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Estradiol and progesterone receptors in the human oviduct

Receptores de estradiol y progesterona en el oviducto humano

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The Fallopian tubė, undergoes morphological cyclic changes throughout the menstrual cycle. These morphofunctional changes occur simultaneously with the cyclic fluctuations of Estradiol (E_2) and Progesterone (P) plasma levels therefore they can be considered mediated by ovarian steroids acting through specific receptors.

The aim of this investigation was to further define the ovarian hormone action on this tissue by studying: 1) Tubal steroid content and its relationship with serum steroid concentration. 2) The steroid receptors content throughout the menstrual cycle in normal Fallopian tube. 3) A protein marker of steroid action on the tissue, and 4) The steroid receptors content in pathological Fallopian tube.

The Fallopian tube retains E_2 and P at or above their plasma level. In spite of the lack of variation in plasma estrone (E_1) during the cycle a significant increase was seen in oviductal tissue during the luteal phase. These data suggest an active process in E_1 formation by the Fallopian tube.

Oviductal tissue bound E_2 and P with high affinity E_2 KD = 3,9 x 10^{-10} M; P KD = 2 x 10^{-9} M. During the late proliferative stage, when plasma E_2 reached a maximum the highest content of Estradiol receptor 179 ± 31 fentomol/mg protein and Progesterone receptor 105 ± 60 fentomol/mg protein were determined in the ampulla.

During the secretory phase a progressive decrease in the nuclear Estradiol receptor was observed (54 fentomol/mg protein).

Estradiol receptor concentrations are higher in the ampulla than in the fimbria and isthmus, Progesterone nuclear receptors have a similar distribution in the three anatomical segments of the Fallopian tube.

E_2 and P are bound by pathological tube with similar KD as observed in normal tissue. Nevertheless, during the proliferative phase, the hydrosalpinx showed significantly lower content of Estradiol receptor than normal tubes. Nuclear progesterone receptor content were lower in hydrosalpinx but not significantly different than in normal tissue.

These findings could explain the low intrauterine pregnancy rate following salpingoneostomy.

INTRODUCTION

The human oviduct undergoes morphological and functional changes during the menstrual cycle. Tubal epithelial cells are highest and more ciliated during the later proliferative phase. At the late luteal phase, cells are flat and microvilli decrease in size, as well as in number (Verhage *et al.*, 1979). In addition tubal fluid secretions vary during the cycle, being heaviest during the follicular phase. (Lippes *et al.*, 1972). These changes suggest that this tissue is a target organ for ovarian steroids. Steroid target tissues have specific hormone receptors, which are intracellular proteins that become "activated" by their specific ligands to acquire high affinity for specific response elements in the genome (Evans, 1988). The presence of specific estradiol (E_2) and progesterone (P) receptors and their interaction have been described in

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the Fallopian tube (Fuentealba *et al.*, 1976; Flickinger *et al.*, 1977).

In this review we present some of the results obtained in our laboratory, which were conducted in order to further specify the ovarian hormone action on the Fallopian tube. The studies were focused on: 1) Tubal steroid content and its relationship with serum steroid concentrations. 2) The steroid receptors content throughout the menstrual cycle in normal Fallopian tube. 3) A protein marker of steroid action on the tissue, and 4) The steroid receptors content in pathological Fallopian tube.

MATERIAL AND METHODS

Fertile patients requesting surgical sterilization at our institution were included in this study. Informed consent to remove one Fallopian tube at operation was obtained from each patient. At laparotomy, the tube on the side of the corpus luteum or ripe follicle was removed, with careful attention given to preserving the ovarian blood suply. The tube was processed to measure tissue content of steroids, 17-β estradiol dehydrogenase (17-β-SDH) activity and steroid receptos (Devoto *et al.*, 1980; Sierralta *et al.*, 1981; Pino *et al.*, 1982; 1984). Determinations were made in total tube or in each of the anatomical segments: isthmus, ampulla and fimbria.

In addition to the histological data of the endometrium, the presumptive day of ovulation was ascertained by the plasma concentration of luteinizing hormone (LH). The samples were classified according to the following criteria: a) early proliferative, before beginning of the E_2 peak (E_2 < 200 pg/ml). b) Late proliferative, plasma E_2 > 200 pg/ml. c) Early secretory, 24 to 96 hours after the LH peak, and d) Late secretory, more than 96 hours after the LH peak.

RESULTS AND DISCUSSION

Steroid Content

In the earlier studies we examined whether or not steroids were retained by the human oviduct. The three anatomical segments were found to retain E_2 and P at or above their plasma levels (Devoto *et al.*, 1980). However, the tissue concentration of these steroids was lower in the Fallopian tube, as compared with the human endometrium.

