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Effects of Oestradiol, the Oestrous Cycle and Pregnancy on Weight, Metabolism and Cytosol Receptors in the Oviduct and Vaginal Complex of the Brushtail Possum (*Trichosurus vulpecula*)

J. D. Curlewis[A,B] *and G. M. Stone*[A]

[A] Department of Veterinary Physiology, University of Sydney, N.S.W. 2006. (Address for correspondence.)
[B] Present address: The Institute of Zoology, Regent's Park, London, NW1 4RY.

Abstract

Weight, RNA, DNA and protein content of the oviduct, vaginal cul-de-sac, lateral vagina and urogenital sinus and oestradiol and progesterone cytosol receptor concentrations in vaginal cul-de-sac, lateral vagina and urogenital sinus were examined after administration of oestradiol to ovariectomized animals and on days 0, 5, 9 and 13 of the non-pregnant cycle and on day 13 of the pregnant cycle. In ovariectomized animals, oestradiol induced an increase in weight, RNA : DNA and protein : DNA ratios and a decrease in DNA : tissue weight ratio for each organ and in addition an increase in total DNA in vaginal cul-de-sac and urogenital sinus. There was no effect of oestradiol on oestradiol cytosol receptor concentration but there was a significant increase in progesterone cytosol receptor concentration in all organs that were examined.

During the oestrous cycle, changes in the wet weight of each organ showed a common pattern with maximum weight at day 0 followed by variable rates of decline until day 13. In oviduct and vaginal cul-de-sac, the decrease in weight was paralleled by a decrease in RNA : DNA and protein : DNA ratios whereas the DNA : tissue weight ratio showed the opposite pattern and total DNA remained unchanged. The changes in the lateral vagina and urogenital sinus were similar except that a significant decline in total DNA was also seen after day 0 and the DNA : tissue weight and protein : DNA ratios in the urogenital sinus and the lateral vagina respectively showed no significant changes. Progesterone cytosol receptor concentration in the lateral vagina and urogenital sinus were high on day 0 and then declined until day 13. In contrast, there were no consistent effects on oestradiol receptor concentration.

Introduction

There are several qualitative reports of the effects of oestradiol on the reproductive tract of the brushtail possum (Tyndale-Biscoe 1966; Hughes and Rodger 1971; Khin Aye Than and McDonald 1976) but in general there have been no detailed studies. The morphological changes which occur during the oestrous cycle and pregnancy have received more attention (Pilton and Sharman 1962; Shorey and Hughes 1973) but very little is known about the concomitant changes in organ weight and metabolic activity. Previously we have reported the changes in weight, metabolic activity and oestradiol and progesterone cytosol receptor concentration of the uterus which occur after oestradiol treatment of ovariectomized animals and during the reproductive cycle of the possum (Curlewis and Stone 1986). In those experiments, oviduct, vaginal cul-de-sac, lateral vagina and urogenital sinus were also collected and the changes in organ weight, RNA and DNA metabolism and oestradiol and progesterone cytosol receptor concentration were examined and are reported in this paper.

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Materials and Methods

Animals

Mature possums were trapped in the Sydney region and maintained in captivity as previously described (Curlewis *et al.* 1985). To study the effects of oestradiol, mature female possums were ovariectomized and at least 1 week later were injected with a priming dose of 10 μg oestradiol. After 2 weeks, five animals were treated with 5 μg oestradiol daily for 3 days and five control animals were treated with vehicle only. Hormones were administered s.c. in 250 μl peanut oil.

For experiments on intact females, vaginal smears were taken on each day to monitor reproductive cycles (Curlewis *et al.* 1985). On at least their second consecutive cycle, animals were randomly allocated to be killed on days 0, 5, 9 or 13 of the non-pregnant cycle or day 13 of pregnancy (days 0, 5, 9, 13 and 13P respectively; $n = 5$ at each stage; for further details see Curlewis and Stone 1986).

Possums were killed between 0900 and 1100 h by exsanguination and cervical dislocation under pentobarbitone sodium anaesthesia, and the genital tract was quickly removed and placed on ice. Except where indicated, all further manipulations were performed at 0–6°C and where possible on crushed ice.

