Relationship between plasma and uterine oestradiol in pseudopregnant rabbits

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Summary. Plasma oestradiol concentrations did not change during hCG-induced pseudopregnancy except for a slight increase on Day 18. However, a marked decline was observed in tissue oestradiol on Day 3 whether expressed on the basis of tissue wet weight or protein. The lowest concentration was found at Day 6 but the Day 18 values were comparable to those of untreated controls.

Introduction

In experimental studies dealing with reproductive endocrinology the pseudopregnant rabbit is a frequently used model. Accordingly, considerable information has been presented on the secretion of oestradiol and progesterone (see Hilliard & Eaton, 1971; Shaikh & Harper, 1972) and on the peripheral plasma levels of progesterone (Hilliard, Scaramuzzi, Penardi & Sawyer, 1974). However, relatively little quantitative information is available on oestradiol levels in peripheral plasma during rabbit pseudopregnancy, for it is generally believed that, unlike those of progesterone, there are few or no variations in plasma oestradiol concentrations.

Since plasma steroid levels may not necessarily reflect the concentrations in the target tissue and since it is reasonable to think that the biological activity will perhaps be governed by concentrations in the tissue, we have focused our attention in the present paper on relationships between plasma and tissue concentrations of oestradiol.

Materials and Methods

Animals

The 25 virgin albino rabbits weighing 2.2–3.0 kg were fed freely with a mixed diet and tap water. Pseudopregnancy was induced in 20 animals by a single injection of 100 i.u. hCG (Gonadex: Leo, Sweden) into the ear vein and 5 untreated animals served as controls. The hCG-injected animals were divided into 4 groups of 5 animals each on the basis of the duration of pseudopregnancy and accordingly were killed by cervical dislocation 3, 6, 12 or 18 days after the injection. Blood was drawn from the left heart ventricle, and after centrifugation the plasma was stored frozen at -18°C until assay. The uterus was dissected out, slit open and blotted dry. The ovarian part of one horn was pooled with the cervical part of the other and *vice versa*, thus providing two comparable tissue samples. One was used for the estimation of tissue oestradiol and the other for fluorometric determination of norepinephrine, which has been reported separately (Thorbert, Batra, Owman, Rosengren & Sjöberg, 1976).

Oestradiol assays

Plasma. The procedure for radioimmunoassay was that described by Lindberg, Lindberg, Martinsson & Johansson (1974). The antiserum was raised in sheep against oestradiol-17 β -6-oxime conjugated with bovine serum albumin and used at a dilution of 1:100 000. After

extraction of 1 ml plasma with diethyl ether, 0.1 ml each of antiserum and [^3H]oestradiol ([2,4,6,7- ^3H]oestradiol-17 β , sp. act. 102 Ci/mmol: New England Nuclear) were added. The mixture was equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal.

The data on the precision and accuracy of this method for the determination of oestradiol-17 β in the rabbit plasma were also obtained. Although the cross-reaction of the antiserum to oestradiol-17 α was only 0.7% (oestradiol-17 β = 100%), it was 11% for oestrone which could not be ignored. We therefore checked for the interference, if any, by oestrone in the extracted plasma. The concentration of oestradiol in plasma extract before and after Sephadex LH-20 chromatography was compared. Sephadex LH-20 was allowed to swell overnight in developing solvent solution (methanol:benzene, 15:85 v/v). This suspension was transferred to glass columns (1.1 cm i.d.) and packed to a gel height of 9 cm. The solvent solution was allowed to run through the column for 30 min before the application of the sample. The dried residue of the ether extract of plasma was dissolved in 0.5 ml solvent solution and 0.2 ml of this was applied on the column and subsequently eluted. Simultaneously 0.2 ml of the dissolved extract was taken into assay tube for direct determination of oestradiol without chromatography. Under the present conditions of chromatography 90% of oestradiol, as revealed by preliminary experiment using radioactive oestradiol, was eluted in the fraction between 12 and 20 ml. This system of chromatography is very effective in separating oestradiol-17 β from oestrone and oestriol as well as from progesterone and 17 α -hydroxyprogesterone (Powell & Stevens, 1973). After evaporating the solvent, the oestradiol concentration in the samples was determined by the usual radioimmunoassay.