In spite of the lack of variation in plasma estrone (E_1) during the cycle, this steroid was concentrated during the luteal phase, suggesting that the human oviduct develops active estrone formation (Table I).

Steroid receptors and protein markers of steroid action in normal Fallopian tube

Our aim was to analyze not only the variations of receptor concentration throughout the menstrual cycle, but also to relate them to a characteristic hormonal response. Therefore, as a response to E_2 action, the presence of the progesterone receptor was measured; since it has been proved in several tissues that E_2 priming is a requirement for progesterone receptor synthesis (Leavitt *et al.*, 1977). On the other hand, P action on this tissue was related to the presence of 17-β-SDH activity, since it has been demonstrated in the human endometrium, that this protein is synthesized after P action (Tseng and Gurpide, 1975).

Estradiol and progesterone receptor concentrations were measured in the cytosol and nuclei of the three segments of the human oviduct (Pino *et al.*, 1982; 1984). All segments of the oviductal tissue bound E_2 and P with high affinity and specificity. Kd values for E_2 and P were 3.9×10^{-10} M and 2×10^{-9} M, respectively. The concentration of both receptors varied troughout the menstrual cycle, showing a clear relationship with plasma concentration of both steroid hormones (Tables II and III).

Cytosolic estradiol receptor concentration was constant throughout the cycle, in the three segments analyzed (Table II), as it has been observed previously in the human oviduct (Flickinger *et al.*, 1977), and contrasting with results obtained in the monkey oviduct (Flickinger *et al.*, 1977, West and Brenner, 1983).

TABLE I

Unconjugated estrone in the Fallopian tube and peripheral blood during the normal menstrual cycle

	ESTRONE			
		Tissue concentration (pg/g wet tissue)		
Stage of the cycle	Plasma Concentration pg/ml	Isthmus	Ampulla	Fimbria
Early proliferative n = 3	50 ± 10	100 ± 40	60 ± 30	70 ± 20
Late proliferative n = 5	70 ± 10	110 ± 10	100 ± 8	110 ± 40
Early secretory n = 8	60 ± 10	190 ± 50*	240 ± 60*	310 ± 60*
Late secretory n = 6	60 ± 8	120 ± 50	170 ± 50*	150 ± 50

The concentration of estrone in the different segments of the Fallopian tube and plasma during the menstrual cycle. Values are means ± standard error.
n: number of observations.
*: significantly different compared to proliferative phase $p < 0,05$.

However, E_2 action on this tissue can be inferred from the changes observed in nuclear estradiol receptor concentration. Since steroid receptors are nuclear proteins (Evans, 1988), the higher content of nuclear estradiol receptor indicates an increasing E_2 action during the proliferative stage, which decreases throughout the secretory stages.

These results strongly suggest that in this tissue, as in experimental models (Evans *et al.*, 1980), the concentration of estradiol receptor is under the regulation of both steroid hormones. In support of this, we have observed positive correlation between nuclear estradiol receptor content and plasma E_2, whereas a negative linear correlation was observed with respect to plasma P (Pino *et al.*, 1984).

On the other hand, the concentration of progesterone receptor seems to be a consequence of E_2 action, since its content clearly increased at the late proliferative stage, in the three anatomical segments of the Fallopian tube (Table III). Thus, during the proliferative phase and for the whole cycle, a positive linear regression was found for plasma E_2 and cytosolic progesterone receptor content (Pino *et al.*, 1984), suggesting that E_2 stimulates the synthesis of progesterone receptor in the oviduct, as proposed in experimental models (Leavitt *et al.*, 1977).

On the other hand, to explain the decrease of cytosolic progesterone receptor during the secretory stages, at least three factors must be considered: a) P receptor inactivation or degradation after hormone action; b) negative effect of P on receptor synthesis (Vu Hai *et al.*, 1977) and c) a decrease of the stimulatory effect of E_2 (Evans *et al.*, 1980). Events a) and b) are sustained by the negative linear correlation that we found between plasma P and cytosolic progesterone receptor concentrations (Pino *et al.*, 1984).

From Table III, it can be appreciated that the progesterone receptor content measured in the soluble fraction was higher than the nuclear progesterone receptor concentration. This has also been observed in other tissues (Walters *et al.*, 1980) and would result from progesterone receptor leakage from nuclei during homogenization, which decreases when the nuclear protein is bound by endogenous hormone. Thus the increase of nuclear progesterone receptor concentration ob-

TABLE II

Estradiol cytosolic and nuclear receptors in the normal Fallopian tube during the menstrual cycle

STAGE OF THE CYCLE	Receptor concentration fmoles/mg protein					
	ISTHMUS		AMPULLA		FIMBRIA	
	Cytosolic	Nuclear	Cytosolic	Nuclear	Cytosolic	Nuclear
Early proliferative	19 ± 4	21 ± 19	46 ± 19	100 ± 35	33 ± 10	62 ± 27
Late proliferative	23 ± 8	79 ± 15*	42 ± 20	179 ± 31*	27 ± 11	131 ± 70*
Early secretory	15 ± 12	23 ± 11	62 ± 15	54 ± 35	35 ± 15	58 ± 12
Late secretory	23 ± 6	8 ± 4	42 ± 27	27 ± 10	23 ± 8	50 ± 11,5

Values are the mean ± SD from 6 determinations. *: Values significantly higher than the corresponding concentration during the early proliferative and secretory stage $P < 0.05$.

