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TISSUE SPECIFIC EFFECTS OF PROGESTERONE ON PROGESTERONE AND ESTROGEN RECEPTORS IN THE FEMALE UROGENITAL TRACT

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Summary—The effect of progesterone administration on progesterone and estrogen receptors in the uterus, vagina and urethra of rabbits was studied. After 24 h of progesterone treatment the concentration of cytosolic progesterone receptors decreased to about 25% of the control value in the uterus, whereas no significant change in receptor concentration was observed in the vagina or the urethra. The concentration of the nuclear progesterone receptor did not change in any of the three tissues studied. The apparent dissociation constant (K_d) of nuclear progesterone receptor increased after progesterone treatment in all three tissues. Although the K_d of the cytosolic progesterone receptor also increased in all tissues, the difference was significant for only the vagina and urethra. The concentration of cytosolic estrogen receptors in the uterus decreased significantly ($P < 0.001$) after progesterone treatment whereas the K_d value increased slightly ($P < 0.05$). In vagina or the urethra, there was no change in either estrogen receptor concentration or K_d values after progesterone treatment. These data clearly showed that the reduction by progesterone of progesterone and estrogen receptor concentrations occurs only in the uterus and not in the vagina or the urethra.

INTRODUCTION

Current models for the action of steroid hormones include a scheme whereby the steroid hormone after entering a target cell bind to cytoplasmic receptors and the receptor complex is then transported to the nucleus. Although certain questions have recently been raised with respect of the validity of this two-step model, a number of previous studies have shown that progesterone receptors are under the control of both estrogen and progesterone [1–3]. It has been shown for example that a single injection of progesterone brings about a rapid and sustained decrease in cytosolic progesterone receptors in uteri of estrogenized guinea pigs [4], rats [5] and rabbits [6], or in chick oviduct [7]. The marked reduction in cytosolic receptors was not reflected as an increase in the nuclear receptors in these studies.

The biological significance of the progesterone induced loss and subsequent, although delayed, replenishment of the receptor is not clear. It is also not known whether the progesterone-induced decrease in progesterone receptors is a general occurrence in organs having progesterone receptors, or is only seen in so-called target organs for sex steroids. We have recently reported the presence of both estrogen and progesterone receptors in tissues of the female lower urinary tract [8, 9]. The concentration of progesterone receptor was found to be similar to that in the vagina, but was lower than that in the uterus [9]. In the present study we examined the effect of progesterone treatment on progesterone and estrogen receptors in the urogenital tissues of estrogenized rabbits.

EXPERIMENTAL

Animals

Virgin albino rabbits (2.8–3.2 kg) were ovariectomized 2–3 weeks before the experiment. Since estrogens increase the synthesis of progesterone receptors, all rabbits for the present experiments were estrogenized. Ovariectomized rabbits were given a single injection of 4 mg polyestradiol phosphate (Estradurin®, Leo, Sweden) i.m. With this treatment steady and relatively high levels of estradiol-17β are maintained for at least 10 days [10]. The control group received no further treatment and the animals were killed after 5 days. The progesterone group (7 rabbits) received an injection of 4 mg progesterone in oil s.c., 4 days after the Estradurin injection and the animals were killed after 24 h. Uterus, vagina and urethra were removed and placed in ice-cold physiological saline solution. The above protocol for estrogenization and progesterone treatment of rabbits is very similar to that used in a previous study [6].

Progesterone receptors

Cytoplasmic and nuclear fractions were prepared essentially as described previously [6, 9]. Briefly, after excess fat and connective tissue had been trimmed, the tissues were blotted dry, weighed and placed in the buffer solution containing 10 mM Tris–HCl (pH 8), 1 mM EDTA, 1 mM dithiothreitol, 2 mM

sodium molybdate and 10% glycerol. The tissues were then homogenized in 10 vol (w/v) of the above buffer with a Polytron homogenizer for 3 × 10 s periods (per g tissue) with intermittent cooling pauses of 20 s. The homogenate was centrifuged at 1000 *g* for 10 min to obtain the nuclear pellet. The supernatant was centrifuged at 90,000 *g* and the pellet discarded while the cytosolic supernatant was saved. The nuclear pellet was washed once and extracted with 0.4 M KCl for 45 min at 4°C. The procedure for the determination of progesterone receptors in the nuclear extract and the cytosolic fractions was essentially that described previously [6] except that both cortisol and testosterone were used to prevent nonspecific binding [9].