Uterine tissue. Uterine oestradiol values were determined by the method described by Batra (1976). After thawing, the tissue was chemically digested by a mixture of NaOH and sodium dodecyl sulphate (SDS) (Batra & Bengtsson, 1976). Generally 2.5 ml of the mixture were used per g wet weight of tissue and the digestion was complete within 2 to 6 h. Removal of SDS was accomplished by Sephadex LH-20 gel chromatography, and 6 ml of the eluate were collected. After evaporating and redissolving the eluate in ethyl acetate, a suitable amount (depending on the predicted concentration of oestradiol) was taken for radioimmunoassay. Recovery of unlabelled oestradiol-17 β was found to be 94.5–99.5% and the coefficient of variation was 4.5% and 9.9% for two different pools of rabbit uterine tissue.

The sensitivity of the method, defined as the amount significantly different from zero at the 95% confidence limit was 5 pg. The blank values using NaOH and SDS were not distinguishable from zero.

Protein concentration in the digested tissue was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin dissolved in the digestion mixture as standard.

Statistical analysis was by Student's *t* test.

Results

The data on the recovery of different amounts of oestradiol added in plasma of non-pregnant rabbit are shown in Table 1. The intra- and inter-assay coefficients of variation, with values ranging from 15 to 65 pg/ml, were 9.87 and 16.2%, respectively ($n = 10$). There was an excellent agreement in the concentration of oestradiol between chromatographed and non-chromatographed extracts of plasma samples (Table 2).

Plasma oestradiol

The levels of oestradiol in plasma changed relatively little during pseudopregnancy, except for a slight rise on Day 18 (Table 3).

Table 1. Recovery of added oestradiol (pg/ml) in plasma from non-pregnant rabbit

<i>n</i>	Amount added	Amount recovered	% recovery
9	20	20.0 ± 1.9	100
10	40	40.2 ± 1.9	99

Tissue oestradiol

When expressed in terms of wet weight of tissue, oestradiol values declined rapidly, the levels being reduced to 29% of the Day 0 values by Day 3 of pseudopregnancy (Table 3). After Day 6 there was an increase which continued until Day 18, and values comparable to those of the controls were obtained. When tissue oestradiol concentrations were determined on the basis of protein content (Table 3), the pattern of changes during pseudopregnancy was similar, although the decrease was less marked.

Table 2. Comparison of oestradiol concentrations in chromatographed and non-chromatographed extracts of six different plasma samples

Sample No.	Oestradiol conc. (pg/ml)	
	Non-chromatographed sample	Chromatographed sample
1	19	20
2	21	21
3	19	19
4	29	24
5	28	30
6	25	24

There was an overall good correlation ($r = 0.83$) between the values ($n = 25$) expressed on the basis of wet weight and that of protein (data not shown).

Table 3. Plasma and uterine oestradiol concentrations (mean ± s.e.m. for 5 animals) in pseudopregnant rabbits

	Untreated controls	Day of pseudopregnancy			
		3	6	12	18
Plasma oestradiol (pg/ml)	31.80 ± 3.97	25.00 ± 3.99	28.80 ± 2.22	34.00 ± 3.54	48.60 ± 3.13*
Uterine oestradiol (pg/g wet wt)	950 ± 175	277 ± 62*	177 ± 17*	562 ± 19	936 ± 145
(pg/mg protein)	4.82 ± 0.84	3.43 ± 0.73	2.04 ± 0.26*	2.76 ± 0.17*	4.42 ± 0.74
Uterine:plasma oestradiol ratio	29.50 ± 3.27	11.60 ± 2.45*	6.23 ± 0.59*	17.20 ± 1.68*	18.80 ± 2.10*
Plasma progesterone (ng/ml)†	0.28 ± 0.07	8.92 ± 0.86*	9.44 ± 1.26*	9.42 ± 2.00*	2.34 ± 0.56*
Uterine progesterone (ng/g wet wt)†	6.12 ± 0.26	8.80 ± 1.18	22.80 ± 3.80*	13.54 ± 1.32*	4.88 ± 0.29*

*Value significantly different from controls at $P < 0.05$.

