SIMILARITIES AND DIFFERENCES IN THE BINDING OF OESTRADIOL-17 β TO RAT OVIDUCT AND UTERUS

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A characteristic property of organs that respond to oestrogens is their ability to take up this hormone from circulation (Jensen & Jacobson, 1962; Talwar, Segal, Evans & Davidson, 1964; Noteboom & Gorski, 1965; King & Gordon, 1966; Baulieu, Alberga & Jung, 1967; Puca & Bresciani, 1969). The hormone is initially bound by 'receptor' proteins present in the cytoplasm, but the hormone receptor complex migrates rapidly to the nucleus (Jensen, Suzuki, Kwashima, Stumpf, Jungblut & DeSombre, 1968; Shyamala & Gorski, 1969). While there is extensive information on characteristics and properties of oestrogen 'receptor(s)' in the uterus, comparatively few data are available on the oviduct. Roy, Roy, Das Gupta, Engineer & Kar (1972) have reported the uptake of this hormone in Fallopian tubes surgically removed from women *post partum* and those with lactational amenorrhoea. Some investigators have suggested a rôle for oestrogens in the genesis of cilia (Brenner, 1967) and in modulation of the sensitivity of the Fallopian tube to adrenergic stimuli (Coutinho & Maia, 1970). This communication reports the similarities and differences in the binding of oestradiol-17 β to the uterus and the oviducts in rats.

When radioactive oestradiol was given systemically to ovariectomized rats, the hormone was observed to be taken up by the oviduct but to a much lower extent (about 50% on a per unit weight basis) than by the uterus. In contrast to the uterus, where the hormone was largely found in the heavy particulate (nuclear) fraction following high speed centrifugation, the subcellular distribution of the hormone was predominantly in the cytosol fraction (Table 1).

In the oviduct, oestradiol-17 β did not undergo much metabolic change. Most of the radioactivity (85 to 89%) extracted from the 600-g pellet and from the cytosol fraction analysed on thin-layer chromatographic plates was identified as oestradiol-17 β .

The binding of the steroid to the rat oviduct was highly discriminatory. Amongst the steroids, only oestradiol-17 β and amongst non-steroids only diethylstilboestrol, competed with [^3H]oestradiol-17 β for binding with the cytosol fraction (Table 2). Oestradiol-17 α , a biologically inactive stereoisomer of oestradiol-17 β , did not compete.

The rat oviduct cytosol 'receptors' had a low dissociation constant (K_d) for oestradiol of 4.31×10^{-11} mol (Text-fig. 1) suggesting a high affinity for this hormone. This was of the same order as that described for rat uterine cytosol 'receptor(s)' by various workers (Toft, Shyamala & Gorski, 1967; Shyamala & Gorski, 1969; Puca & Bresciani, 1969; Ellis & Ringold, 1971).

The number of binding sites in cytosol per rat oviduct (fresh weight, 5 to 7 mg) was approximately 2×10^{-14} mol or 0.33×10^{-14} mol/mg wet weight of

Table 1. Subcellular distribution of [^3H]oestradiol-17 β in oviducts and uterus of ovariectomized rats

Fraction	Oviducts* (% radioactivity)	Uterus (% radioactivity)
600-g pellet	30.0 $\pm$ 2.7	72.5
105,000-g pellet	3.0 $\pm$ 0.58	4.3
Cytosol	64.9 $\pm$ 2.7	23.2

A dose of 10 μCi (0.0278 μg) of [2,4,6,7- ^3H]oestradiol-17 β in 0.1 ml propane-1,2-diol was administered intraperitoneally to ovariectomized rats. The oviducts and uteri were removed after 60 min and homogenized in 0.32 M-sucrose tris-EDTA buffer (10 mM-tris-HCl, pH 7.4 and 1.5 mM-EDTA). The homogenate was centrifuged at 600 g for 15 min and at 105,000 g for 75 min. The radioactivity in the subcellular fractions was extracted with acetone, the extracts were evaporated to near dryness and counted in toluene-based scintillation fluid containing 4 g PPO and 0.5 g POPOP/litre. In a typical experiment, the total counts per min were 20,485 in uterus (131 mg fresh weight for both horns) and 819 in oviduct (13 mg).

* The results are the mean $\pm$ S.E. of nine experiments.

tissue. The number of binding sites reported by Shyamala & Gorski (1969) in the immature rat uterus were 8.4×10^{-13} mol. As the immature rat uterus weighs approximately 20 to 30 mg, it is surmised that the number of oestradiol-binding sites in the oviduct is approximately one-tenth of the number in the uterus. The binding sites are calculated from the initial slope of the Scatchard plot and represent sites of high affinity. The data in Tables 1 and 3, however, show that on a wet weight basis the radioactivity in the oviduct is roughly half

Table 2. Specificity of binding of oestradiol-17 β to rat oviduct cytosol fraction

Treatment	Total bound (counts/min)	% bound
Cytosol + [^3H]oestradiol-17 β	800	100
Cytosol + oestradiol-17 β + [^3H]oestradiol-17 β	322	40.2
Cytosol + diethylstilboestrol + [^3H]oestradiol-17 β	283	35.4
Cytosol + oestrone + [^3H]oestradiol-17 β	795	99.4
Cytosol + oestradiol-17 α + [^3H]oestradiol-17 β	788	98.3
Cytosol + testosterone + [^3H]oestradiol-17 β	777	97.1
Cytosol + progesterone + [^3H]oestradiol-17 β	733	91.6

Aliquots (0.2 ml, 0.40 mg protein) of rat oviduct cytosol in tris-EDTA buffer (pH 7.4) were incubated with the indicated non-radioactive steroid hormones (1×10^{-5} M) for 15 min; followed by [2,4,6,7- ^3H]oestradiol-17 β (1×10^{-8} M = 1 μCi) for 60 min at 37° C. A 10- μl vol (10 μg approximately) of Dextran-blue was added to each tube and the incubate was passed through a Sephadex G-25 column (1.1 cm $\times$ 15 cm) in the cold. The radioactivity in the protein-bound hormone eluted with the breakthrough peak was counted.

