Vol. 83, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 14, 1978 Pages 327-333

ESTROGEN ACTION IN THE MOUSE UTERUS: AN ADDITIONAL NUCLEAR EVENT

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Received May 22,1978

Summary: An in vivo injection of ^{3}H-estradiol to an ovariectomized mouse resulted in the appearance of two increases of nuclear receptor: the first at 1 hr and the second occurring at approximately 7-8 hrs post-injection. These findings were substantiated by quantifying cytoplasmic and nuclear receptor levels with the exchange assay after injections of unlabeled estradiol. Cytosol receptor levels were depleted at 0.5-1 hr and replenished from 2-10 hr with values at 15-24 hr significantly greater than those at zero time. Nuclear receptors decreased precipitously to near control values after the initial translocation event. At 7-8 hr a second smaller nuclear increase occurred which then declined to near zero levels. Both of these nuclear events appear to be dose related. Similar experiments using cycloheximide did not abolish either of these events, but did significantly diminish the cytosol receptor replenishment.

INTRODUCTION

Recent studies have suggested that estrogen action in the uterus involves two responses (1): an early response occurring from 1-4 hr and a later response at 6-24 hr. Estrogenic compounds such as 17β-estradiol or diethylstilbestrol are considered potent estrogens since they are able to elicit both the early and late responses. A number of reports have linked early estrogen action with estrogen receptor translocation to the nucleus at 1-2 hr (2). The second response at 6-24 hr is associated with estrogen receptor retention in the uterine nuclei (3,4). The possibility that the " true uterine growth " phase of estrogen action occurring at 6-24 hr could also be linked to a second nuclear receptor increase has been investigated. In this preliminary report, we

0006-291X/78/0831-0327$01.00/0

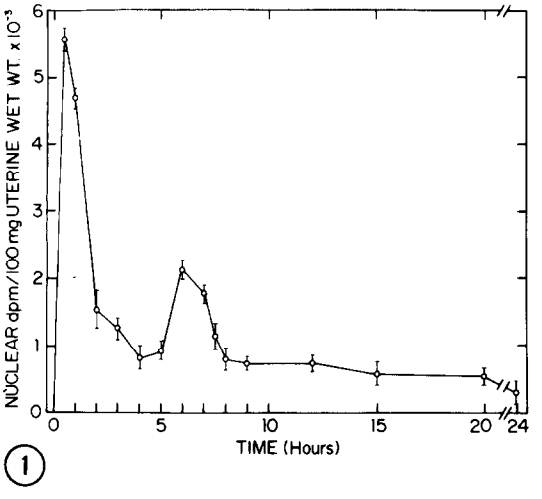
demonstrate using the ovariectomized mouse uterus that there are two nuclear receptor increases, the first occurring at 1-2 hrs and the second event at 7-8 hrs. These results raise the possibility that a second nuclear accumulation of estrogen-receptor complex is involved in sustained hormone action.

METHODS and MATERIALS

Female CD-1 mice were obtained from the Charles River breeding laboratories (Wilmington, MA.). Where appropriate, animals were ovariectomized 5 or 7 days prior to sacrifice. Immature mice were bred in the NIEHS animal facility. 17β Estradiol (2,4,6,7-^{3}H) (New England Nuclear) or 17β-estradiol (Steraloids, Inc.) were administered intraperitoneally as saline solutions (0.15 M NaCl) containing 2% ethanol. Cycloheximide (Sigma Chem.) was administered identically except the solution was 10% with respect to ethanol. When unlabeled estradiol was injected, the cytosol receptor binding was assayed by the protamine sulfate precipitation technique (5) and nuclear receptor levels were determined using the nuclear exchange assay (6). Direct ^{3}H-estradiol uptake was assessed as previously described (7). The diphenylamine reaction as modified by Burton (8), was used to determine the DNA content of the nuclear samples.

