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The cytosol receptors for progesterone in the different parts of rabbit fallopian tube and uterus during ovum transport

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Abstract. Progesterone receptors were determined in the cytosol from the ampulla, ampullary-isthmic junction and isthmus of rabbit fallopian tube and uterus of estrus and pregnant rabbits. The receptor levels when compared among its various anatomical segments, were the same in ampulla, isthums and uterus but maximum in ampullary-isthmic junction. Significant differences were observed in mated animals at 14, 24, 34, 48, 70 and 144 h after coitus. The receptor concentrations in portions of the fallopian tube showed no significant change between 14 and 24 h after coitus, except for a decrease in ampullary-isthmic junction at 24 h. At 34 h the concentration of receptor further decreased in all parts of the tube. At 48 and 70 h after coitus, receptor concentrations decreased gradually in ampulla and ampullary-isthmic junction, while isthmus showed a gradual increase. At 144 h, the receptor concentration showed no further change in ampulla and ampullary-isthmic junction; however, isthmus showed a decline. The uterine receptor concentration declined steadily from estrus till 70 h after coitus, however, it was increased at 144 h. The dissociation constant (K_d) of cytosol receptor in all the tissues at estrus and during early pregnancy was found similar. The implications of these changes in relation to the normal ovum transport have been correlated in this paper.

Keywords. Progesterone; receptors; fallopian tube; ovum transport; rabbit.

Introduction

The process of egg transport is an important event in reproduction and is regulated by steroid hormones (Chang and Harper, 1966; Harper 1966; Boling, 1969). The concentration and intracellular distribution of receptors have been reported to vary according to the physiological state of the animals (Martil and Psychoyos, 1976; Lee and Jacobson, 1971; Clark *et al.,* 1972). An estimation of progesterone-receptor levels and a study of their regulation in the fallopian tube and uterus may help in our understanding of the molecular events in the critical period after ovulation.

Earlier studies have shown the presence of progesterone binding macromolecules in rabbit uterus (Rao *et al.,* 1973; Faber *et al.,* 1973; McGuire and Banso, 1972; Davies *et al.,* 1974). The presence of specific receptors for progesterone in human (Fuentealba *et al.,* 1975), chick (Toft and O'Malley, 1972; Sherman *et al.,* 1970) and rabbit oviducts (Muechler *et al.,* 1976) has also been reported. However, El-Banna and Sacher (1977) could not detect cytosolic progesterone receptors in the rabbit and oviduct tissue.

In the present investigation progesterone receptor levels in the cystosol of different segments of rabbit fallopian tube have been studied in relation to ovum transport

and their possible correlation with plasma-estradiol and progesterone levels have been examined.

Materials and methods

Animals

Sexually mature (virgin) albino rabbits (2.5 to 3.0 kg) of our Institute colony kept under uniform husbandry conditions at 26±2°C were used in this study. The animals were divided into 7 groups; each group consisted of 6 animals. The first group was sacrificed as such while the others were mated with two fertile bucks and the mating was confirmed by the presence of spermatozoa in vaginal smears. Animals of each group were sacrificed at 14, 24, 34, 40, 70 and 144 h after mating.

Preparation of cytosol

The tissues were removed, chilled in ice-cold Tris-EDTA buffer (0.01 M Tris-HCl and 0.001 M EDTA, 0.25 M sucrose, pH 7.4). Both oviducts and uteri were excised, trimmed to remove the adhering fat and rinsed in cold Tris-EDTA buffer. Oviducts of all groups were separated into ampulla, ampullary-isthmic junction and isthmus as described elsewhere (Puri and Roy, 1980a). From each group different parts of the oviducts and uteri were weighed separately, minced and homogenized in 10 vol of cold buffer using an all-glass homogenizer at 0-4°C. Homogenates were centrifuged at 800 *g* for 10 min to get the nuclear pellet which was used for the measurement of DNA (Burton, 1956). The supernatant was centrifuged at 105,000 *g* for 1 h in an IEC/B-60 (International Equipment Co., Needham Heights, Massachussets, U.S.A.) ultracentrifuge. The cytosol was mixed with dextran-coated charcoal suspension (0.05% of dextran T-70 from Pharmacia, Uppasala Fine Chemicals, Sweden and 0.5% Norit A, prepared with 0.01 M Tris-HCl, 0.001 M EDTA buffer, pH 7.4) in the ratio of 1:1 (v/v). The suspension was agitated for 60 min in the cold (0-4°C) and centrifuged at 800 *g* for 10 min. Supernatants thus obtained were immediately used for the assay of progesterone binding.

