Changes in the concentration of prostaglandins in preovulatory human follicles after administration of hCG

M. A. Lumsden, R. W. Kelly*, A. A. Templeton†, P. F. A. Van Look‡, I. A. Swanston and D. T. Baird

*Department of Obstetrics and Gynaecology, and *MRC Unit of Reproductive Biology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, U.K.*

Summary. The concentrations of prostaglandins F-2 α , E, D-2 and 13,14-dihydro-15-keto PGE were measured in follicular fluid collected from women undergoing routine laparoscopy following induction of follicular development with clomiphene and hCG. Laparoscopy was performed before, or at 12, 24 or 36 h after administration of hCG. Prostaglandins were measured as the methyloxime derivative by radioimmunoassay. Peaks in PGE and PGF-2 α concentration occurred at 12 and 36 h with a significant nadir at 24 h, whereas PGD-2 production was very low at 36 h. The concentration of PGF-2 α rose significantly between 0 and 36 h and was greatest in follicles yielding oocytes, suggesting a possible role for this prostaglandin in the mechanism of follicle rupture.

Introduction

Although there is extensive evidence in experimental animals that prostaglandins (PGs) may be involved in ovulation, the data for women are fragmentary and incomplete. The concentration of PGE-2 and PGF-2 α in follicular fluid rises immediately before ovulation (Bauminger & Lindner, 1975; Ainsworth, Baker & Armstrong, 1975; Tsang, Ainsworth, Downey & Armstrong, 1979), probably in response to the preovulatory LH surge. In the rabbit this increase is prevented by administration of an inhibitor of prostaglandin synthetase, e.g. indomethacin (Grinwich, Kennedy & Armstrong, 1972; Holmes *et al.*, 1983). If the biological action of PGF-2 α is neutralized by an intrafollicular injection of antiserum (Armstrong, Moon & Zamecnik, 1974), follicular rupture does not occur although luteinization of the granulosa cells (as indicated by the subsequent secretion of progesterone) occurs normally. It seems unlikely, therefore, that, although prostaglandin E can stimulate steroid synthesis by granulosa cells in culture (Marsh, 1975), the LH-induced rise in PG synthesis is necessary for progesterone production, although it may play a role in activation of the oocyte in certain species (Karim & Hillier, 1979).

There is no direct evidence that prostaglandins are involved in ovulation in women. The concentration of prostaglandins in follicular fluid is highly variable but at least one study has reported an increase in the concentration of PGF-2 α in fluid of the preovulatory follicle (Darling, Jogee & Elder, 1982), and 36 h after an injection of hCG, mature follicles contain more PGF-2 α than do immature follicles (Feichtinger *et al.*, 1982). When follicular fluid was collected from infertile women at various intervals after the onset of the preovulatory LH surge, the resumption of meiosis in the oocyte was correlated with the increasing concentration of PGF-2 α (Seibel, Swartz, Smith, Levesque & Taymor, 1984).

† Present address: Department of Obstetrics & Gynaecology, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD, U.K.

‡ Present address: Human Reproduction Research Programme, WHO, CH-1211 Geneva 27, Switzerland.

In the present study we investigated the concentrations of PGE, PGF-2 α , PGD-2 and 13,14-dihydro-15-keto PGE (PGEM) in follicular fluid collected at timed intervals after administration of hCG to induce ovulation.

Patients and Methods

Details of the fixed schedule of follicular stimulation have already been published (Templeton *et al.*, 1984). Briefly, patients were recruited from the gynaecological clinic where they presented for routine laparoscopic sterilization. The study was explained to them in detail and written informed consent was obtained. Patients on the oral contraceptive pill could not enter the study until one spontaneous menstrual period had occurred. The length of the cycle which preceded induction of ovulation was adjusted by giving 10 mg norethisterone (Primulot N, Schering) daily for between 7 and 14 days starting on Day 22 of the cycle (Day 1 being the first day of the spontaneous menstrual period). Follicular development was stimulated by administration of clomiphene citrate (Serophine, Serono), 150 mg/day, from Days 7 to 11 after stopping the norethisterone treatment (Day 1 being the first day after stopping norethisterone). On Day 16 follicular diameter was measured by ultrasound scanning and the patients were admitted to hospital. Venous blood was then withdrawn every 8 h until the time of operation to measure the concentrations of LH, oestradiol and progesterone. Patients were assigned to 1 of 4 groups. In 3 groups 4500 units hCG were given on Day 17 and laparoscopy carried out 12, 24 or 36 h later. In the fourth group, laparoscopy was carried out on Day 17 without prior administration of hCG.

