experiments were designed to study the translocation of the pituitary estrogen–receptor complex into the nucleus in rats under in vivo and in vitro conditions. Furthermore, the effect of castration on the translocation process in the hypothalamus and pituitary of female rats was investigated. We were prompted to report these data since they add further evidence that estrogen stimulated translocation of the pituitary 17β-estradiol receptor from the cytosol to the nucleus is a primary event in the mechanism of action of this steroid.

2. Materials and methods

Rats of the Sprague Dawley strain (Him: OFA (SD) SPF, Forschungsinstitut für Versuchstierzucht, University of Vienna, Austria) were used throughout this study. The animals were housed in a temperature-controlled room lighted for 12 h/day and unrestricted access was provided to food and water. Intact adult female animals weighing 175–200 g and rats 30 days after castration, respectively, were used in the in vivo translocation experiments. 17β-Estradiol, 1.5 μg in 0.5 ml 2.5% aqueous alcohol, was injected i.p. 20, 40, 60 and 120 min before killing the rats. Control animals were treated with estrogen-free aqueous alcohol. In the experiments with castrated animals the rats were sacrificed 60 min after the injection. The animals were killed by decapitation, hypothalami and pituitaries were excised and placed in chilled TMK-buffer (0.01 M Tris, 0.0015 M $MgCl_2$, 0.01 M KCl, pH 7.2). The posterior lobes of the pituitaries were removed and discarded. All further procedures were done at 0–4°C. Six glands/ml TMK-buffer were homogenized by 10 strokes at 500 rev/min in a glass–Teflon Potter Elvehjem type homogenizer. The homogenate was centrifuged at 800 × *g* for 10 min. The pellet which contained the nuclear fraction was washed 3 times by resuspension in 5 ml TMK-buffer

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and recentrifugation. Nuclei obtained from 36 anterior pituitaries were resuspended in 10.8 ml TMK-buffer. The purity of the preparation was checked by phase-contrast, fluorescence and electron microscopy, which revealed intact nuclei with only little contamination. The supernatant of the 800 × *g* centrifugation of the total homogenate was spun in a Beckmann Spinco LS 2-65 B preparative ultracentrifuge at 110 000 × *g* (av.) for 60 min in order to obtain the cytosol fraction.

Binding assays of the cytosol preparations at various times after estrogen administration were performed as described previously [6]. Briefly, 0.4 ml cytosol samples were incubated with 17β-[^{3}H] estradiol at concentrations ranging from 2.0×10^{-9} to 3.5×10^{-11} M in the presence and absence of a 1000-fold excess of unlabeled estrogen. After incubation overnight 0.2 ml of a charcoal suspension (0.6% charcoal Norit-A, 0.06% Dextran T-70, 0.1% gelatine, 0.1 M phosphate buffer, pH 7.4) was added. The incubation mixture was centrifuged and the radioactivity of the supernatant was determined.

Quantitative estimation of the nuclear receptor–hormone complex was performed according to methods described previously [8] by incubating 1.0 ml nuclei suspensions obtained at various times after the estrogen injection with 17β-[^{3}H]estradiol at concentrations ranging from 0.5×10^{-9} M to 1×10^{-8} M, in the presence and absence of a 1000-fold excess of unlabeled 17β-estradiol to account for unspecific binding. The incubation was performed in triplicates at 37°C for 30 min. The reaction was stopped by transferring the incubation tubes to an ice-bath and adding 3 ml chilled TMK-buffer. After centrifugation at 800 × *g* the pellet was washed 2 times with 3 ml TMK-buffer by resuspension and recentrifugation. The pellet was extracted with 3 ml absolute ethanol by vortexing and was left at room temperature for 10 min. Thereafter the tubes were centrifuged at 3000 × *g* for 10 min, and the radioactivity of the supernatant determined.

The number of 17β-estradiol-binding sites and apparent affinity constants were estimated by Scatchard plot analysis [7].