Tissue Preparation

After removal of the surrounding connective tissue, the genital tract was divided into the component organs as described in Fig. 1. The lumen of all organs except oviduct was opened and blotted dry following which the wet weight was recorded. Tissue samples were then stored in liquid nitrogen for up to 2 months for the receptor studies and up to 4 months for the remainder.

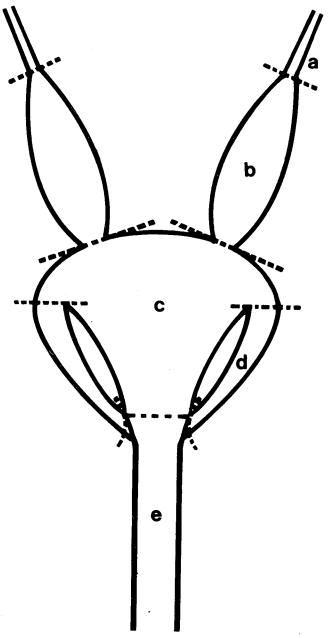

Fig. 1. Schematic drawing of the reproductive tract of the brushtail possum. Divisions were made along the broken lines as indicated. *a*, oviduct; *b*, uterus; *c*, vaginal cul-de-sac; *d*, lateral vagina; *e*, urogenital sinus.

Experimental Design

The effects of oestradiol and the reproductive cycle on organ weight, RNA : DNA, protein : DNA and DNA : tissue weight ratio and total DNA of the oviduct, vaginal cul-de-sac, lateral vagina and urogenital sinus were determined. In addition, changes in oestradiol and progesterone cytosol receptor level were examined in all tissues except oviduct. In the oestradiol administration experiment, several samples of lateral vagina collected for estimation of nucleic acid content were lost so some results for this organ have been omitted.

DNA, RNA and Protein Estimation

Finely minced tissue (oviduct, 50–200 mg; other organs, 200–500 mg) was homogenized in 6·0 ml ice-cold water using an Ultra-Turax homogenizer (Janke and Kunkel, Staufen, F.R.G.). Protein and

nucleic acids were precipitated in $HClO_4$ (0·2 mol/l) and the pellet washed twice with cold $HClO_4$ (0·2 mol/l). Nucleic acids were extracted with $HClO_4$ (0·5 mol/l) at 90°C for 20 min and the final pellet was digested in NaOH (0·25 mol/l) at 90°C for 20 min to yield the protein fraction.

Measurement of DNA was by the method of Burton (1956), RNA by the method of Mejbaum (1939) and protein by the method of Lowry *et al.* (1951) using calf thymus DNA, yeast RNA (both from Sigma Chemical Co., St Louis, Mo. U.S.A.) and bovine serum albumin (Cohn fraction V; C.S.L., Melbourne, Australia) as the respective standards.

Cytosol Receptor Assays

The concentration of cytosol receptors for oestradiol and progesterone was measured as described previously for possum uterus (Curlewis and Stone 1986). In brief, minced tissue (200–500 mg) was homogenized in 6·0 ml buffer using an Ultra-Turax homogenizer, centrifuged at 12 000 *g* for 10 min and the precipitate set aside for DNA estimation. The supernatant was centrifuged at 100 000 *g* for 60 min to give the cytosol. An aliquot of cytosol was set aside for protein estimation and the remainder was stripped of endogenous steroids with 1/10 volume of 2·8% charcoal–0·28% dextran (both w/v). For the oestradiol receptor assay, aliquots of stripped cytosol were incubated with a saturating concentration of [^{3}H]oestradiol (10 *n*mol/l) for 20 min at 30°C and were then stored for a further 18–20 h at 0°C. Non-specific binding was determined in a parallel incubation to which diethylstilboestrol (6 μmol/l) had been added. Unbound steroid was removed by charcoal–dextran as above and the [^{3}H]oestradiol receptor complex was further purified by agar gel electrophoresis. For the progesterone receptor, aliquots of stripped cytosol were incubated with cortisol (5 μmol/l) for 20 min and then with [^{3}H]promegestone (10 *n*mol/l) for a further 20 h at 0°C. Non-specific binding was determined in a parallel incubation in which (promegestone 6 μmol/l) had been added. The incubations were then treated as for the oestradiol receptor. Specific binding was estimated as the difference between total and non-specific binding. Estimates were corrected for procedural losses and dilution and then converted to picomoles and femtomoles bound. Results are expressed as picomoles or femtomoles of steroid bound per milligram of tissue DNA or cytosol protein.

