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OESTRADIOL RECEPTOR LEVELS IN THE HUMAN FALLOPIAN TUBE DURING THE MENSTRUAL CYCLE AND AFTER MENOPAUSE

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SUMMARY

Using an oestradiol receptor assay method for measuring occupied and unoccupied receptor sites, receptor levels were measured in the soluble and particulate fractions obtained from the cytoplasm of different segments of the human Fallopian tube during the menstrual cycle. Higher levels of receptor were associated with the ampullar region than with the isthmus and in both cases the level of total receptors associated with the cytoplasmic particulate fraction were elevated in the proliferative phase when compared to the secretory phase. The levels of nuclear oestradiol were higher in the isthmus region than in other regions of the tube.

INTRODUCTION

A previous report [1] indicated that after the *in vivo* administration of ^{3}H-oestradiol there was a greater retention of the steroid in the isthmus than in the ampulla of the human Fallopian tube. This difference was observed during the menstrual cycle but not after menopause. No difference was observed in either the retention of ^{3}H-oestradiol or the measured levels of receptors along the tube in ovariectomized rabbits [2]. However, after oestradiol treatment a similar retention pattern was obtained to that found throughout the human menstrual cycle.

The aim of the present study was to measure the concentration of receptor sites (occupied and unoccupied) in several segments along the human Fallopian tube and compare these concentrations in the proliferative and secretory phases of the menstrual cycle.

METHOD

The measurement of oestradiol receptor sites

The assay method, which has been previously described [6], consists of incubating the receptor preparations with several concentrations of ^{3}H-oestradiol. The ^{3}H-oestradiol bound to a low affinity sites is then removed by incubation with charcoal. From Scatchard analyses the number of unoccupied binding sites is then determined. However, this design did not permit the measurement of both occupied and unoccupied sites. More recent assay methods for the oestradiol receptors in the immature rat uterus (Katzenellenbogen *et al.*)[4] and progesterone receptors in the guinea-pig uterus (Milgrom *et al.*)[5] have enabled the determination of both occupied and unoccupied sites. The latter techniques involved establishing assay conditions under which the receptor preparation was stable and from a knowledge of the rate of dissociation of the steroid from the oestradiol–receptor complex an incubation time period could be determined during which total exchange of the added ^{3}H-oestradiol could occur.

In the present study the receptor preparation is apparently stable for 4–6 h at 30°C although by 8 h a small loss (5%) is observed. The rate constant (k_{-1}) for the dissociation of the complex at 30°C, when incubated in the presence of either an excess of oestradiol or with charcoal [7], is $2{\cdot}32 \times 10^{-4}$ s^{-1} and $2{\cdot}15 \times 10^{-4}$ s^{-1} respectively. This is equivalent to a $t_{1/2} = 50$ min. An assay incubation period of 4 h was chosen which permitted $\cong 96\%$ of the bound steroid to exchange with the added steroid with no loss of binding sites due to the instability of the receptor preparation. This design was validated by incubating a receptor preparation with increasing concentrations of oestradiol (0–1 ng/ml) to occupy varying proportions of the binding sites. The preparation was treated with charcoal to remove unbound oestradiol and then assayed for binding sites. The number of binding sites detected were uninfluenced by the concentration of oestradiol. Incubation in the absence of oestradiol in the first incubation step led to a loss of binding sites. A detailed description of the assay method will be presented elsewhere [20].

Tissue fractionation

The Fallopian tube was fractionated according to the flow chart presented in Fig. 1. The dashed lines indicate a simplified procedure introduced after 2/3rds of the study was completed. The buffer used to extract the cytosol pellet was also used to extract the nuclear pellet. This buffer, which contained 0·1 M KCl, was able to extract all the receptors from the cytosol pellet. Since it is unlikely that such a buffer would extract nuclear receptors [15–17] it was assumed that the recovered binding sites from the nuclear fraction represented extracted receptors from cytoplasmic contaminants in the pellet. The nuclear

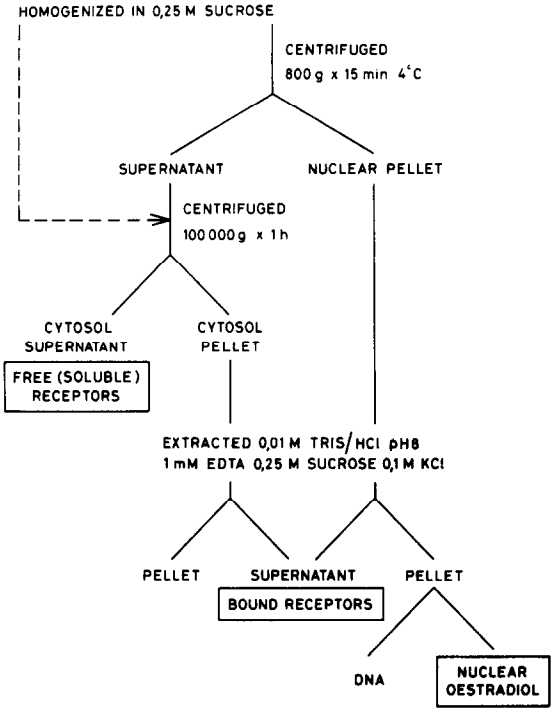

Fig. 1. Flowsheet for fallopian tube fractionation.

