Concentrations of unconjugated oestradiol and progesterone in blood plasma and prostaglandins F-2 α and E-2 in oviducts of hamsters during the oestrous cycle and in early pregnancy

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Summary. Blood and tissue samples were obtained from hamsters during the oestrous cycle and for the first 4 days of pregnancy. Maximum steroid and PG concentrations occurred around ovulation with continuously declining levels afterwards, indicating that ovarian steroids, at least in part, may be responsible for oviducal PG synthesis.

Introduction

The patterns of changes of oestradiol and progesterone in peripheral blood of hamsters during the oestrous cycle is well established (Leavitt & Blaha, 1970; Lukaszewska & Greenwald, 1970; Norman & Greenwald, 1971; Shaikh, 1972; Baranczuk & Greenwald, 1973; Labhsetwar, Joshi & Watson, 1973; Ridley & Greenwald, 1975; Saidapur & Greenwald, 1978; Bast, 1979), but there is little information for the early stage of pregnancy when ovum transport takes place (Leavitt & Blaha, 1970; Lukaszewska & Greenwald, 1970; Baranczuk & Greenwald, 1974; Blaha & Leavitt, 1974). These ovarian steroids occur in specific patterns in the blood and this is important for the regulation of normal ovum transport by exerting several effects on the secretory and muscular activity of the oviduct (Hafez, 1973). It has also been reported that administration of oestrogens influences the rate of ovum transport through the oviduct of various species (Greenwald, 1967) including the hamster (Bastiaans, 1973).

Other agents involved in reproductive processes are the prostaglandins (PGs) including PGF-2 α and PGE-2 (for review see Labhsetwar, 1974). When PGF-2 α is administered i.p. to hamsters ovum transport is accelerated (Thomas, Bastiaans & Rolland, 1980). The relationship between oviducal tissue levels of PGF and ovum transport in rabbits has already been documented by Saksena & Harper (1975) and Rajkumar, Garg & Sharma (1979). The above mentioned observations led to the question of whether a relationship exists between ovarian steroids in plasma and oviducal PGF-2 α and PGE-2 tissue levels and we therefore measured (1) steroid levels in plasma of cyclic and pregnant hamsters; (2) the changes of oviducal tissue levels of PGs; and (3) the patterns obtained for steroids and PGs were compared for similarity.

Materials and Methods

Female hamsters (*Mesocricetus auratus*) weighing between 80 and 120 g were housed in temperature (22–24°C) and light (05:00–19:00 h) controlled quarters. The hamsters were checked for at least 3 consecutive oestrous cycles using the characteristic postovulatory vaginal discharge for

selection. Only animals with a regular oestrous cycle of 4 days were used. Pregnancy was achieved by caging a male with a female hamster during the night of ovulation. Mating was considered successful when spermatozoa were present in the vaginal smear and the day was defined as Day 1 of pregnancy.

The 4-day oestrous cycle and the first 4 days of pregnancy were divided into 24 and 15 sampling periods, respectively. Blood and tissue samples were collected at 03:00–05:00 h, 09:00–11:00 h, 15:00–17:00 h, and 21:00–23:00 h of each day examined with the exception of cycle and pregnancy Day 1, when the first sampling period was not used. During the cycle, samples were also collected on the odd hours between 01:00 and 08:00 h of Day 1 and those between 09:00 and 24:00 h of Day 4. For each sampling period, two different groups of approximately 4–6 hamsters were selected. After an i.p. injection of pentobarbitone sodium (Nembutal: Abbott S.A., Saint-Rémy-sur-Avre, France), 90 mg/kg body weight, one group of hamsters was bled by right ventricular heart puncture, whereas autopsy was performed on the other group of animals for removal of the oviducts. Approximately 4 ml blood were collected in a polystyrene tube containing 18.5 μ mol EDTA as anticoagulant. After centrifugation for 10 min at 1200 *g* at room temperature, the plasma samples of each animal were kept frozen at -20°C until used for determination of steroid concentrations. The oviducts were obtained by opening the abdomen and dissected free from the ovaries and uterus while kept in ice-cold saline solution (9 g NaCl/l). After blotting, the oviducts were quickly frozen in liquid nitrogen to prevent any further synthesis of PGs and stored at -70°C . PG concentrations were determined within 10 days after autopsy.