Statistical Methods

For the oestradiol administration experiment, difference from the control group within each parameter and organ was tested by Student's *t*-test. Where the effect of the stage of the reproductive cycle was studied, significance of treatment effects was examined by analysis of variance of $\log_{10}$ transformed data. The effect of stage of the cycle was partitioned into four non-orthogonal contrasts. These were linear, quadratic and cubic responses over days 0, 5, 9 and 13 and the contrast day 13 *v.* day 13P. The method of Carmer and Seif (1963) was used to calculate the coefficients used to partition variance.

Results

Effects of Exogenous Oestradiol on Ovariectomized Animals

The results in Table 1 indicate that wet weight and RNA : DNA and protein : DNA ratios of each organ increased significantly after oestradiol treatment. In addition, the DNA : tissue weight ratio decreased significantly in the vaginal cul-de-sac, decreased but failed to reach significance in the oviduct and remained unchanged in the urogenital sinus. Total DNA content increased in the vaginal cul-de-sac and urogenital sinus but not in the oviduct. Oestradiol cytosol receptor concentration was not affected by oestradiol administration but there was a significant increase in progesterone receptor concentration in all organs.

Changes during the Reproductive Cycle

Oviduct

Results and summary of the analysis of variance are shown in Table 2. Weight and RNA : DNA and protein : DNA ratio were greatest at day 0 following which there was a linear decrease to day 13. The DNA : tissue weight ratio (not shown) showed the opposite pattern while total DNA remained unchanged. There were no effects of pregnancy on any parameter.

Vaginal complex

Results and summary of the analyses of variance for parameters measured in the vaginal cul-de-sac, lateral vagina and urogenital sinus are shown in Table 3 respectively. In general, a similar pattern of response was observed in each organ with maximum weight, RNA : DNA and protein : DNA ratio at oestrus followed by variable rates of decline until day 13.

Table 1. Effect of oestradiol administration on organ weight, RNA : DNA, and protein : DNA ratio, total DNA, and oestradiol and progesterone cytosol receptor concentration in organs of the reproductive tract of ovariectomized possums

Animals were treated with either peanut oil (C) or 5 μg oestradiol in peanut oil (E) for 3 days and killed on day 4. Results are means ± s.e.m. for five animals. Difference from the mean within each parameter and organ was tested using Student's *t*-test. *$0{\cdot}05 > P > 0{\cdot}01$; **$0{\cdot}01 > P > 0{\cdot}001$; ***$0{\cdot}01 > P$; n.d., not determined

Organ	Treatment	Wet weight (g)	RNA : DNA	Protein : DNA	Total DNA (mg)	Progesterone receptor (pmol/mg DNA)	Oestradiol receptor (pmol/mg DNA)
Oviduct	C	0·04 ± 0·01	0·4 ± 0·02	5 ± 0·4	0·5 ± 0·11	n.d.	n.d.
	E	0·09 ± 0·00	0·9 ± 0·06***	11 ± 0·7***	0·5 ± 0·05	n.d.	n.d.
Vaginal cul-de-sac	C	0·90 ± 0·12	0·4 ± 0·03	12 ± 1·1	4·5 ± 0·60	0·10 ± 0·04	2·44 ± 0·83
	E	4·83 ± 0·99	1·4 ± 0·08	30 ± 3·2***	8·6 ± 1·06*	0·71 ± 0·16**	4·95 ± 1·66
Lateral vagina	C	0·26 ± 0·04	n.d.	n.d.	n.d.	0·15 ± 0·03[A]	4·48 ± 0·78[A]
	E	0·81 ± 0·17	n.d.	n.d.	n.d.	0·66 ± 0·14**	4·02 ± 0·72
Urogenital sinus	C	1·52 ± 0·12	1·0 ± 0·04	21 ± 0·6	3·9 ± 0·31	0·14 ± 0·03	2·40 ± 0·56
	E	3·46 ± 0·38	1·8 ± 0·08***	36 ± 2·5***	6·7 ± 0·16***	0·37 ± 0·7*	2·54 ± 0·42

[A] One sample lost.

