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# EARLY CHANGES IN RABBIT UTERINE PROGESTERONE RECEPTOR CONCENTRATIONS AND UTEROGLOBIN SYNTHESIS AFTER PROGESTERONE ADMINISTRATION

Tuula Torkkeli

Department of Biochemistry and Department of Clinical Chemistry,
University of Oulu, SF-90100 Oulu 10, Finland

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SUMMARY

Uterine cytosol and nuclear receptors were measured at various time intervals after one dose or two consecutive doses of progesterone and the receptor values compared with the course of induction of uteroglobin, a progesterone-regulated uterine protein. The data of this study suggest (i) that progesterone action involves receptor consumption and (ii) that in the rabbit uterus the biologic response is not a direct function of available cytosol or nuclear receptor sites.

The first step in the action of steroid hormones is the combining of the hormone with a cytoplasmic receptor protein. The receptor-steroid complex is then translocated to the nucleus where it regulates specific gene expression (1,2). According to this model, target tissue responsiveness to a steroid hormone is a reflection of distribution and concentration of the receptors between cell cytoplasm and nucleus (3-5). Progestins are known to decrease the concentration of their own receptors (5-9), while estrogens seem to promote the synthesis of cytosol progesterone receptors (1,2,6,10-13). Based on the latter findings, many previous studies of acute progesterone action on the mammalian uterus have been carried out under conditions where target tissue progesterone receptor levels have been artificially elevated by estrogen priming (1,2,6,7,14,15). Since estradiol is a powerful antiprogestin, at least in the rabbit uterus (16), an estradiol priming period may interfere with the subsequent action of progesterone. It was, therefore, the purpose of the present study to investigate the action of progesterone alone on the rabbit uterus, with special emphasis on acute changes in cytosol and nuclear progesterone receptors and their relationship to the biologic response, uteroglobin synthesis. As progestins are known to acutely depress progesterone receptor levels in many experimental systems (5-9) a second dose of progesterone was also administered at 24 h after the initial steroid injection, in order to find out whether the biologic

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response to progesterone is strictly proportional to the available cytosol and nuclear receptor sites.

## MATERIALS AND METHODS

Chemicals. Progesterone (4-pregnene-3,20-dione) was purchased from Steraloids, Inc., Wilton, NH, U.S.A., and hexylene glycol (2-methyl-2,4-pentanediol) from Fluka AG, Buchs, Switzerland. Tritium-labeled (spec. act. 53 Ci/mmol) ORG 2058 (16 α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione) was purchased from the Radiochemical Centre, Amersham, U.K.,and non-radioactive ORG 2058 was gift from Dr. E. de Jager (Organon, Oss, The Netherlands). Other chemicals were from Merck AG, Darmstadt, GFR, or the Sigma Chemical Co., St. Louis, MO, U.S.A., and were of the highest available purity grade.
Animals. Estrous New Zealand white rabbits weighing about 3 kg were used. Progesterone was injected intravenously in ethanol/sesame oil (1/9, v/v, 0.5 ml). Details of the treatments are specified in the legends to the figures.
Determination of uteroglobin. Radioimmunoassay (17) was used to measure uteroglobin content in the uterine fluid obtained by flushing of the uterine horns with 0.15 mol/l NaCl (17).
Measurement of cytosol and nuclear progesterone receptors. Tritium-labeled synthetic progestin, ORG 2058, was employed for receptor measurements, which were performed as previously described in detail (14,18). In short, a 105,000 x g supernatant fraction was used for cytosol receptor assays, where 6 different concentrations of [$^3$H] ORG 2058 (1-35 nmol/l) were used. The results were calculated by the method of Scatchard (19). Uterine nuclei were isolated by a hexylene glycol technique (18,20), and their receptor content measured by exchange procedure (14,18,21) using [$^3$H] ORG 2058 (20 nmol/l) as the ligand. In order to diminish non-specific binding of [$^3$H] ORG 2058 in the exchange assay, bovine serum albumin, (1 g/l), (Miles laboratories) was included in the washing buffer (18). Non-specific binding of labeled ORG 2058 in the receptor assays was estimated by parallel use of a 100-fold molar excess of nonradioactive ORG 2058 along with [$^3$H] ORG 2058. The average cellular receptor content was calculated assuming that each uterine cell contained 5.3 pg DNA (22).
Other methods. DNA was measured by the diphenylamine reaction (23) and the protein content according to Lowry et al. (24).