Radioimmunoassays

Oestradiol and progesterone measurements in blood plasma were based on a technique for human serum samples (Thomas, Corbey & Rolland, 1977). Chromatographic purification on Sephadex LH-20 columns (Pharmacia Fine Chemicals AB, Uppsala, Sweden) of plasma extracts of hamsters was necessary to obtain reliable results with this material. The modifications introduced included (1) the addition of 17 Bq [2,4,6,7- ^3H]oestradiol (sp. act. 4.0 TBq/mmol) and 17 Bq [1,2,6,7- ^3H]progesterone (sp. act. 3.4 TBq/mmol) (both from The Radiochemical Centre, Amersham, U.K.) to 2 ml plasma; (2) solvent extraction with 2×10 ml diethyl ether; and (3) the chromatographic separation of progesterone (fraction I) and oestradiol (fraction III) from immunoreactive material present in the dried plasma extracts. Sephadex LH-20 (0.5 g) was packed into 0.5×30 cm glass columns and three separate effluent fractions (1.6, 0.5, and 3.0 ml, respectively) of toluene/methanol (9:1, v/v) mixture were collected consecutively and used for assay determinations. The recoveries of [^3H]progesterone and [^3H]oestradiol were greater than 80%. Fraction II always contained <5% of the radioactivity added to the plasma samples and was discarded. The assay sensitivities were 8 fmol oestradiol/tube and 64 fmol progesterone/tube. Method blanks were always below the limit of sensitivity. Intra- and inter-assay variabilities were calculated (Rodbard, 1974) from duplicate measurements of one hamster plasma pool in 10 consecutive assays and were 7.2 and 12% for oestradiol, and 8.3 and 12% for progesterone, respectively.

The concentrations of PGF-2 α and PGE-2 in oviducal tissue were determined as described previously (Thomas, van den Berg, de Koning Gans & Lequin, 1978a, b). The frozen tissue (25–40 mg) was weighed and homogenized in 1 ml assay buffer (0.05 M-Tris(hydroxymethyl)amino-methane-HCl, pH 7.4 at $0-4^{\circ}\text{C}$, with 0.15 M-NaCl, 0.1% gelatin (w/v), and 0.31 μ mol calcium acetyl salicylate to prevent PG synthesis (Vane, 1971)). A small mechanically-driven Potter–Elvehjem glass homogenizer was used at 1100 rev/min for 1.5 min at $0-4^{\circ}\text{C}$ and the tube was rinsed once with 1 ml assay buffer. Cross-contamination of the homogenizer with radioactive material was avoided by adding PG tracer (17 Bq [5,6,8,11,12,14,15(n)- ^3H]PGF-2 α , sp. act. 5.92 TBq/mmol) to the combined tissue homogenate after homogenization. The addition of a single tritiated PG was sufficient to monitor the similar procedural losses of both PGF-2 α and PGE-2 throughout the following steps (Thomas *et al.*, 1978b). The homogenates were acidified with 0.1 ml 1 N-HCl to pH

3.5, extracted with 2×10 ml ethyl acetate, and the solvent residue was applied to gel filtration on Sephadex G-25. An aqueous, low molecular mass effluent was collected containing the PGs with recoveries of [^3H]PGF-2 α between 50 and 70%. The selected effluent was applied to specific radioimmunoassays for PGF-2 α and PGE-2 and the results obtained were corrected for percentage recovery. Cross-reactivity at 50% [^3H]PG displacement for the anti-PGF-2 α antiserum was 17% with PGF-1 α . The anti-PGE-2 antiserum showed 3.2% cross-reaction with PGE-1. The other PGs tested (E, F, A, B) give values of $<0.1\%$ with the antisera used. The PGE-2 assay used [5,6,8,11,12,14,15(n)- ^3H]PGE-2 with a sp. act. of 5.92 TBq/mmol. Sensitivities of the two assays were 1.4 fmol PGE-2 and 8.5 fmol PGF-2 α /tube. Method blanks were below these sensitivity limits. Intra- and inter-assay variabilities (Rodbard, 1974) were calculated from a urine pool stored at -70°C . After 10 consecutive assays the PGE-2 determinations showed 6.4 and 15%, and the PGF-2 α assay 5.3 and 11% intra- and inter-assay variability, respectively. Since the complete procedure for sample handling, including tissue homogenization, cannot be repeated in each assay, a urine pool with a proven high degree of stability was taken to monitor the precision of the PG assays used.

Results

Oestrous cycle

The results are shown in Text-fig. 1. Oestradiol values were <55 pmol/l around ovulation (occurring between 24:00 h of Day 4 and 04:00 h of Day 1) and in the morning of Day 1. A maximum oestradiol level of 991 ± 84 pmol/l (mean $\pm$ s.e.m.) was observed between 15:00 and 16:00 h on Day 4. The concentrations of progesterone were very low on Days 3 and 4 until 12:00 h, with a minimum of 0.32 nmol/l between 03:00 and 05:00 h on Day 4. Maximum progesterone levels (79.5 ± 5.4 nmol/l) were detected between 19:00 and 22:00 h on Day 4. PGF-2 α and PGE-2 showed rather similar patterns during the cycle. Minimum PG levels (1.11 ± 0.18 nmol PGF-2 α and 0.016 ± 0.002 nmol PGE-2/g oviduct) were detected between 09:00 and 17:00 h on Day 2. The highest concentrations were observed from 19:00 h on Day 4 until 08:00 h on Day 1, with a maximum level for PGF-2 α (12.8 ± 0.88 nmol/g oviduct) occurring at 21:00–22:00 h on Day 4 and for PGE-2 (0.224 ± 0.034 nmol/g oviduct) between 05:00 and 06:00 h on Day 1.

