Increased sensitivity and accumulation of estradiol in the rat oviduct during early pregnancy

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We have previously reported that a single injection of estradiol-17ß (E_2) given on day 3 of pregnancy (P3) is far more effective for accelerating oviductal transport in the rat, than treatment given on day 1 (P1). In order to quantify this change, dose-response curves were established for six different doses of E_2 (range 0.031 to 1.00 µg per animal) given on P1, P2 or P3. In addition, a possible mechanism was explored by comparing the plasmatic and oviductal levels of E_2 between 30 and 180 min following treatment with E_2 on P1 or P3. As the interval from ovulation to treatment was increased, the transport of a larger number of embryos was accelerated and a smaller dose was required. The minimal effective dose decreased 30-fold from P1 to P3, the oviducts accumulated 20% to 90% more E_2 on P3 than on P1, tissue levels were 6- to 48-fold higher than plasma levels and the latter did not differ between P1 and P3.

It is concluded that the oviduct exhibits increased sensitivity and responsiveness to E_2 on P3 and this is associated with greater accumulation of the hormone in the organ, not attributable to higher E_2 plasma levels.

Key words: *Early pregnancy, estradiol treatment, oviductal transport.*

INTRODUCTION

Estradiol administration consistently accelerates oviductal embryo transport in pregnant rats. Its effectiveness is related to the dose, mode and time of administration (Ortiz *et al*, 1979; Forcelledo *et al*, 1986). As for the time of administration, the effect becomes more intense as treatment is postponed from day 1 (P1) to day 3 (P3) of pregnancy (Ortiz *et al*, 1991). This may reflect a functional change of the genital tract during early pregnancy, a change in the bioavailability of the administered dose or simply the fact that the eggs are much closer to the uterus on P3 than at earlier intervals (Alden, 1942; Moore and Croxatto, 1988). Teleologically one would expect increased responsiveness of the oviduct to the signals that time the passage of embryos to the uterus as the right moment for this event approaches.

In the present study we established the dose-response curves of embryo transport to E_2 on P1, P2 or P3 and the blood and tissue levels of E_2 were compared after treatment on P1 or P3. The data are consistent with the occurrence of a functional change of the oviduct in early pregnancy.

MATERIAL AND METHODS

Animals and treatment

Rats of the Sprague-Dawley strain were kept under controlled environmental conditions as

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described previously (Ortiz *et al*, 1979). Vaginal smears were taken daily and after two consecutive 4-day cycles, proestrous females were caged overnight with fertile males. Mating was confirmed the next morning by the presence of sperm in the vaginal smear and that day was designated day 1 of pregnancy (P1).

In all experiments, estradiol-17 ß (E_2) (Sigma) was given at 17:00 as a single s.c. injection, dissolved in 0.1 ml propylene glycol. Control animals received the vehicle alone.

Experiment 1. This experiment was designed to compare the oviductal sensitivity and responsiveness to E_2 on days 1 to 3 of pregnancy. Rats on P1, P2 or P3, were separated into 7 groups of 5 animals (n = 35 rats/day of pregnancy). Each female received 0.031, 0.062, 0.125, 0.25, 0.5 or 1 µg of E_2 and was killed 24 h later to assess the effect of treatment on ovum transport.

Experiment 2. This experiment was designed to determine the plasma disappearance rate of E_2 after treatment on P1 or P3. Groups of 6 to 8 rats received 0.125 µg or 1 µg E_2 on either of these days. Blood samples were collected from the tail vein, immediately before and at 30, 60 and 180 min after injection. Each sample was centrifuged and the plasma obtained was stored at -20°C until the assay of E_2.

Experiment 3. This experiment was designed to compare oviductal accumulation of E_2 attained after treatment on P1 or P3. The plasma and oviductal levels of the hormone were assessed at two intervals following treatment with a high or a low dose of hormone. Groups of 8 to 12 rats received 0.125 µg or 1 µg E_2, on P1 or P3, and were bled from the abdominal aorta, under ether anesthesia, 30 or 180 min after treatment. The oviducts were dissected free of other tissue and were flushed to remove the luminal contents and ensure that E_2 measured corresponded to tissue levels. After recording the wet weight the tissues were frozen at –20°C until the assay of E_2 content. Blood samples were processed as above.