† Data from Thorbert *et al.* (1976).

Tissue/plasma oestradiol ratio

Since the plasma oestradiol levels changed relatively little, the change in the ratio of tissue oestradiol (per g wet weight) to plasma oestradiol (per ml) essentially reflected the alterations in tissue oestradiol values during pseudopregnancy (Table 3).

Discussion

Plasma oestradiol concentrations reported in the present study are in good agreement with those recently found by Wu, Blasco, Flickinger & Mikhail (1977) in the non-pregnant rabbit. The finding of relatively little change in plasma oestradiol concentrations during pseudopregnancy in the rabbit in general agrees with the data of Challis, Davies & Ryan (1973) who studied changes in ovarian hormones in pregnant rabbits. Since plasma progesterone levels in pseudopregnancy (Hilliard *et al.*, 1974; Thorbert *et al.*, 1976) and in pregnancy (Challis *et al.*, 1973) alter greatly, it must be concluded that there is no evident influence of progesterone on oestradiol secretion. This conclusion is supported by the data of Eaton & Hilliard (1971) who found no change in ovarian oestradiol secretion between ovaries with and without corpora lutea.

It is therefore noteworthy that the present results show a considerable alteration in tissue oestradiol levels in pseudopregnancy, in spite of a relatively constant level of this steroid in the plasma. However, the levels of progesterone in plasma and tissue of pseudopregnant rabbits do alter considerably (Thorbert *et al.* 1976), although in an inverse relationship to those of tissue oestradiol (Table 3), and it is natural to ask whether plasma and/or tissue progesterone concentrations influence the tissue oestradiol levels and to what extent.

The lowest concentration of oestradiol in tissue, whether expressed in terms of protein or wet weight, was found at Day 6 in this study, and coincided with the highest tissue progesterone value. The tissue levels of oestradiol rose considerably at Day 12 in spite of an unchanged level of plasma progesterone (or oestradiol), but coincident with the decrease in tissue progesterone. When tissue progesterone decreased further (Day 18), tissue oestradiol increased. The decrease in tissue progesterone at Day 12 without any change in plasma progesterone might be due to a progesterone-induced decay of progesterone receptors as discussed previously (Thorbert *et al.*, 1976). These results clearly indicate that the tissue oestradiol is influenced by progesterone and support the finding that progesterone reduces oestradiol receptors in the uterus (Jacobson, Keyes & Bullock, 1972; West, Verhage & Brenner, 1976). A similar interaction between oestradiol and progesterone after administration of steroids has recently been demonstrated for the rabbit uterus (Batra, Sjöberg & Thorbert, 1978) and other reproductive tract tissues (Resko, Boling, Brenner & Blandau, 1976).

The changes in uterine oestradiol metabolism during rabbit pregnancy might influence the concentration of oestradiol in this organ. However, the much increased activity of oestradiol dehydrogenase during pregnancy in the rabbit is due to oestradiol itself and not to an effect of progesterone (Jütting, 1970). It is therefore difficult to imagine how tissue oestradiol changes with no change in plasma oestradiol could be explained by a consideration of metabolic factors.

In a previous study we reported there was an inverse relationship between tissue progesterone and neuronal norepinephrine in the rabbit uterus (Thorbert *et al.*, 1976). Administration of oestradiol for 7 or 14 days markedly increases uterine norepinephrine content and this increase persists for at least 14 days without further treatment (Falck, Owman, Rosengren & Sjöberg, 1969a). However, with a combined progesterone and oestradiol treatment uterine norepinephrine is reduced and returns to control values (Falck *et al.*, 1969b). During pseudopregnancy a marked decrease in uterine norepinephrine was observed on Days 3, 6, and 12, with the lowest level occurring on Day 6, coinciding with the peak in tissue progesterone (Thorbert *et al.*, 1976). That observation, together with the present data, would suggest that the decreased level of nor-

epinephrine which occurs in the rabbit uterus during pseudopregnancy is caused by the action of not only increased progesterone but also decreased oestradiol tissue concentrations.

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