Table 2. Wet weight, RNA, DNA and protein metabolism in oviduct obtained from possums at days 0, 5, 9 and 13 of the non-pregnant cycle (days 0, 5, 9 and 13 respectively) and day 13 of the pregnant cycle (day 13P)

$0{\cdot}01 > P > 0{\cdot}001$; *$0{\cdot}001 > P$; n.s., not significant

Stage of cycle	Wet weight (mg)	RNA : DNA	Protein : DNA	Total DNA (mg)
0	147 ± 16	1·6 ± 0·10	14 ± 1·7	0·61 ± 0·66
5	95 ± 19	0·9 ± 0·04	10 ± 0·5	0·53 ± 0·11
9	71 ± 9	0·8 ± 0·06	10 ± 0·3	0·48 ± 0·08
13	61 ± 6	0·8 ± 0·03	9 ± 0·5	0·36 ± 0·04
13P	83 ± 6	0·8 ± 0·08	9 ± 0·3	0·53 ± 0·03

Summary of analyses of variance

Source of variation	d.f.	*F* values for above parameters			
Linear	1	22·2***	71·6***	12·4**	n.s.
Non-linear	2	0·6	8·2**	1·7	n.s.
13 *v.* 13P	1	2·8	0·3	0	n.s.

In the vaginal cul-de-sac and lateral vagina but not the urogenital sinus, DNA : tissue weight ratio was low at day 0 and then increased until day 13 (results not shown). Total DNA in the lateral vagina and urogenital sinus was high on day 0 and then declined to day 13. Pregnancy effects on these parameters were not seen.

Progesterone cytosol receptor level in the lateral vagina and urogenital sinus, but not vaginal cul-de-sac, was high on day 0 and then declined until day 13. In contrast, there were no consistent effects on oestradiol cytosol receptor level. In the vaginal cul-de-sac, there was a highly significant increase in oestradiol receptor level between days 0 and 13

Table 3. Wet weight, RNA, DNA and protein metabolism and oestradiol and progesterone cytosol receptor concentration in vaginal cul-de-sac, lateral vagina, and urogenital sinus obtained from possums at days 0, 5, 9 and 13 of the non-pregnant cycle (days 0, 5, 9 and 13 respectively) and day 13 of the pregnant cycle (day 13P)

Results are means ± s.e.m. for five animals. *0·05 > P > 0·01; **0·01 > P > 0·001; ***0·001 > P; n.s., non significant

Stage of cycle (day)	Wet weight (g)	RNA : DNA	Protein : DNA	Total DNA (mg)	Progesterone receptor (pmol/mg DNA)	Oestradiol receptor (pmol/mg DNA)
			Vaginal cul-de-sac			
0	7·7 ± 2·41	2·9 ± 0·43	34 ± 1·8	12·2 ± 3·31	0·56 ± 0·189	1·62 ± 0·602
5	2·1 ± 0·39	1·0 ± 0·10	8 ± 1·1	7·5 ± 1·50	0·85 ± 0·210	2·00 ± 0·276
9	1·6 ± 0·26	0·8 ± 0·08	3 ± 0·7	8·2 ± 1·41	0·57 ± 0·250	1·62 ± 0·392
13	1·2 ± 0·07	0·9 ± 0·05	14 ± 1·0	6·7 ± 0·70	0·39 ± 0·050	1·97 ± 0·234
13P	2·1 ± 0·35	0·9 ± 0·08	14 ± 0·8	10·7 ± 1·58	0·32 ± 0·041	1·24 ± 0·167
			Lateral vagina			
0	1·5 ± 0·31	2·6 ± 0·37	38 ± 4·8	2·8 ± 0·80	2·29 ± 0·318	5·91 ± 0·906
5	0·7 ± 0·15	1·4 ± 0·17	31 ± 3·0	1·6 ± 0·34	1·14 ± 0·120	4·23 ± 0·492
9	0·4 ± 0·09	1·1 ± 0·12	28 ± 1·2	1·2 ± 0·24	1·16 ± 0·306	3·10 ± 0·702
13	0·4 ± 0·04	1·0 ± 0·06	29 ± 2·3	1·3 ± 0·14	0·88 ± 0·156	2·78 ± 0·296
13P	0·5 ± 0·04	1·0 ± 0·05	26 ± 3·2	1·5 ± 0·11	0·58 ± 0·091	2·48 ± 0·449
			Urogenital sinus			
0	6·7 ± 1·79	2·1 ± 0·25	39 ± 3·7	12·1 ± 1·02	1·08 ± 0·104	2·16 ± 0·894
5	2·7 ± 0·30	1·4 ± 0·63	27 ± 0·8	5·7 ± 0·71	0·86 ± 0·218	2·77 ± 0·370
9	2·6 ± 0·09	1·3 ± 0·15	26 ± 1·0	5·8 ± 0·32	0·49 ± 0·148	2·43 ± 0·245
13	2·3 ± 0·23	1·2 ± 0·10	25 ± 0·9	5·4 ± 0·54	0·38 ± 0·088	1·98 ± 0·251
13P	2·5 ± 0·17	1·0 ± 0·14	26 ± 1·5	5·8 ± 0·36	0·34 ± 0·093	2·17 ± 0·342