Determination of progesterone binding to the cytosol receptor

The receptor assay was carried out according to the method of Vu Hai and Milgrom, (1978) by incubating 25 nM [^{3}H]-progesterone (1∝, 2∝, (n)-[^{3}H]-progesterone (specific activity 49 Ci/mmol) purchased from the Radiochemical Centre, Amersham, England) at 0-4°C for 60 min (Set A); the non-specific binding was determined by parallel incubation in the presence of a 250-fold excess of cold progesterone (Set B). Unlabelled Cortisol at a concentration of 1 μM was used to minimize the interaction of progesterone with corticosteroid binding globulin.

Preliminary experiments demonstrated that the concentration [^{3}H]-progesterone (25 nM) used was sufficient for saturating both oviductal and uterine cytosol receptors (data not shown). The optimum incubation time at 0-4°C for [^{3}H]-progesterone exchange was also determined in all the segments of the tube and uterus (data not shown). It appeared that cytosols from ampulla and ampullary-isthmic junction segments attained equlibrium after 30 min of incubation while isthmus and uterus did so at 60 min. The maximum specific binding remained stable till 2 h of incubation. Therefore, 1 h was chosen as the optimum time for progesterone cytosol receptor assay at 0-4°C.

At the end of the incubation, progesterone bound to its receptor was separated by incubating with dextran-coated charcoal for 10 min in the cold; then it was centrifuged at 800 *g* for 10 min. The supernatants were decanted into vials and counted for radioactivity.

Radioactivity measurement

Radioactivity determinations were carried out in a Packard Model 3330 liquid scintillation spectrometer; the vials contained 0.6 ml of the sample in an aqueous phase plus 12 ml of scintillation fluid (naphthalene 52 g; 2.5 diphenyloxazole, 3.25 g l,4-bis-[2(5-phenyl-oxazolyl)] benzene, 65 mg; methanol 150 ml; toluene 300 ml and dioxane,300 ml). The efficiency of counting was 52%. Quenching corrections were done using the internal standard technique.

Determination of the dissociation constant

K_d was determined by incubating cytosols from various tissues with different concentrations of [^{3}Hl-progesterone (1-10 nM for 1 h at 0.4°C (Set A). Parallel incubations were also done containing at 250-fold excess of cold progesterone and 1 μM of Cortisol (Set B). Specific binding was calculated as the difference between bound radiolabelled hormone of these two types of incubations. K_d was calculated from a Scatchard plot (Scatchard, 1949) of specific progesterone binding. The results were expressed as f mol of bound progesterone per μg DNA and were statistically analysed using the analysis of variance. The difference between the mean values of two groups was calculated by the method of least significant difference (*P* value of 0.05 or less was considered to be significant).

Results

The differences in the binding of [^{3}H]-progesterone and the calculated K_d in different segments of the oviduct and uterus are given in table 1. It appears that at estrus

Table 1. Progesterone receptors in the cytosol in the different parts of the fallopian tube and uterus of rabbits in estrus.

Tissue	Number of animals	$K_d \times 10^{-9}$ M	fmol bound progesterone per μg DNA
Fallopian tube			
i) Ampulla (A)	6	2.25±0.09	20.95±2.38
ii) Ampullary-isthmic junction (AIJ)	6	2.10±0.04 (vs A, P>0.05)	33.16±1.30 (vs A, P<0.01)
iii) Isthmus (I)	6	2.15±0.09 (vs A and AIJ P>0.5)	23.8±0.52 (vs A, P>0.05) (vs AIJ, P<0.01)
Uterus	6	1.75±0.26 (vs A, I and AIJ P>0.05)	25.95±0.85 (vs A, P>0.05) (vs AIJ, P<0.02) (vs I, P>0.05)

Values are mean ±S.E.

similar levels of receptors are obtained in ampulla, isthmus and uters but the concentrations are maximum in ampullary-isthmic junction. The dissociation constants in the uterus and in all the three segments of the tube showed no significant difference.

The pattern of receptor distribution in the above tissues during different post-coital periods are presented in table 2. The ampulla and ampullary-isthmic junction

Table 2. Progesterone cytosol receptors in different parts of the fallopian tube and uterus during the transport of ovum.

(Results are expressed as fmol bound progesteron/μg DNA)

Tissue	*Post-coitum* (h)					
	14	24	34	48	70	144
Fallopian tube						
i) Ampula	17.39±2.01	14.34±3.69	7.53±0.73	5.72±0.28	5.47±0.35	5.92±0.37
ii) Ampullary-isthmic junction	29.71±3.24	16.39±2.38	9.59±0.54	7.14±0.56	5.76±0.53	7.95±1.61
iii) Isthmus	15.54±2.47	17.23±2.35	8.78±1.55	11.82±1.58	14.54±2.60	7.13±0.25
Uterus	17.20±1.95	11.76±0.34	9.25±2.56	8.62±2.62	5.51±0.24	9.97±1.11

Values are mean ±S.E.; number of animals in each group =6.