Aliquots (300 μl) of cytosol or nuclear-extract were added to assay tubes containing [^{3}H]progesterone (approx. 8000 cpm) and different concentrations (0.2–2.5 nM) of progesterone together with a 250-fold excess of cortisol and testosterone. After 24 h of incubation, 0.4 ml dextran–charcoal solution (0.05% dextran-80 and 0.5% charcoal) was added to each assay tube. The contents were mixed for 5 s and allowed to stand for 1 min. The tubes were then centrifuged at 1500 *g* for 5 min. The supernatant was decanted and 0.5 ml, after mixing with scintillation cocktail (Opti-Flour Packard), was counted in a Packard Tri-Carb liquid scintillation spectrometer. All procedures were carried out at 2–4°C. Protein concentration in the cytosol and nuclear-extract was determined by the method of Lowry *et al.* [11].

Estradiol receptors

The procedure for the determination of concentrations of estradiol receptor was identical to that described for progesterone receptors except that [^{3}H]estradiol-17β was used in concentrations between 0.1–1.5 nM in the various assay tubes and 5α dihydrotestosterone was used to eliminate nonspecific binding.

Chemicals

[2,4,6,7-^{3}H]Estradiol-17β (114 Ci/mmol) and [1,2,6,7-^{3}H] progesterone (105 Ci/mmol) were purchased from New England Nuclear Corporation, Boston, MA, U.S.A. Estradiol polyphosphate (Estradurin) was provided by AB Leo, Helsingborg, Sweden and progesterone by ACO, Solna, Sweden.

Calculations and statistics

The concentration of receptor sites and the apparent dissociation constant (K_d) were determined by Scatchard analysis. The mean values from different groups or batches were compared using Mann–Whitney U-test.

RESULTS

Data from preliminary experiments indicated that compared with 30°C (1–2 h incubation) progesterone receptor concentrations measured at 4°C after 24 h of incubation were generally higher in both cytosolic and nuclear fractions. Similar findings were recently reported for estrogen receptor assay in the rabbit uterus [12].

Typical curves on the data obtained for cytosolic progesterone receptors of the uterus, vagina and urethra of control and progesterone-treated rabbit are shown in Fig. 1. After 24 h of progesterone administration, the concentration of cytosolic pro-

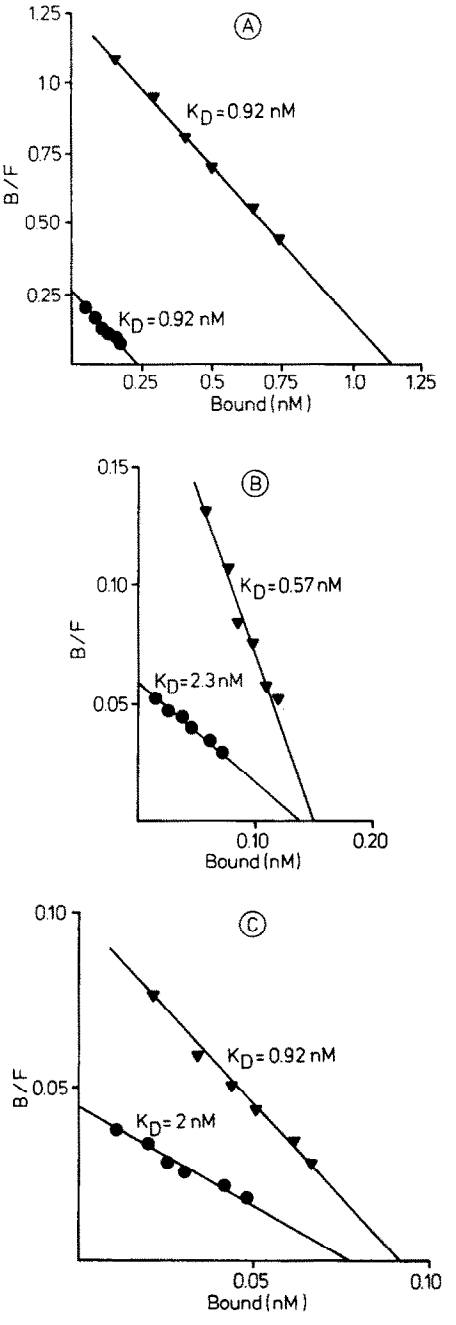

Fig. 1. Representative Scatchard plots of progesterone receptors in cytosolic fractions of uterus (A), and vagina (B) and urethra (C) from control (▼) and progesterone-treated (●) rabbits.