Experiments on the in vivo translocation were also done with 15-day old female rats. Groups of 30 animals each were injected s.c. with 25 μg 17β-estradiol in 0.5 ml physiological saline solution containing 10% ethanol kept at 37°C. Control animals were killed after an injection with an estrogen-free solution. The estrogen-treated animals were sacrificed 30 min and 60 min, respectively, after the injection. Preparation of cytosol samples, incubation with 17β-[^{3}H] estradiol and sucrose-gradient centrifugation was carried out as described previously [6].

In experiments on the in vitro translocation 30 female rats weighing 175–200 g were used 3 days after ovariectomy. Cytosol and nuclei fractions were obtained as described for the in vivo experiments. The cytosol (4.5 ml) was incubated with 17β-[^{3}H] estradiol (1×10^{-9} M) for 2 h. Saturation of binding sites was recorded after 90 min incubation at 4°C (our unpublished data). Thereafter, the cytosol was treated with 2.25 ml charcoal suspension for 20 min and the ^{3}H-labeled cytosol was used for further experiments after centrifugation at 3000 × *g* for 10 min. The nuclear pellet was resuspended in TMK-buffer and aliquots were filled into incubation tubes. Subsequent to centrifugation (800 × *g*) these nuclear pellets were resuspended in aliquots of ^{3}H-labeled cytosol samples. After mixing incubation was done in a water bath shaker at 25°C for 3, 5, 10, 15 and 30 min. The reaction was stopped by adding 8 vol. TMK-buffer at 0–4°C. The mixture was centrifuged at 3000 × *g* for 10 min and the radioactivity of the supernatant was determined. The pellet was extracted with 3 ml absolute ethanol to estimate nuclear bound radioactivity.

3. Results and discussion

The translocation of the pituitary cytosol 17β-estradiol–receptor complex to the nucleus was monitored by estimating the number of estrogen-binding sites in the cytosol and by determining the nuclear binding capacity after the administration of a single dose of 17β-estradiol (fig.1). Apparent affinity constants of 2.3×10^{10} M^{-1} and 1.0×10^{9} M^{-1}, respectively, of cytosol and nuclear binding sites were estimated. A rapid depletion of cytosol 17β-estradiol receptor sites was recorded. About 90% of the pituitary cytosol binding sites are lost after administration of estrogen. The depletion period reaches a low about 20 min after estrogen. Thereafter a slow replenishment of binding sites occurs and

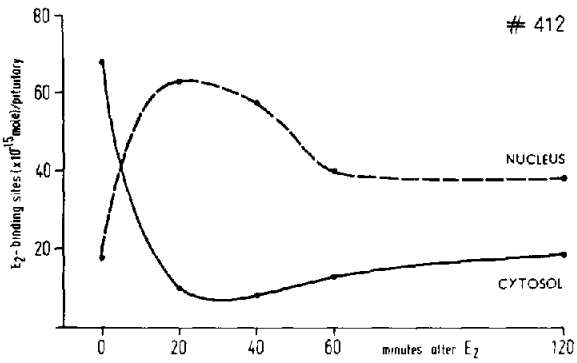

Fig.1. Translocation of the pituitary 17β-estradiol receptor complex from the cytosol to the nucleus after a single s.c. injection of 1.5 μg estrogen in adult female rats.