## RESULTS

Cytosol and nuclear progesterone receptor concentrations measured in this study in uteri of vehicle-treated estrous rabbits (83,000 and 30,000 receptors/cell, respectively) were higher than those previously found (14,18,25) in our laboratory using identical assay techniques. This difference may be due to the fact that older rabbits than previously were used in the present study.
Administration of a single intravenous dose of progesterone (5 mg/kg) to estrous rabbits resulted in a rapid decline in the cytosol receptor content (from 83,000 to 21,000 receptors/cell, Fig. 1) and in a concomitant initial nuclear receptor accumulation (from 30,000 to 40,000 receptors/cell, Fig. 2) at 0.5 h after progesterone injection. After the initial rise, the nuclear receptor content dramatically dropped to about one-third of the pretreatment levels (12,000 receptors/cell) and continued to decrease slightly until 24 h (Fig. 2). In con-

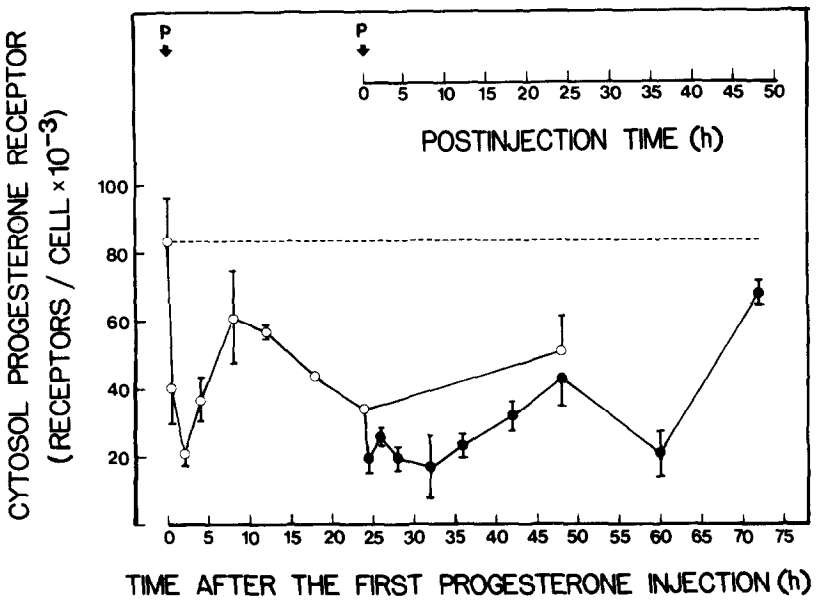


Figure 1. Changes in cytosol progesterone receptor levels in the rabbit uterus in response to a single dose (-o-) or to two consecutive doses (-•-) of progesterone (P, 5 mg/kg, intravenously). The steroid was given in ethanol/sesame oil (1/9, v/v, 0.5 ml). Measurement of cytosol progesterone receptor concentration was performed as outlined in the text. The dashed line represents the control level in vehicle-treated estrous rabbits (mean of 6 animals), and the points with vertical bars show the means ± S.E.M. of 3 experiments. In two cases the S.E.M. values were smaller than the width of the symbols. The time scale in the insert refers to the time elapsed after the second injection of progesterone.

trast, there was a clear replenishment phase in the cytosol receptor concentration between 2-8 h after progesterone treatment (Fig. 1). This replenishment was, however, incomplete and between 8-24 h, the cytosol receptor concentration again decreased to about 40% of the initial level at 24 h. Increased luminal fluid uteroglobin concentration was first detected at 4 h and peaked at 12 h after the first dose of progesterone, when it was about 20-fold higher than that in the controls (Fig. 3). This uteroglobin accumulation was short-lived and subsided to close to the control levels within 36 h of hormone administration.