Table 1. Progesterone receptor concentration and apparent dissociation constant (K_d) of cytosolic (C) and nuclear (N) fractions in uterus, vagina and urethra of control and progesterone-treated rabbits

	Control ($n = 8$)		Progesterone-treated ($n = 7$)	
Tissue	Receptor (10^{-15} mol/mg protein)	K_d (nM)	Receptor (10^{-15} mol/mg protein)	K_d (nM)
Uterus (C)	674.0 ± 61.4	0.92 ± 0.06	187.3 ± 9.3*	1.12 ± 0.09
Uterus (N)	195.3 ± 24.8	0.63 ± 0.04	220.2 ± 11.9	0.94 ± 0.08*
Vagina (C)	125.3 ± 9.5	0.57 ± 0.04	102.2 ± 15.6	2.30 ± 0.27*
Vagina (N)	69.9 ± 13.0	0.39 ± 0.04	63.7 ± 8.5	0.82 ± 0.04*
Urethra (C)	71.2 ± 3.4	1.04 ± 0.12	72.0 ± 9.7	2.39 ± 0.33*
Urethra (N)	45.4 ± 7.3	0.53 ± 0.05	31.3 ± 5.0	0.95 ± 0.08*

Values are means ± SEM. Significance of differences between control and progesterone-treated group is indicated by *$P < 0.005$.

gesterone receptor sites decreased to about 25% of the control value in the uterus, whereas no significant change in receptor concentration was observed in the vagina or the urethra. The concentration of the nuclear receptor did nto change in any of the tissues studied (Table 1). In Table 1 the mean K_d values are also shown along with the concentrations of progesterone receptors in both cytosolic and nuclear fractions. The concentration of cytosolic receptor in the uterus was about 5 and 9 times higher than that in the vagina and urethra respectively. The values of K_d for progesterone receptors in nuclear fractions increased significantly ($P < 0.005$) in all three tissues after progesterone treatment. The K_d values for the cytosolic receptors from vagina and urethra were also considerably higher ($P < 0.005$) in the progesterone-treated group than in control. Although the K_d for uterine cytosolic receptor was slightly higher in progesterone treated rabbits (1.12 nM) than in controls (0.92 nM), the difference was not statistically significant.

Scatchard analysis of the data obtained for cytosolic estrogen receptor measurements are shown in Fig. 2 and the calculated mean values of receptor concentration and K_d are shown in Table 2. The concentration of receptor in the uterus decreased considerably ($P < 0.005$) after progesterone treatment and there was no significant change in the K_d value.

In the vagina and urethra there was no significant difference in either estrogen receptor concentration or their K_d between controls and progesterone-treated rabbits (Table 2).

DISCUSSION

The marked decrease in the cytosolic concentration of progesterone receptors in the uterus after progesterone treatment is in agreement with the data published previously and consistent with the concept of down-regulation of progesterone receptor by progesterone [4–6]. However, as seen from the present data no decrease in progesterone receptor concentration could be detected in either the vagina or the urethra. These data are somewhat paradoxical as they show, for the first time, tissue specificity in the

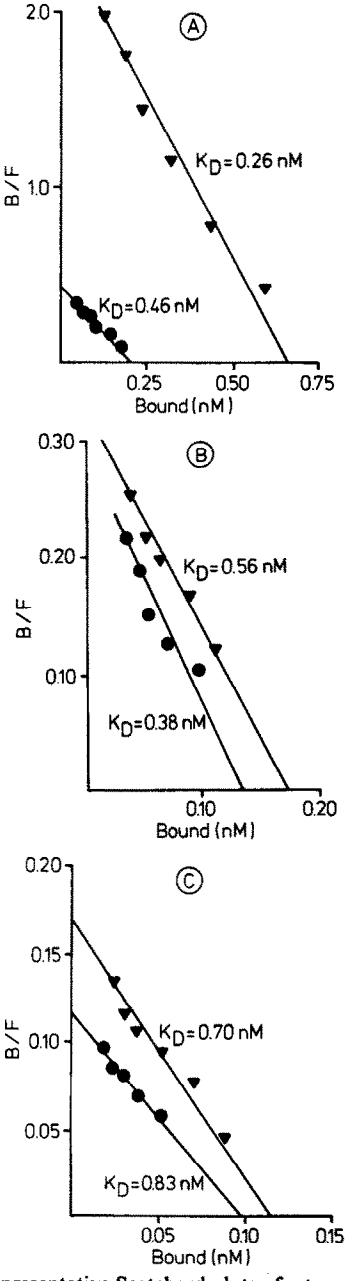

Fig. 2. Representative Scatchard plots of estrogen receptors in cytosolic fractions of uterus (A), vagina (B) and urethra (C) from control (▼) and progesterone-treated (●) rabbits.