Assessment of transport

Animals were killed with an overdose of ether. Oviducts and uterine horns were separated leaving the interstitial segment attached to the oviduct. After flushing each organ separately with saline (0.9% NaCl), the number and condition of the embryos recovered were assessed.

A statistically significant reduction in the number of oviductal embryos and/or an early recovery of ova from the uterus were considered evidence of accelerated oviductal transport.

Radioimmunoassay of estradiol

Plasma and tissue E_2 concentrations were measured by RIA as previously described (Forcelledo *et al*, 1986). The minimal detectable dose was 6 pg/tube, the interassay coefficients of variation (CV) were 5% and 32%, and the intraassay CV were 12% and 20% for the high and low pool, respectively. The recovery was about 90%.

The oviducts of two rats were pooled before processing. Each pool was homogenized in 1 ml of PBS using a glass-teflon homogenizer. One 0.5 ml aliquot was extracted twice with 5 volumes of fresh diethyl ether. After evaporation, the residue was suspended in 1 ml of PBS and divided in two aliquots of 0.5 ml to be processed in duplicate as described for plasma samples.

Reagents for E_2 assay were provided by the WHO Programme for the Provision of Matched Reagents for the Radioimmunoassay of Hormones in Reproductive Physiology.

Statistical analysis

Results are presented as means ± SEM's. Differences in the total number of eggs recovered were analyzed by Student's *t* tests and the differences in tissue or plasma E_2 content across treatments were ascertained by analysis of variance (ANOVA) and non-paired Student's *t* tests. *p* values under 0.05 were considered statistically significant (Conover, 1980).

RESULTS

Influence of time of treatment on the acceleration of oviductal egg transport in response to E_2

The mean number of eggs recovered from the genital tract 24 h after treatment is shown in Figure 1. Recovery of ova from the genital tract of control animals ranged from 12.0 ± 0.7 to 13.2 ± 0.7 and was not influenced by the day of vehicle administration. All ova in control groups were in the oviducts until P4. In rats treated with E_2, the number and location of embryos differed according to the dose and the day of E_2 treatment. The number of eggs recovered from the oviduct in E_2-treated groups diminished significantly after treatment with 1 μg on P1 and P2 and 0.031 μg on P3. In addition, eggs were found prematurely in the uterus in animals treated with 0.025 μg on P2. Thus, the minimal effective dose decreased at least 30-fold from P1 to P3. Accelerated transport through the oviduct after treatment on P1 or P2 was accompanied by loss of eggs, but after treatment on P3 at least 60% of accelerated ova were retained in the uterus. These results confirm that, during early pregnancy, the transport of a larger number of embryos is accelerated and smaller doses of E_2 are required, as the interval from ovulation to treatment is prolonged.

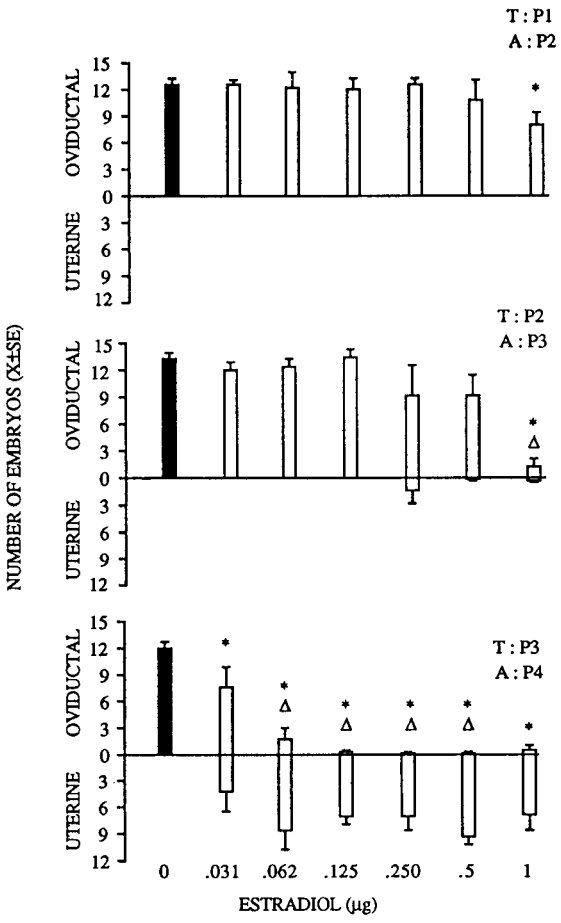

Fig 1. Mean number of embryos recovered from oviductal or uterine flushings 24 h after a single s.c. injection of estradiol-17 beta in 100 μl propylenglycol (open bars) or vehicle alone (black bars). Animals treated (T) at 17:00 h on days P1, P2 or P3, respectively, and autopsied (A) 24 h later. Each bar, mean of 5 animals. * = significantly different ($p<0.05$) from control. Δ = significantly different ($p<0.05$) from group treated with same dose and autopsied the previous day.