TABLE III

Progesterone cytosolic and nuclear receptors in the normal Fallopian tube during the menstrual cycle

STAGE OF THE CYCLE	Receptor concentration fmoles/mg protein					
	ISTHMUS		AMPULLA		FIMBRIA	
	Cytosolic	Nuclear	Cytosolic	Nuclear	Cytosolic	Nuclear
Early proliferative	90 ± 30	23 ± 8	180 ± 90	26 ± 8	165 ± 90	23 ± 8
Late proliferative	540 ± 300*	113 ± 50*	660 ± 285*	105 ± 60*	270 ± 150	90 ± 45*
Early secretory	180 ± 90	68 ± 38*	300 ± 90	60 ± 22*	330 ± 60	75 ± 30*
Late secretory	90 ± 30	68 ± 22*	210 ± 30*	71 ± 30	150 ± 90	64 ± 15

Values are the mean ± SD from 6 determinations. *: Values significantly higher than the corresponding concentration during the early proliferative stage.

served from the late proliferative stage throughout the secretory stages would indicate increased P action on this tissue.

Finally, the oviductal response to P action can be related to the increased activity of 17-β-SDH observed during the secretory phase (Sierralta *et al.*, 1981). The increased enzyme activity is followed by an increased content of E_1 during the secretory phase (Table I), suggesting that enhanced oxidation of E_2 occurs in the oviduct, as in the endometrium (Tseng and Gurpide, 1975).

Steroid receptors in pathological Fallopian tube

Present knowledge about the reproductive phenomena that take place in the Fallopian tube is limited to partial explanation of events like ovum pick up, ovum and sperm transport and early cleavage of the embryo.

The understanding of tubal function has increasing importance in clinical infertility, since it can lead to improvement in the treatment of tubal infertility or to

TABLE IV

Histologic and biochemical findings in Hydrosalpinx in 9 infertile patients
Comparison of estradiol and progesterone receptor content in hydrosalpinx and normal tube

Patient Histology	Steroid receptor content fmol/mg protein			
	E_2		P	
	Cytosol	Nuclear	Cytosol	Nuclear
1) Loss of tubal folds with atrophic epithelium	13,5	53,4	29,3	ND[a]
2) Loss of tubal folds with low epithelium, thick muscular wall	2	ND	84	105
3) Loss of tubal folds; low epithelium	–[b]	–	113	30,3
4) Loss of tubal folds; low epithelium	–	–	50	30
5) Tubal folds preserved with normal epithelium	15,9	42,7	696	210
6) Loss of tubal folds; area of normal epithelium	–	–	40,5	65,4
7) Loss of tubal folds; low epithelium	32,9	29,2	33,7	170
8) Loss of tubal folds; atrophic epithelium	9,2	70,9	428	170
9) Loss of tubal folds; flat epithelium	14,2	22,1	84,9	72,7
Hydrosalpinx n = 9	14,6 ± 4,2*	43,6 ± 8,6*	173,3 ± 77,3*	94,8 ±24,5
Normal Fallopian tube n = 7	60 ± 25	120 ± 30	465 ± 108	143 ±25

[a]: ND, not detectable

[b]: –, not performed

*: Significantly different as compared with normal tube ($p < 0,05$).

the development of new contraceptives acting at the tubal level.

The low intrauterine pregnancy rate of hydrosalpinx following restoration of tubal patency by microsurgery is well known and it has been associated with damage of the endosalpinx caused by the infection (Winston, 1980).

The biochemical evaluation of hydrosalpinx from infertile patients showed the presence of both estradiol and progesterone receptors, at the cytosol and nuclear level. The binding constant for these specific proteins was of the same magnitude as observed in normal tissue. However, the mean levels of cytosolic and nuclear estradiol and progesterone receptors in hydrosalpinx were significantly lower than those of normal Fallopian tube (Devoto *et al.*, 1984). When individual results from hydrosalpinx were considered, a lack of correlation between estradiol and progesterone receptors was noted (Table IV). This could result from the depletion of ephitelial cells observed in hydrosalpinx, since immunocytochemical studies in the macaque oviduct have detected that estradiol receptors are mainly located within secretory cells (Brenner *et al.*, 1990).

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