Summary of analyses of variance

Source of variation	d.f.	F values for above parameters					
Linear	1	46·7***	69·7***	129·7***	n.s.	n.s.	n.s.
Non-linear	2	4·0*	30·3***	17·5***	n.s.	n.s.	n.s.
13 *v.* 13P	1	3·1	0	0	n.s.	n.s.	n.s.
Linear	1	31·7***	32·2***	n.s.	9·5**	13·4**	12·4**
Non-linear	2	2·7	4·2*	n.s.	1·4	0·8	0·5
13 *v.* 13P	1	0·2	0·2	n.s.	0·4	2·3	0·4
Linear	1	79·0***	17·6***	26·6***	60·1***	11·2**	n.s.
Non-linear	2	12·1***	0·9	3·3	14·1***	0·2	n.s.
13 *v.* 13P	1	0·3	0·8	0·3	0·4	0·1	n.s.

but only when protein was used as the base parameter (results not shown). In the urogenital sinus, the level also tended to increase after day 0 but in the lateral vagina the opposite response was observed. Pregnancy was without effect on receptor level except in the vaginal cul-de-sac where it was highest in non-pregnant animals.

Discussion

The increase in weight of all organs after oestradiol administration is in agreement with qualitative observations made by other authors (Hughes and Rodger 1971; Khin Aye Than and McDonald 1976). The accompanying increases in RNA : DNA and protein : DNA ratio are indicative of increased metabolic activity. In the oviduct and vaginal cul-de-sac, the increase in weight appears to be associated with hypertrophy but water imbibition could also account for the decrease in DNA : tissue weight ratio. In the vaginal cul-de-sac and urogenital sinus, evidence for hyperplasia (i.e. significant increase in total DNA) was seen and this would also contribute to the weight increase in these organs.

In all tissues, progesterone cytosol receptor level was low after ovariectomy but increased after oestradiol administration. A similar pattern has been reported for both possum uterus (Curlewis and Stone 1986) and the uterus of many other species (Milogrom *et al.* 1970; Feil *et al.* 1972; Reel and Shih 1975; Owen *et al.* 1982) and probably represents synthesis of new receptor in response to oestradiol. For the uterine oestradiol cytosol receptor, the level is usually diminished in ovariectomized animals (Feherty *et al.* 1970; Steggles and King 1970), becomes depleted immediately after oestradiol treatment but then increases over 24 h eventually to exceed that seen in ovariectomized animals. Replenishment of the oestradiol cytosol receptor is due to synthesis of new receptor and possibly receptor recycling (Mester and Bailieu 1975). In the present study, oestradiol had no effect on oestradiol receptor level in any of the tissues examined. However, Young and McDonald (1982) have shown that oestradiol administration elevated oestradiol receptor level in possum vaginal cul-de-sac endothelium and endometrium. This has been confirmed for the oestradiol receptor in whole uterus (Curlewis and Stone 1986). Reasons for this difference between uterus and the vaginal tissues are not immediately apparent but it is possible that there may be a slow rate of receptor synthesis in vaginal cul-de-sac, lateral vagina and urogenital sinus and possibly an increase in receptor level would have occurred at some time later than 24 h after the cessation of oestradiol treatment.