RESULTS and DISCUSSION

Our interest in the CD-1 mouse as a model for studying hormonal carcinogensis has prompted us to in vestigate some of the basic properties of the estrogen receptor system in the mouse uterus. The pattern of uterine nulcear radioactivity after an injection of ^{3}H-estradiol (3.0 μg/Kg) is shown in Fig 1. There is an appreciable loss of radioactivity from the nuclear compartment after the initial translocation. The second increase occurs at 7-8 hr and represents approximately 20% the size of the first peak. Similar experiments using 24 day old immature mice gave the same pattern whether uterine wet weight or DNA were the normalzing indices. The amount of DNA per assay time point remained relatively constant over the experimental time studied. Injection experiments (not shown) that were performed with an increased estrogen dose (isotopically diluted to 10 μg/Kg) produced a dimunition of both nuclear increases. The distribution pattern of cytosolic and nuclear receptor as assessed by the exchange assay after an injection of unlabeled estradiol is shown in Fig 2. In this case, the nuclear distribution was similar to that determined by the ^{3}H-



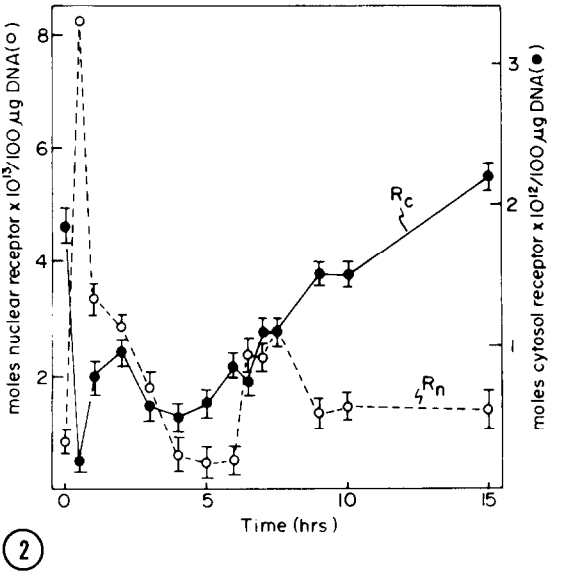


Fig. 1. Sexually mature CD-1 mice, ovariectomized for 5 days were injected I.P. with 3 µg/Kg dose (12.7 µCi) of ^{3}H-estradiol. Three animals were sacrificed for each time point. The nuclear fraction was isolated and radioactivity determined. Data points represent the mean ± S. E. of triplicate determinations.

Fig. 2. Sexually mature mice castrated for 5 days (wt 30 grm.) were injected I.P. with a 10 µg/Kg dose of 17β-estradiol. The compound was injected in a 100 µl volume of a saline solution containing 2% ethanol. At various times after the injection the animals (2 per time point) were sacrificed and the uterine nuclear and cytoplasmic estrogen receptor levels determined. Individual assays are described in Methods and Materials. Data points represent the mean ± S. E. of triplicate determinations for receptor binding at each time point.

estradiol injection (Fig 1.). The receptor values of the saline injected controls never varied greater than 2% over the course of the experimental period and showed no indication of nuclear increases or cytoplasmic depletion.

In order to determineif both these nuclear events were dose dependent, the experiments described in Fig 3 were performed using increasing doses of injected 17 -estradiol. It is apparent that both the first and second nuclear events increased in magnitude as a function of the increase in the dose of hor-

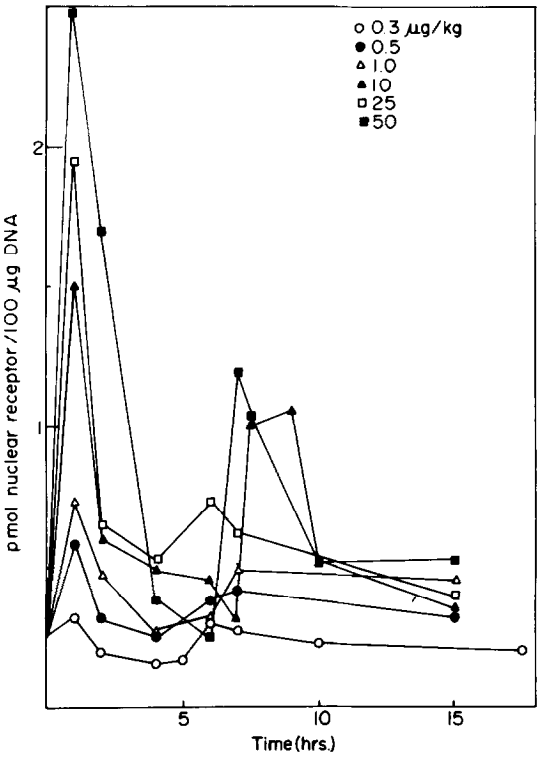


Fig. 3. CD-1 Mice which were 5 day castrate, were injected with the following doses of 17β-estradiol (○) 0.3 µg/Kg, (●) 0.5, (△) 1.0, (▲) 10, (□) 25, (■) 50. The procedures were the same as described in the legend to Fig 1.

mone. The second event appears as a rise at 7-8 hr which then plateaus to 15 hr. This rise and plateau occurred when 1.0 µg/Kg or less dose of estradiol was injected. At greater doses there was a substantial rise and fall in these receptor levels to values at 15 hr that are slightly above control.