Plasma disappearance rate of E_2

The E_2 plasma levels attained at 30, 60 and 180 min after injecting 0.125 or 1 μg of E_2 on P1 or P3 are shown in Figure 2. Plasma disappearance rates of E_2 were best described by a function of one exponential (r = 0.989 - 0.998). On both days, a significant effect of dose and time of sampling was recognized, but the maximal plasma levels and their decays were similar for each dose.

Oviductal accumulation of E_2

The concentration of E_2 was from 6- to 48-fold higher in the oviducts than in plasma and with 1 μg it was at least twice as high as with 0.125 μg of E_2 (Fig 3). The oviducts accumulated 20 to 90% more E_2 on P3 than on P1. The difference was significant at 30 and 180 min after the lower dose and at 180 min after the higher dose of E_2. The largest difference between P1 and P3 was observed at the latter point.

From 30 to 180 min after administration of 1 μg E_2, the plasma levels decreased 80% in both groups, while in the same interval, the oviductal E_2 concentration decreased 25% on P1 and was maintained at the same level on P3. Following treatment with 0.125 μg E_2, the E_2 content of the oviduct was higher at 180 min than at 30 min in both groups although plasma E_2 levels had decreased during the same period.

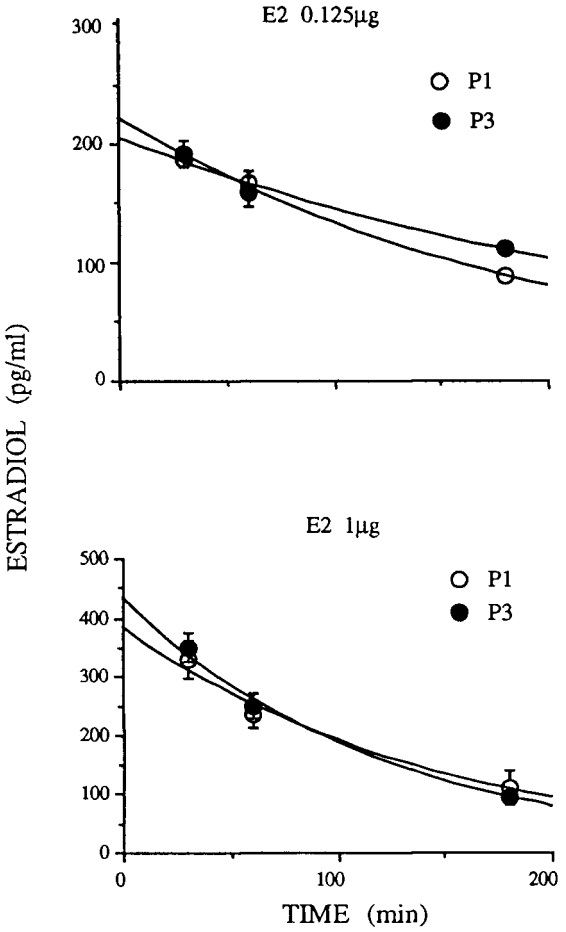

Fig 2. Plasmatic estradiol (E_2) levels of rats on days P1 or P3 after a single s.c. injection of 0.125 or 1 µg E_2. Circles, means of 8 rats (0.125 µg) or 6 rats (1 µg) sampled serially at 30, 60 and 180 min. Vertical lines, SEM's. ANOVA did not show difference in E_2 values between P1 and P3.