The changes in weight and metabolic activity of the oviduct and the vaginal complex during the oestrous cycle followed the opposite pattern to that reported previously for the uterus where cellular hypertrophy and increased metabolic activity result in a linear increase in uterine weight from day 0 to day 13 (Curlewis and Stone 1986). Changes in the parameters measured in the oviduct were suggestive of hypertrophy and high metabolic activity at day 0 but, by day 5, regressive changes which persisted until day 13 are evident. The weight of the individual organs of the vaginal complex was also maximal at day 0 and then declined until day 13. In the vaginal cul-de-sac and lateral vagina, changes indicative of hypertrophy were seen on day 0 while for the lateral vagina and urogenital sinus, evidence of hyperplasia was also seen. In those eutherian species in which there is a long oestrous cycle, hyperplasia and/or hypertrophy of the oviduct is induced by oestradiol at oestrus and atrophy during the luteal phase is due to the absence of oestradiol or the antagonistic effects of progesterone in the presence of oestradiol (for a review, see Brenner and West 1975). Hormonal control of the oviduct and vaginal complex of the possum appears similar. Plasma oestradiol concentration is high at oestrus and then declines to a very low level on days 5 to 13 (Curlewis *et al.* 1985). In view of the effects of exogenous oestradiol reported above, it is clear than endogenous oestradiol alone could account of the observed changes in the oviduct and vaginal complex during the oestrous cycle.

When the cytosol receptor data are considered, a uniform pattern which would support a role for oestradiol in modulation of the oestradiol receptor was not seen. Oestradiol cytosol receptor level in the lateral vagina was highest on day 0 then declined during the cycle, but in the vaginal cul-de-sac and urogenital sinus, the level either increased or showed no change. In contrast with these findings, Young and McDonald (1982) observed high oestradiol cytosol receptor level in the endothelium of the vaginal cul-de-sac at oestrus and a low level during the luteal phase of the cycle. This difference may be related to the experimental regimes used

in each study. Young and McDonald (1982) ovariectomized animals at each stage of the cycle and then collected tissues 3 days later for the receptor assays. In this way they hoped to avoid any effect of endogenous oestradiol on measurement of cytosol receptor level but their results would be confounded by the expected steroid effects on receptor synthesis over this period. In the present study, tissues were collected from entire animals so that endogenous oestradiol which is present in the peripheral circulation on day 0 may have masked the cytosol receptor or translocated the receptor to the nucleus thus giving a low estimate of cytosol receptor level.

The effect of pregnancy on oestradiol cytosol receptor level in the vaginal cul-de-sac at day 13 of the cycle is difficult to explain. This effect was just significant at the 5% level and was not accompanied by any functional changes. It may, however, be related to the development of the pseudo-vaginal canal which develops in this tissue at parturition under the influence of progesterone.

Progesterone cytosol receptor level in the lateral vagina and urogenital sinus was highest at oestrus and decreased later in the cycle. In the vaginal cul-de-sac, receptor level followed the same trend when expressed in terms of tissue DNA but this effect failed to reach significance. It was also shown that oestradiol administration increased progesterone receptor level in the vaginal cul-de-sac, lateral vagina and urogenital sinus so it seems likely that the high receptor level on day 0 may have been induced by oestradiol and the subsequent decline after oestrus due to the absence of oestradiol. Alternatively, the decline in progesterone receptor level after day 0 may be a result of increasing plasma progesterone concentration (Curlewis *et al.* 1985) which could mask the receptor or translocate receptor to the nucleus thus reducing the measured level. In addition, progesterone may affect replenishment of its own receptor. The changes in progesterone cytosol receptor level in the vaginal tissues of the possum suggest a role for progesterone in modulation of cellular activity in these organs. If such a role exists, then it must be associated with the regression of these organs.

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