Table 2. Estrogen receptor concentrations and apparent dissociation constant (K_d) of the cytosolic fractions from the uterus, vagina and urethra

	Control ($n = 8$)		Progesterone-treated ($n = 7$)	
	Receptor (10^{-15} mol/mg protein)	K_d (nM)	Receptor (10^{-15} mol/mg protein)	K_d (nM)
Uterus	365.8 ± 35.8	0.31 ± 0.05	167.7 ± 14.4*	0.45 ± 0.05
Vagina	128.2 ± 8.0	0.51 ± 0.07	119.5 ± 10.8	0.40 ± 0.03
Urethra	86.4 ± 9.3	0.74 ± 0.11	69.1 ± 5.3	0.94 ± 0.13

Values are means ± SEM. Significance of differences between control and progesterone-treated group is indicated by $*P < 0.005$.

effect of progesterone on progesterone receptor dynamics. Data from a previous study showed that the various biochemical characteristics of the progesterone receptor in the guinea pig vagina were not different from those of the uterine progesterone receptor [13].

Progesterone treatment in the present study, however, led to a decrease in the affinity of the nuclear progesterone receptor (higher K_d values) from all three tissues. Progesterone treatment even caused a reduction in affinity in cytosolic receptor from vagina and urethra but not uterus. We are unaware of any published data showing a decrease in progesterone receptor affinity by progesterone treatment. Although no information on the possible influence of endogenous steroids on receptor measurement in urinary tract tissue is available, our previous data on uterine progesterone receptor indicated a lack of such an influence [6].

The biological significance of the progesterone induced loss of cytosolic progesterone receptor is not clear. However, this loss does not occur in all target organs after progesterone treatment. This together with the fact that the existence of an authentic cytosolic receptor has recently been questioned [14–16] raises doubts about the biological importance of down-regulation of cytosolic progesterone receptor after progesterone treatment. This scepticism is also supported by the data of Isomaa *et al.*[17] showing no relationship between progesterone receptor concentration, as altered by estrogen/progesterone treatment, and biological response measured in terms of the synthesis of uteroglobin.

A significant decrease in the affinity of nuclear receptor in all three tissues and also a significant reduction in the affinity of cytosolic receptor in vagina and urethra was clearly shown. Since, in contrast to cytoplasmic receptor, binding to nuclear receptor is essential for interaction with specific nuclear acceptor sites and subsequent gene expression, changes in binding affinity of the nuclear receptor may be of significance in the regulation of biological response. A decrease in receptor affinity for the hormone should indicate a decrease in the sensitivity of the target cell to hormonal action. A consequence of this would be that the response of the tissue following a second exposure to hormone or in the presence of continuous low concentration of the hormone, would successively decrease. This appears to be a logical self-limiting mechanism against a continuous exposure to hormone.

Progesterone treatment in the present study also markedly decreased cytosolic concentration of estrogen receptors in the uterus in agreement with the previous findings [18, 19]. However, in contrast to the uterus, no change in estrogen receptor concentration in the vagina or the urethra was observed. Here again tissue specificity of progesterone action on the regulation of estrogen receptors is indicated. From their recent data on progestin-induced reduction of estrogen receptor, Takeda and Leavitt[20] have suggested that progesterone-induced receptor turnover may be responsible for the down regulation of estrogen receptors. It is possible that progesterone induces a significant turnover of estrogen receptor in the uterus, an organ possessing a high sensitivity to progesterone compared with the vagina or urethra. It is also possible that a certain minimum number of progesterone receptor is required to induce down-regulation of estrogen and its own receptor. This requirement is probably fulfilled in the uterus but not in the vagina or the urethra.

In spite of the fact that all female mammalian species secrete both estrogen and progesterone, although in varying amounts, the response of individual target organs is not always identical. In most instances, progesterone and estrogen seem to be antagonistic to each other. The antagonistic action of progesterone to estrogen is usually thought to be mediated by a reduction of estrogen receptor which is clearly seen in the case of the uterus. In the absence of a progesterone effect on estrogen receptors as found in the present study, progesterone might, therefore, be thought to have no antiestrogenic influence in the vagina or the urethra. Indeed, no clear evidence is available for an antiestrogenic effect of progesterone in the vagina or the urethra although both organs are considered to be target organs for estrogen [8, 9, 21].

Provided that progesterone can be shown to have no antiestrogenic effect on the vagina (or the urethra), a combination of progesterone with estrogen in estrogen replacement therapy should be highly preferred. The combination of progesterone with estrogen would in that case reduce the unwanted effects of estrogen on proliferation of the endometrium, and not the beneficial effect of estrogen on the vagina or

the urethra since it did not seem to reduce estrogen receptors in these tissues in contrast to the uterus.

Progesterone treatment also slightly reduced the affinity of the uterine estrogen receptor. This was, however, not observed in the case of the vagina or urethra.

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