26% of the control levels are attained 120 min after estrogen treatment. Concomitantly, a 3.5-fold increase over control levels of nuclear binding was noted and maximal nuclear receptor concentration was registered 20 min after the application of 17β-estradiol. At 60 min 63% of maximal nuclear binding was noted. Thereafter a slow loss of 17β-estradiol from the nucleus was found and 120 min after estrogen administration, nuclear binding was still elevated 2-fold over control levels. It is interesting to note that the loss of bound estrogen from the nucleus between 20 min and 40 min after estrogen is faster than the replenishment of cytoplasmatic binding capacity during the same period. Data shown in fig.1 suggest that long-term retention of the nuclear estrogen–receptor complex may be necessary for delayed actions of the steroid hormone. In addition, the concomitant loss of bound 17β-estradiol from the nucleus and the replenishment of cytosol binding protein may indicate a direct relationship between these two events. These results provide further evidence that an in vivo injection of 17β-estradiol translocates a cytoplasmic receptor complex into the nucleus. The time-course of the accumulation of nuclear estrogen–receptor complex described in the present communication is different from patterns reported for immature rats recently [8]. Specific nuclear uptake was maximal 1 h after estrogen application and about 6-times higher than control levels. This discrepancy may be due to the fact that the translocation process is dependent upon the developmental stage of the animal, since changes in the pituitary nuclear uptake of 11β-[6,7-^{3}H] methoxy-17-ethinylestradiol in rats at different ages were described recently [9].

Data shown in table 1 suggest that the estrogen stimulated loss of cytoplasmic receptor content depends on endogenous steroid hormone levels. A depletion of 17β-estradiol-binding sites in the pituitary cytosol to 7.5% and 1.6% of control levels, respectively, is recorded in intact and castrated adult female rats 60 min after a single s.c. injection of estrogen. Similar effects can be noted in experiments with the hypothalamus (data not shown). The cytosol receptor is depleted to 23.1% and 8.6% of control levels, respectively, in intact and ovariectomized rats. On the other hand no significant changes of the apparent affinity constants are registered (table 1).

In an attempt to evaluate the biological role of the pituitary 4–5 S 17β-estradiol-binding protein found in the cytosol at early stages of development of the rat [6] we investigated the estrogen stimulated loss of this binding component in 15-day old female rats (fig.2). Thirty minutes after a single s.c. injection of 25 μg 17β-estradiol only some 60% of control levels of the 4–5 S moiety could be found. At 60

Table 1
Apparent affinity constants (K) and number (n) of cytosol 17β-estradiol receptor sites of anterior pituitary in adult female rats before and 30 days after castration

	K ($\times 10^{10}$ M^{-1})		n ($\times 10^{14}$ mol/mg protein)	
	Intact	Castrated	Intact	Castrated
Control	2.3	3.2	18.6	31.0
Estrogen-treated	3.8	1.1	1.4	0.5

Data were obtained in control animals and rats 60 min after s.c. of 1.5 μg 17β-estradiol

min an almost complete depletion was noted. The effect seems to be dose dependent, since the injection of 5 μg 17β-estradiol provokes only a 20% loss and a single s.c. administration of 0.5 μg estrogen does not affect the 4–5 S binding component (our unpublished data). On the other hand, a complete loss of the 8 S moiety was observed 30 min after a single injection of 0.5 μg, 5 μg and 25 μg, respectively, of 17β-estradiol. These data suggest a translocation of the 8 S and 4–5 S cytosol receptors. But, the present data do not allow to rule out that the 4–5 S moiety may be identical with fetal rat estradiol binding plasma protein [14]