portions of the tube show no significant change at 14 h after mating; however, the value in isthmus is slightly decreased. At 24 h, although ampulla and isthmus showed no significant alteration over the 14 h group, the ampullary-isthmic junction value is decreased ($P<0.01$). Further at 34 h the values in all the portions significantly declined ($P<0.01$). Thereafter, the receptor concentration in ampulla and ampullary-isthmic junction showed no further change till 70 h after coitus; however isthmus showed a trend to increase. 144 h after mating ampulla and ampullary-isthmic junction showed no significant alteration over the 70 h group but the value in isthmus decreased significantly. The level in the uterine receptor showed a steady decline till 70 h; however, it increased at 144 h over the 70 h group. At different hours after mating the dissociation constants ranged from $1.75 \pm 0.2 \times 10^{-9}$ M to $2.61 \pm 0.35 \times 10^{-9}$ M and were not altered significantly in all the tissues studied.

Discussion

The above findings showed that significant differences occur in the progesterone receptor concentrations in different parts of the fallopian tube and the uterus. These differences cannot be attributed to the changes in the kinetics of hormone binding to the receptor as no significant difference in K_d was observed. However, the differential sensitivity and retention of this hormone could be a responsible factor.

When progesterone receptors of estrus animals were compared with those of mated animals at different times of mating, significant alterations in different parts of the tube and uterus were noted. All the portions of the tube showed an almost similar pattern of receptor concentration till 144 h after coitus. The K_d of progesterone receptors in different segments during early pregnancy also showed no significant change, although the values of K_d as well as receptor concentrations reported here are at variance with those described earlier (Muechler *et al.,* 1977). The discrepancies are primarily, because we used cytosol from which progesterone was removed and different incubation conditions in the exchange assay of receptors.

At 14 and 24 h after mating, when ova are present in ampulla and ampullary-lsthmic junction portions of the tube respectively (Gupta *et al.,* 1970; Polidoro *et al.*, 1973), there is no change in receptor concentrations in these portions, corresponding to the unaltered plasma level of progesterone at these times (Spilman and Wilks, 1976). However, the decrease in receptors in ampullary-isthmic junction at 24 h does not correspond to the plasma level. The possibility of involvement of the tissue progesterone level in this decrease cannot be overlooked (Vu Hai *et al.,* 1978). After several hours of blockage of the egg at ampullary-isthmic junction they begin to move into the isthmus between 24 and 36 h after coitus (Polidoro *et al.,* 1973). Therefore, at 34 h when the movement of eggs from ampullary-isthmic junction into the isthmus occurs (Spilman and Harper, 1975; Pauerstein *et al.,* 1974) the sudden fall in receptor concentration in all the parts as obtained here could be correlated with its necessity to release the tubal lock at ampullary-isthmic junction for the transport of the egg. Further, the low level of receptors as compared with control in all the portions of the tube and uterus between 34 and 144 h after mating coincided with increased plasma progesterone levels (Spilman and Wilks, 1976) and the gradual movement of the egg through the isthmus into the uterus (Spilman and Harper, 1975).

On the basis of the above findings and their correlation with plasma progesterone concentration (Spilman and Wilks, 1976), it is concluded that, as plasma progesterone increases, the receptor concentration in tissues decreases. This decrease in cytosol receptors with increasing plasma progesterone could be correlated in either of two ways: (i) cytosol receptors are translocated towards the nucleus; (ii) inactivation of receptor is occurring under the influence of increased progesterone level. Nuclear translocation of the receptor does not fully explain here the disappearance of receptors from the cytosol, as nuclear receptors do not increase at these times (Puri and Roy, 1980b). Thus, the total number of receptors in the above tissues decreases as the plasma progesterone level increases. This inactivation of cytosol receptors has also been described earlier (Milgrom *et al.,* 1972, 1973; Freifeld *et al.,* 1974; Luu Thi *et al.,* 1975; Vu Hai *et al.,* 1977). However, the mechanism of inactivation of progesterone receptors by progesterone is as yet not clear. Our findings further suggest that altered levels of progesterone receptors in different segments of the oviduct and uterus during ovum transport may regulate its action between the time of ovulation and implantation. Furthermore, no definite correlation of plasma estradiol level with the receptors can be offered, as the hormonal level remained unaltered in the post-ovulatory period (Spilman and Wilks, 1976).

The present studies do not support the findings of El-Banna and Sacher (1977) that either progesterone receptors are absent in the tube or that they are already saturated

with endogenous progesterone, which do not exchange with added [^{3}H] –progesterone during the incubation; these authors, it appears, used too low concentrations of the hormone and further they did not follow the removal of endogenous hormone by incubation with dextran-coated charcoal before the assaying of receptors. However, our results clearly indicate the presence of progesterone receptors in the fallopian tube.

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