In order to gain more information about the relationship of cytosol and nuclear progesterone receptor concentrations to the biologic response, a second dose of progesterone (5 mg/kg, i.v.) was given at 24 h after the first steroid injection. The cytosol progesterone receptor content was at this time 40% and the nuclear progesterone receptor level only 20% of the respective pretreatment values (Figs. 1 and 2). Changes in cytosol and nuclear progesterone receptor

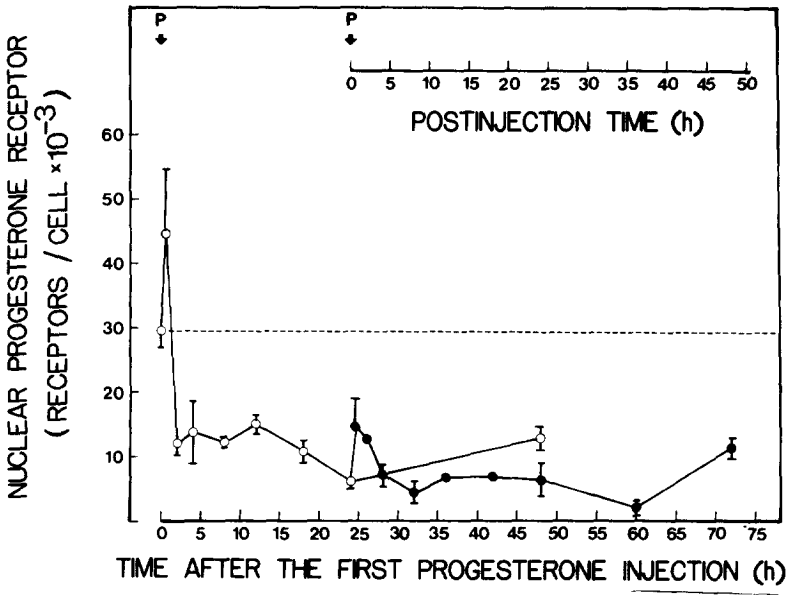


Figure 2. Changes in nuclear progesterone receptor levels in the rabbit uterus after administration of a single dose (-o-) or two consecutive doses (-•-) of progesterone (P, 5 mg/kg, intravenously). The steroid was given in ethanol/sesame oil (1/9, v/v, 0.5 ml). Determination of nuclear progesterone receptor content was conducted as described in the text. The dashed line represents the control level in vehicle-treated estrous rabbits (mean of 6 animals), and the points with vertical bars show the means ± S.E.M. of 3 experiments. In some cases the S.E.M. values were smaller than the width of the symbols. The time scale in the insert refers to the second dose of progesterone.

concentrations elicited by the second dose of progesterone were not completely identical with those after the first steroid dose, with two major differences: the nuclear receptor accumulation lasted for a longer time (at least 2 h) and the replenishment of cytosol receptor content occurred more slowly than after the first steroid injection. Moreover, the cytosol receptor content seemed to reach even higher values at later time points (Fig. 1).

In spite of the much lower cytosol and nuclear receptor concentrations prior to the second than the initial progesterone administration (Figs. 1 and 2), induction of uteroglobin secretion was not diminished, but was more pronounced after the second than after the first progesterone injection (peak values at 12 h: 21 vs. 39 µg/uterus, Fig. 3). The time courses for uteroglobin accumulation in the uterine luminal fluid were similar after both progesterone treatments, although uteroglobin levels seemed to stay elevated for a longer time after the second steroid dose.

## DISCUSSION

The receptor changes elicited in the present study by an acute progesterone administration in estrous rabbits were clearly different from those previous-