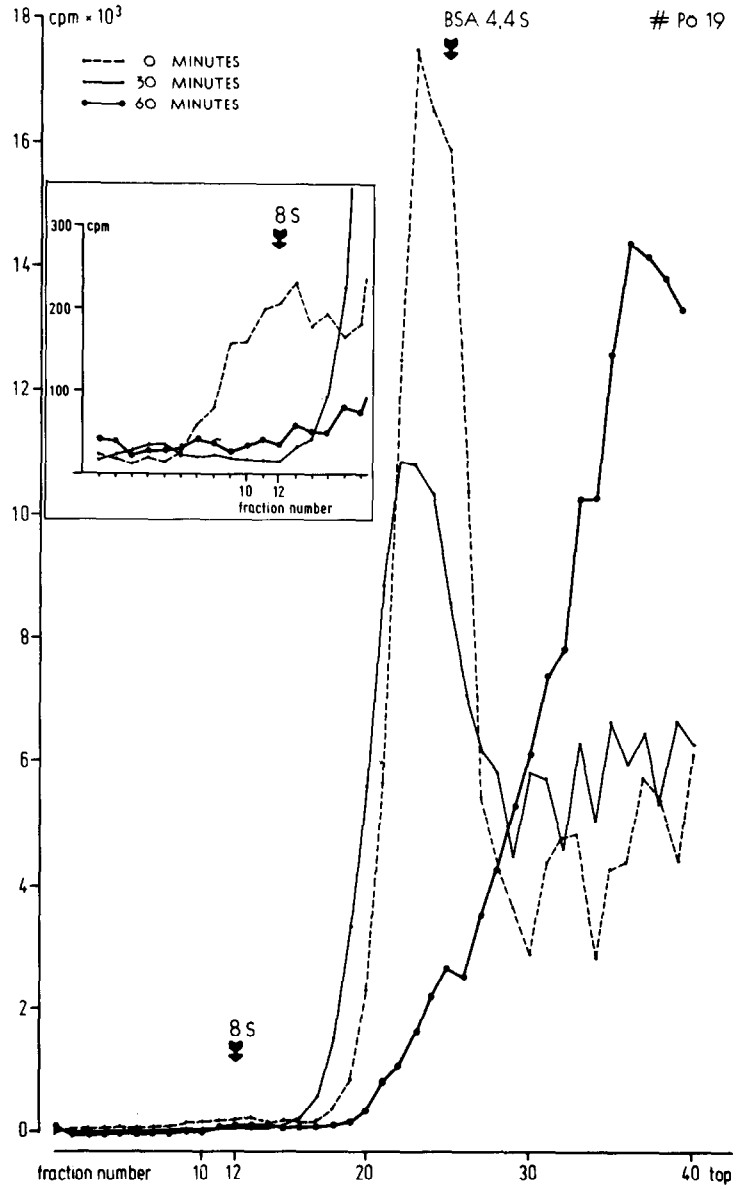

Fig.2. Sucrose-gradient analysis of pituitary 17β-estradiol-binding protein of 15-day old rats at various times after a single s.c. injection of 25 μg 17β-estradiol.

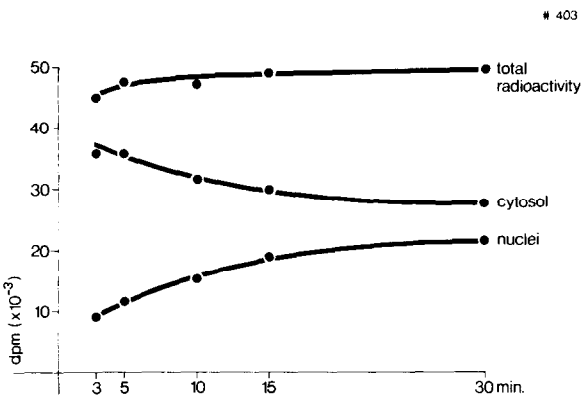

Fig.3. In vitro translocation of the pituitary 17β-estradiol receptor complex obtained from adult female rats 3 days after castration.

due to a possible contamination of freshly prepared pituitaries with plasma proteins.

Similarly, data recorded for in vitro experiments performed with cytosol and nuclear fractions obtained from adult female rats (fig.3) indicate nuclear translocation of the cytosol 8 S binding protein, which was previously shown to be the sole cytoplasmic estrogen receptor in adult rats [6]. Discrepancies observed in the patterns of in vivo (fig.1) and in vitro (fig.3) translocation experiments may be due to differences in the transformation step of the cytosol receptor [1] prior to the translocation.

Data of the present experiments combine to suggest that the translocation of the 8 S moiety of the 17β-estradiol–receptor complex of rat anterior pituitary from the cytosol to the nucleus may be one of the primary steps involved in the modulation by estrogen of LH–RH stimulated gonadotropin release [12] and in the estrogen provoked prolactin release, respectively, [13]. But, further work is necessary to elucidate this mechanism and to prove the biological role of the 4–5 S 17β-estradiol-binding protein found in the cytosol of immature rats.

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