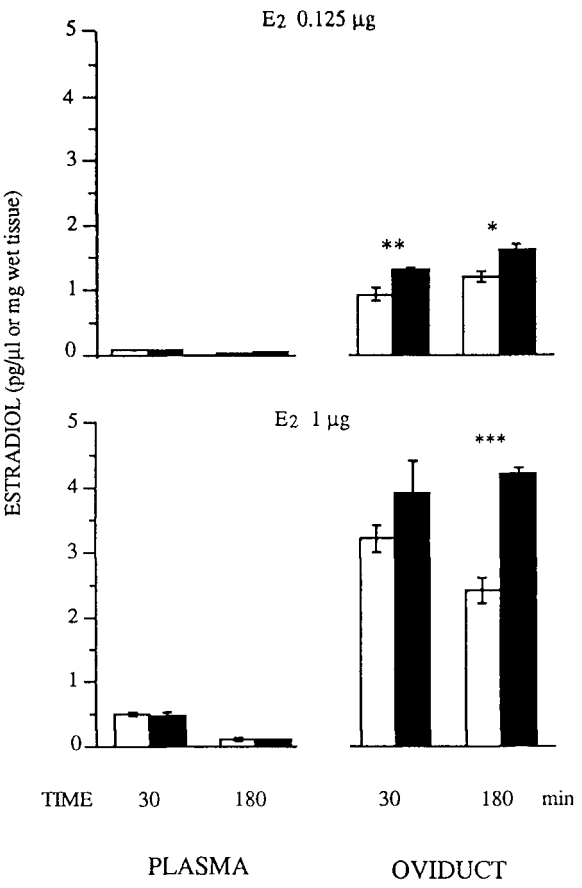

Fig 3. Plasmatic and oviductal concentration of estradiol (E_2) at 30 min and 180 min after a s.c. injection of 0.125 µg or 1 µg E_2 to rats on P1 (open bars) or P3 (black bars). Bars, means and SEM's of 12 and 8 animals for 0.125 and 1 µg, respectively. *, **, *** = P3 values significantly higher ($p<0.05$, $p<0.01$, $p<0.001$, respectively) than those of P1.

Thus, the increased sensitivity and responsiveness to E_2 was associated with a larger accumulation of the hormone in the oviduct unaccounted for differences in E_2 plasma levels.

DISCUSSION

The effectiveness of E_2 for accelerating embryo transport increased notoriously as treatment was postponed from P1 to P3. Increased effectiveness was inferred from the diminution of the minimum effective dose and from the larger number of ova accelerated. The former represents a 30 fold increase in sensitivity and the latter represents at least a 3 - 7 fold increase in responsiveness.

These results confirm and expand a previous comparison of the effectiveness of a single dose of 1 µg of E_2 for accelerating oviductal transport when given on different days to early pregnant rats (Ortiz *et al*, 1991).

A potential explanation for this different effectiveness of E_2 was explored in the present work.

Plasma estradiol levels attained within the first three hours after treatment were not different on P1 versus P3. Thus the increased effectiveness of E_2 given on P3 is not explained by increased bioavailability of the hormone at this time as compared to P1. The concentration of E_2 in the oviduct was many folds higher than in plasma and under the conditions of this experiment, it was clearly influenced by the dose of E_2 and the day of

pregnancy. The oviductal tissue content of the hormone was 20% to 90% higher on P3 than P1. Therefore, the most likely explanation for the increased effectiveness of E_2 on P3 resides in the ability of the oviduct to accumulate and retain a larger amount of hormone on this day as compared to P1. These results represent a differential capacity of the oviduct to accumulate E_2 *in vivo*. Since tissue accumulation and retention of E_2 require the presence of its receptors, we speculate that the rat oviduct may have a larger amount of total available receptors on P3 than on P1.The only measurements of E_2 receptors in the oviduct of the early pregnant rat, we are aware of, were done using biochemical methods to determine nuclear receptors rather than the total receptor population (Fuentealba *et al*, 1988). In those studies a marked increase in nuclear receptor content was observed on day 4 concomitant with an elevation of E_2 levels in plasma.

We conclude that the rat oviduct presents a substantial increase in its sensitivity and responsiveness to E_2 during early pregnancy which is associated with increased ability of the organ to accumulate and retain the hormone. The physiologic significance of this observation cannot be advanced convincingly at this time but we suspect it may be related to the need to ensure responsiveness to the signals that control the time of passage of the morulae into the uterus. This process normally starts in the evening of P4 but can start earlier when the rat oviduct is transporting transferred embryos which are more advanced than native embryos (Ortiz *et al*, 1989). Presumably, embryonic signals and/or systemic neuroendocrine signals set forth by mating, act on the oviduct to increase its capacity to accumulate E_2.

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