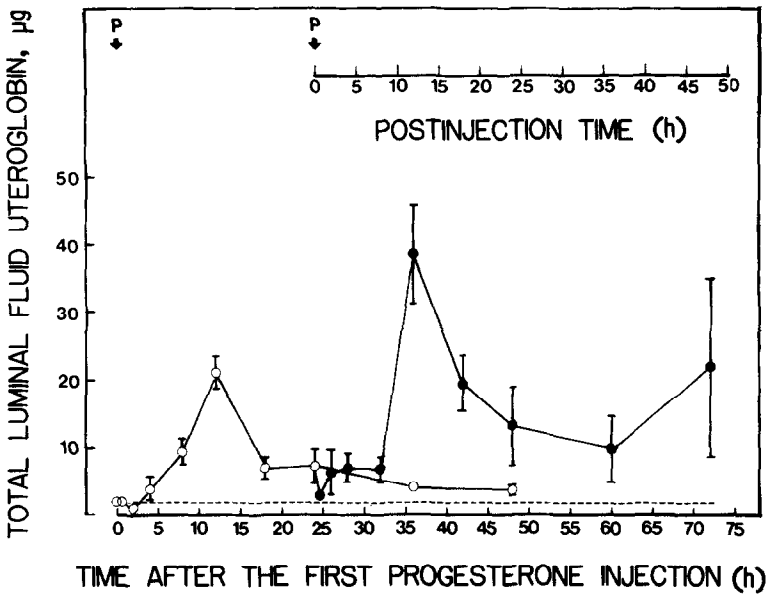


Figure 3. Luminal fluid uteroglobin concentration in the rabbit uterus after administration of a single dose (-o-) or two consecutive doses (-•-) of progesterone (P, 5 mg/kg, intravenously). The steroid was given in ethanol/sesame oil (1/9, v/v, 0.5 ml). Uteroglobin was measured by radioimmunoassay (17). The dashed line represents the control level in vehicle-treated estrous rabbits (mean of 6 animals), and the points with vertical bars show the means ± S.E.M. of 3 experiments. In some cases the S.E.M. values were smaller than the width of the symbols. The time scale in the insert refers to the second dose of progesterone.

ly found in estrogen-primed rabbits (14). The most striking difference was the dramatic decrease in the nuclear progesterone receptor content after a short-lived receptor accumulation in estrous rabbits (Fig. 2), which did not occur at all in the estrogen-pretreated animals (14). This finding is in harmony with our previous results indicating that under a chronic progesterone exposure, concomitant estradiol treatment seems to counteract the depressive effect of progesterone on the nuclear progesterone receptor content (25). A long term progesterone action in rabbit uterus is associated with nuclear receptor consumption or prosessing (25), which seems to be an important intermediate step between receptor translocation and the onset of biologic action of progesterone. The findings of the present study seem to support this hypothesis, since after an initial receptor accumulation, there was a clear decline in the nuclear receptor content following both the first and second progesterone administration.

The depression of uterine cytosol progesterone receptors at 24 h after progesterone administration, previously observed in estrogen-primed rabbits (14),

was also found in estrous rabbits. In the estrogen-primed rabbit, a single dose of progesterone brings about a rapid and long-lasting (at least 24 h) decline in the cytosolic cellular content of progesterone receptors, without a clear replenishment phase (14). By contrast, in estrous rabbits, this receptor reduction by 24 h from progesterone administration was preceded by a clear but incomplete replenishment phase between 2-8 h of hormone injection. A similar replenishment of cytosol progesterone receptors has been previously found in adrenalectomized-ovariectomized rats (26), where the replenishment was also followed by a decrease of cytosol receptor level between 12-24 h after progesterone administration. It is noteworthy that the cytosol progesterone receptor concentration did not decline to unmeasurable values after each steroid dose, in spite of a very high progesterone dose, but a level of about 20,000 receptors/cell was maintained. Interestingly, the same cytosol receptor concentration is maintained even after a 5-day progesterone administration (25), and it may represent the progesterone receptor concentration required for maintenance of the action of this steroid.

A single intravenous dose of progesterone brought about a 20-fold increase in the luminal fluid uteroglobin concentration, which peaked at 12 h from steroid administration. It is of interest to note that in spite of markedly reduced cytosol and nuclear progesterone receptor concentrations at the time of injection, the second dose of progesterone was able to elicit a more pronounced response in terms of uteroglobin secretion (Fig. 3). In this respect, the present results concerning the rabbit uterus are different from those achieved in the rat uterus, in which a prior progesterone administration reduced cytosol progesterone receptor content by 50% and concomitantly blunted the biologic action of a subsequent progesterone dose (26). Whether this disparity is due to species differences, or is because of other factors involved in the regulation of these two biologic actions of progesterone in rat and rabbit uteri, remains to be established.

Maximal induction of uteroglobin synthesis and secretion has been achieved after a 5-day chronic progesterone treatment (17). As shown in this study, the early changes in progesterone-induced uteroglobin synthesis are relatively small and short-lived in nature (Fig. 3). Thus a total of about 40 μg of uteroglobin was maximally obtained even after the second progesterone injection, while a 5-day treatment with a similar amount of progesterone has lead to luminal fluid uteroglobin concentrations that were milligrams in total quantity (17,25). The present data do not, however, exclude the possibility that uteroglobin synthesis occurs in two phases after a single dose of progesterone, the first phase only being studied in this work.

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