Pregnancy

As shown in Text-fig. 1, steroid levels in plasma showed moderate fluctuations during the entire period. Oestradiol values were between 73 ± 15 and 213 ± 29 pmol/l, whereas the concentrations of progesterone gave levels between 21.3 ± 3.2 and 41.3 ± 7.0 nmol/l during the first 4 days of pregnancy. In the same period, the levels of PGs in oviducts showed a decrease, from the time of ovulation early on Day 1, to low levels on Days 3 and 4. The minimum PGF-2 α concentration (2.39 ± 0.43 nmol/g) was measured at 15:00–17:00 h on Day 3 and the lowest PGE-2 value (0.027 ± 0.001 nmol/g) at 21:00–23:00 h on Day 4.

Discussion

The patterns of oestradiol and progesterone during the cycle as demonstrated in this study essentially confirm earlier observations (Leavitt & Blaha, 1970; Lukaszewska & Greenwald, 1970; Norman & Greenwald, 1971; Shaikh, 1972; Baranczuk & Greenwald, 1973; Labhsetwar *et al.*, 1973; Ridley & Greenwald, 1975; Saidapur & Greenwald, 1978; Bast, 1979), although the increased frequency of sampling as compared with most of these studies permitted us to give a more detailed description of peripheral oestradiol levels; e.g. for the oestradiol profile on cycle Day 3 (Baranczuk & Greenwald, 1973; Labhsetwar *et al.*, 1973).

Text-fig. 1. The mean $\pm$ s.e.m. concentrations of steroid hormones in peripheral blood and tissue concentrations of prostaglandins in oviducal tissue of hamsters (4–6/group) at frequent intervals throughout the oestrous cycle and during the first 4 days of pregnancy.

Little information on PG levels in oviducal tissue of several species is available; PGs have been identified in the human oviduct by immunohistological techniques (Ogra, Kirton, Tomasi & Lippes, 1974; Vastik-Fernandez, Gimeno, Lima & Gimeno, 1975) and by radioimmunoassays (Oyamada, 1981), whereas Saksena & Harper (1975) and Rajkumar *et al.* (1979) have measured PGF concentrations in oviducts of rabbits treated with hCG. The present data show that PGF-2 α and PGE-2 are present in the oviducal tissue of hamsters.

Ogra *et al.* (1974) suggested the existence of an endocrine control mechanism (i.e. ovarian steroids) that governs the production of PGF-2 α in the human oviduct. Saksena & Harper (1975) suggested that the sudden increase of oviducal PGF in rabbits 10 h after the induction of ovulation with hCG was caused by an augmented ovarian oestradiol secretion. The measurements reported now demonstrate that the PGF-2 α and PGE-2 concentrations in oviducts of cyclic hamsters show significant rhythmic patterns which resemble the peripheral plasma levels of oestradiol throughout the oestrous cycle. Similar findings on cyclic variations have also been reported for PGF levels in uterine tissue of hamsters (Saksena & Harper, 1972a), rats (Saksena & Harper, 1972b) and guinea-

pigs (Poyser, 1972). Maximum uterine PGF levels coincided with high endogenous levels of oestrogens (Poyser, 1972; Saksena & Harper, 1972a), or following oestradiol administration (Saksena & Harper, 1972a). Exogenous oestradiol significantly increased the PGF levels of the uterus, ovary and oviduct of the rabbit while progesterone depressed them to basal levels (Saksena & Harper, 1975). If one relates these results to the present data, it appears reasonable to speculate that the elevated endogenous oestradiol level in the evening of cycle Day 3 stimulates synthesis of PGF-2 α and PGE-2 in the oviduct, although this oestradiol-induced effect apparently lasts longer for PGF-2 α than for PGE-2. It would also be in agreement with the same hypothesis that the main oestradiol peak occurring in the afternoon of Day 4 concurrently with the high progesterone levels is able to stimulate the oviducal PG synthesis even more, leading to the substantial increase of PGF-2 α and PGE-2 to maximum levels in the evening of Day 4. In accordance with these findings are the observations made during the first stage of pregnancy: the low plasma levels of both oestradiol and progesterone may well explain the continuous decline of the oviducal PG levels towards the minima observed on Days 3 and 4 of pregnancy.

The results presented do not provide a complete explanation for the suggested relationship between ovarian steroids and oviducal PGs. Further experimentation involving treatments with oestrogen and PG synthetase inhibitors is necessary.

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