The appearance of the second nuclear peak coincident with the significant rise in cytosol receptor replenishment is evident in Fig 2. The possibility that additional cytoplasmic receptor produced this increase was tested by injecting 2 mg/Kg of cycloheximide 1 hr before the estradiol injection. This procedure has been reported by Cidlowski and Muldoon to be effective in blocking cytosol receptor replenishment (9). Results of those experiments are ill-

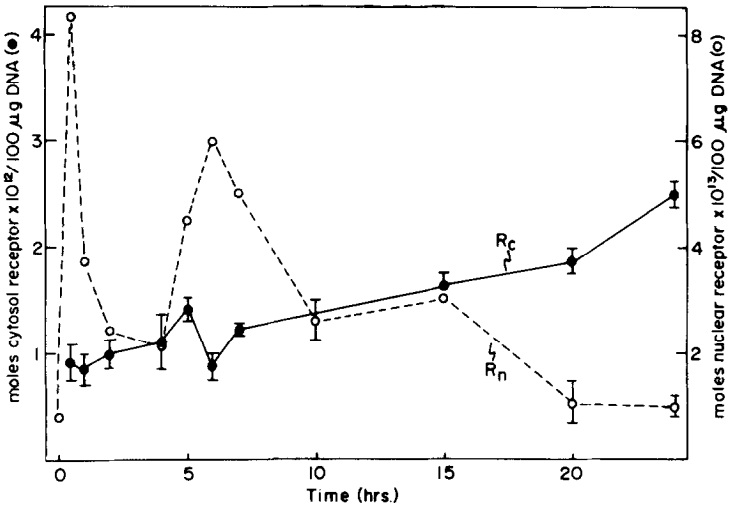


Fig. 4. Castrate (7 day) CD-1 mice were injected with 2 mg/Kg doses of cycloheximide and 1 hr later with 5 µg/Kg of 17β-estradiol. Zero time controls received the cycloheximide injection 1 hr prior to sacrifice. Receptor levels were analyzed as described in Methods and Materials.

ustrated in Fig 4. Comparing Fig 4 with Fig 2, it appears that the cycloheximide did not alter the general pattern of nuclear receptor translocation. Quantification of the cytosol receptor levels at 7-8 hrs could not account for the second nuclear increase in Fig 4.

This study suggests that in the gonadectomized mouse uterus, estrogen action may involve two translocations of receptor complex to the nucleus. The initial event at 1 hr is quite similar to that previously described for the immature rat uterus (2,3,4) or the castrate mouse uterus (10). However, the second nuclear event in the mouse uterus is a new finding. The fact that a portion of the cytosol receptor replenishment can be blocked with cycloheximide (9) was used to determine if the second nuclear increase observed in these studies required synthesis of new cytosolic receptor (9) or recycling of nuclear receptor (11). The results shown in Fig 4 indicate that a portion of the second nuclear increase (38%) could be accounted for by cytosol while the remainder is cytosol independent and may be formed from the recycling of nuclear receptor. These

findings are consistent with a model proposed by Cidlowski and Muldoon (12).

It is difficult at the present time to determine an exact role or function for this second nuclear peak. However, we have evidence (Korach et al, manuscript in preparation) that in the mouse uterus estriol is only capable of producing the first translocation event at 1 hr and not the second nuclear increase. Since studies have shown estriol to be a "short acting estrogen" (1), this would suggest the second nuclear increase is linked with "true uterine growth" (13) in the mouse uterus. Another possibility is that events or factors are occurring at 7-8 hr in the nucleus which could stabilize the hormone nuclear receptor complex, thereby resulting in an increased level at that time, which could be critical for uterine growth. We are presently involved with salt extraction procedures, rates of nuclear receptor processing and RNA polymerase patterns in order to determine the exact role of this second event in estrogen action in the mouse uterus.

ACKNOWLEDGMENT

The authors would like to thank Ms. Patricia Mydlow for her expert technical assistance in performing the assays.

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