TEXT-FIG. 1. Scatchard plot of the binding of oestradiol-17 β to the cytosol fraction of rat oviduct. Aliquots (0.2 ml) of the cytosol fraction were incubated for 60 min at 4° C in the presence of increasing concentrations of [2,4,6,7- 3 H]oestradiol-17 β . The bound radioactivity in each aliquot was measured by passage through a Sephadex G-25 column. $K_d = 4.31 \times 10^{-11}$ mol.

that in the uterus. There would therefore be appreciable binding of oestrogens in the oviduct of low affinity.

The binding of oestradiol to rat oviduct 'receptors' is subject to inhibition by compounds such as 1-[2-*p*(α -(*P*-methoxyphenyl)- β -nitrostyryl) phenoxy]-ethyl pyrrolidine monocitrate (CN-55, 945-27), that antagonize the binding of this hormone in the uterus (Table 3). In this sense, the properties of uterine and oviduct 'receptors' are similar.

These studies demonstrate the presence of 'receptors' for oestradiol in the oviduct that bind the hormone specifically and with high affinity.

Table 3. Effect of Parke Davis compound (CN-55, 945-27) on the binding of [3 H]oestradiol-17 β by rat oviduct and uterus

Treatment	Total tissue uptake (counts/min)	
	Oviduct	Uterus
Tissue + [3 H]oestradiol-17 β	9114	19243
Tissue + 5 μ g CN-55, 945-27 + [3 H]oestradiol-17 β	6846	16560
Tissue + 10 μ g CN-55, 945-27 + [3 H]oestradiol-17 β	4834	11352
Tissue + 20 μ g CN-55, 945-27 + [3 H]oestradiol-17 β	3049	5240

Minced segments (15 mg each) of oviducts and uteri collected from ovariectomized rats were incubated in the presence or absence of CN-55, 945-27 in 1 ml Eagle's essential medium for 30 min at 37° C, followed by incubation for 60 min at 37° C with 10 μ Ci (0.0278 μ g) of [2,4,6,7- 3 H]-oestradiol-17 β . After washing with 0.9% saline, the tissues were homogenized and the radioactivity was extracted with acetone and measured.

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REFERENCES

- BAULIEU, E. E., ALBERGA, A. & JUNG, I. (1967) Récepteurs hormonaux. Liaison de divers oestrogènes a des protéines utérines. Une méthode de dosage de l'oestradiol. *C.r. hebd. Séanc. Acad. Sci., Paris*, D, **265**, 501.
- BRENNER, R. M. (1967) Electron microscopy of estrogen effect on cytogenesis and secretory cell growth in rhesus monkey oviduct. *Anat. Rec.* **157**, 218.
- COUTINHO, E. M. & MAIA, H. (1970) The influence of ovarian steroids on the response of human Fallopian tube to neurohypophyseal hormones *in vivo*. *Am. J. Obstet. Gynec.* **108**, 194.
- ELLIS, D. J. & RINGOLD, H. J. (1971) *The uterine estrogen receptor. A physicochemical study*. In: *Biochemical Endocrinology*, Vol. III, The Sex Steroids, Molecular Mechanisms. Ed. K. E. McKerns. Appleton-Century-Crofts, New York.
- JENSEN, E. V. & JACOBSON, H. I. (1962) Basic guides to the mechanism of estrogen action. *Recent Prog. Horm. Res.* **18**, 387.
- JENSEN, E. V., SUZUKI, T., KWASHIMA, T., STUMPF, W. E., JUNGBLUT, P. W. & DESOMBRE, E. R. (1968) A two step mechanism for the interaction of estradiol with rat uterus. *Proc. natn. Acad. Sci. U.S.A.* **59**, 632.
- KING, R. J. B. & GORDON, J. (1966) The localization of [6,7-³H]oestradiol-17 β in rat uterus. *J. Endocr.* **34**, 431.
- NOTEBOOM, W. D. & GORSKI, J. (1965) Stereospecific binding of estrogens in the rat uterus. *Archs Biochem. Biophys.* **111**, 559.
- PUCA, G. A. & BRESCIANI, F. (1969) Association constant and specificity of estradiol binding receptor molecule. *Nature, Lond.* **223**, 745.
- ROY, S. K., JR, ROY, S. K., DAS GUPTA, P. R., ENGINEER, A. D. & KAR, A. B. (1972) Biochemical composition and *in vitro* uptake of estradiol-17 β 6,7-³H by the Fallopian tube—a preliminary study. *Am. J. Obstet. Gynec.* **112**, 299.
- SHYAMALA, G. & GORSKI, J. (1969) Estrogen receptors in the rat uterus. Studies on the interaction of cytosol and nuclear binding sites. *J. biol. Chem.* **244**, 1097.
- TALWAR, G. P., SEGAL, S. J., EVANS, A. & DAVIDSON, O. W. (1964) The binding of estradiol in the uterus: a mechanism for derepression of RNA synthesis. *Proc. natn. Acad. Sci. U.S.A.* **52**, 1059.
- TOFT, D., SHYAMALA, G. & GORSKI, J. (1967) A receptor molecule for estrogens: studies using cell free system. *Proc. natn. Acad. Sci. U.S.A.* **57**, 1740.