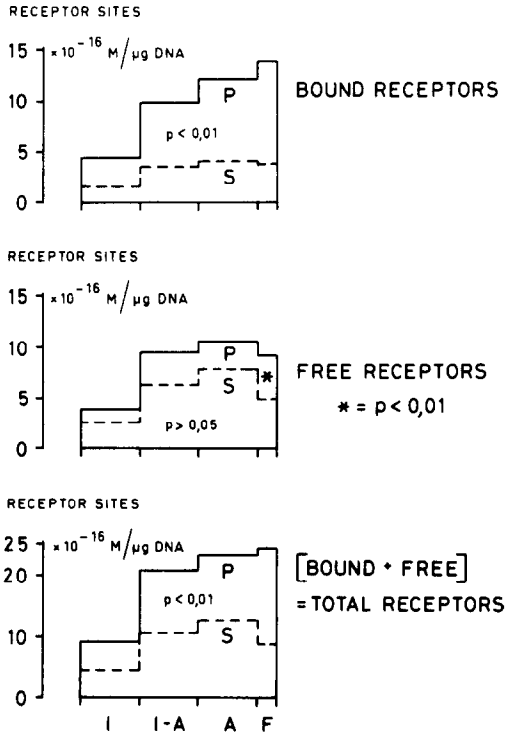

Fig. 2. Oestradiol receptor levels in the human fallopian tube over the menstrual cycle (P = proliferative- and S = secretory-phase).

oestradiol levels were measured by a radioimmunoassay method [19].

RESULTS AND DISCUSSION

The measurement of receptor sites along the fallopian tube during the menstrual cycle

The receptor assay method was applied to the measurement of free (soluble), bound (bound to cytoplasmic organelles) and total (free + bound) receptors along the Fallopian tube. In one tube (not shown) a gradation in receptors was observed from low levels in the isthmus to peak levels in the isthmus-ampulla and ampulla. Using this tube as a guide, a study was implemented which involved measurement of the receptors in four regions of the tube; the isthmus (I), isthmus-ampulla (I-A), ampulla (A) and Fimbria (F) in the proliferative phase (7 tubes) and secretory phase (10 tubes). These tubes were from women diagnosed with myomas and displasia of the cervix. The differences between tube segments and between phases of the cycle were analysed by means of a comparison of selected effects [9]. A log normal distribution was assumed throughout [10] and all values are expressed as geometric means (Fig. 2). The differences in free, bound and total receptors are significantly greater in the ampulla region (including the isthmus-ampulla and fimbria) than in the isthmus ($P < 0{\cdot}001$). Furthermore, there is a significantly greater concentration of bound and total receptors in the proliferative phase. No significant differences were observed between the phases in regard to the levels of the free receptors (with the exception of the fimbria). This represents a fall in the proportion of receptors bound from an average of 53% in the proliferative phase to 38% in the secretory phase. The receptor levels were also determined in four menopausal or postmenopausal women whose last menstrual period ranged from 8 months to 14 yr prior to the date of surgery. In all tubes was a gradation in receptors, from low levels in the isthmus to higher levels in the ampullar region.

The biochemical significance of the receptors bound to the cytoplasmic organelles is not clear. The receptor has been reported to be associated with mitochondria, microsomes [8, 12, 13] and lysosomes [14]. A more detailed study on the localization of the receptor would clarify its role within this cell compartment.

A comparison between the receptor and nuclear oestradiol levels along the fallopian tube

The nuclear oestradiol levels in the buffer-extracted pellet from the various segments of 8 Fallopian tubes (two obtained during the proliferative and 6 during the secretory phase) are presented in Table 1. A significantly higher concentration of oestradiol was observed in the isthmus than in other regions of the tube. These results are in agreement with the *in vivo* retention studies after administration of ^{3}H-oestradiol in the human Fallopian tube [1]. Current hypotheses favour a close relationship between the cytoplasmic receptor and nuclear receptor (and therefore nuclear oestradiol). The implication is that the capacity of

Table 1. Nuclear oestradiol levels in fallopian tube segments ($\times 10^{-16}$ M/μg DNA)

Isthmus	Isthmus-Ampulla	Ampulla	Fimbria
3·19	1·33	1·77	1·95

1 v 1-A, A, F $p < 0.01$. 1 v 1-A $p < 0.001$.

the tissue to retain oestradiol is a function of the concentration of oestradiol receptor. In this study it is observed that there are low concentrations of receptors and high concentrations of nuclear oestradiol in the isthmus when contrasted with the ampulla where the reverse is noted. These results suggest that there are other steps associated with the concentration of the steroid within the tissue which can regulate the degree of its retention. The possible involvement of nuclear acceptor sites for the cytoplasmic receptor [17, 18] or nuclear receptor sites [11, 16] must now be considered.

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