At the time of laparoscopic sterilization, the size and site of follicles was noted. Each follicle was aspirated, the ovum if present was identified and removed and an aliquant sample of the fluid was mixed with methyl oximating solution. Oestradiol and progesterone were measured by radioimmunoassays, the details of which have been described previously from this laboratory (Scaramuzzi, Corker, Young & Baird, 1975; McNatty *et al.*, 1976).

Prostaglandin measurement

Prostaglandins were measured by radioimmunoassay after conversion to methyl oximes. Antisera were raised against the methyl oximes of respective PGs conjugated with human serum albumin. Samples were methyl oxidized by adding an equal volume of methoxyamine hydrochloride (0.12 M) in acetate buffer (1.0 M) pH 5.6–5.8. Samples were then left overnight at room temperature to complete oximation. ³H-Labelled PGs (Amersham International, Amersham, U.K.) were derivatized in the same manner as above. Although PGF-2 α does not form an oxime, samples of PGF-2 α measurement were treated as above. Sensitivities of the assay (pg for 50% displacement of bound counts) were PGE-2, 32.0; PGD-2, 5.5; PGEM, 17.0; and PGF-2 α , 22.9. Significant cross-reactions of PGs as methyl oximes with the antisera were as follows: PGF-2 α antiserum with PGF-1 α , 7.2%, with PGF-3 α , 2.9%, with PGF-2 β , 3.5%, with PGE-2, 1.1% with 6-oxo PGF-1 α , 1.05%, with 13,14-dihydro-PGF-2 α , 1.0%, and with all other PGs tested <0.2%; PGE-2 (methyl oxidized) antiserum with E-1, 53%, with PGE-3, 31%, with PGB-2 <0.2%, with 15-oxo PGE-2, 0.25%, with 20-hydroxy PGE-2, 3.7%, with 8-iso PGE-2, 2.9%; 13,14-dihydro-15-oxo-PGE-2 (methyl oxidized) antiserum with 13,14-dihydro-PGF-2 α , 0.19%, with 15-oxo-PGF-2 α , 0.94%, with 15-oxo-PGE-2, 11.7%, with PGE-2, 0.05%, and with PGF-2 α , 0.02% (13,14-dihydro-PGE-2 was not tested). All the above cross-reactivities were determined by assessing 50% inhibition of binding by corresponding PGs as their methyl oximes. Intra-assay precision (relative standard deviation) was 12.3% for PGE, 5.0% for PGD-2, 10.8% for PGF-2 α and 4.6% for 13,14-dihydro-15-oxo-PGE. Interassay variation (relative standard deviation) was 13.3% for PGE, 8.9% for PGD-2, 13.9% for PGF-2 α and 15.0% for 13,14-dihydro-15-oxo-PGE-2.

Steroid assays

Oestradiol and progesterone were measured by radioimmunoassay. Antisera were raised against oestradiol-6(O-carboxymethyl) oxime and progesterone-11-hemisuccinate conjugated with bovine serum albumin (Scaramuzzi, Corker, Young & Baird, 1975). The method of measurement was that described by McNatty, Hunter, McNeilly & Sawers (1975) and McNatty *et al.* (1976). Assay sensitivity at sample dilution 1:1000 was 50 ng/ml for progesterone and 25 ng/ml for oestradiol. Intra-assay coefficient of variation was $3.57 \pm 0.45\%$ for progesterone and $4.15 \pm 0.29\%$ for oestradiol. The inter-assay coefficient of variation was $7.74 \pm 0.95\%$ for progesterone and $8.45 \pm 0.60\%$ for oestradiol. ^3H -labelled steroids were obtained from Amersham International, Amersham, U.K., and non-radiolabelled steroids from Sigma Chemicals, Poole, Dorset, U.K.

Statistical analysis

Since the prostaglandin concentrations were not normally distributed, non-parametric statistics were used (Wilcoxon signed rank test). Comparison between the concentration of steroid hormones in the 4 groups was made using Student's *t* test.

Results

Of the 65 patients recruited to the study, 5 had ovulated at the time of laparoscopy, an endogenous LH surge had occurred in 4 and no follicles greater than 16 mm were present in 9, leaving 47 women available for the study. Although 89 follicles were aspirated (36, 27, 10 and 16 follicles at 0, 12, 24 and 36 h, respectively) in the present study, only the results of 49 large follicles, i.e. with a volume > 4 ml (i.e. 19–20 mm diameter) are presented as it is unlikely that smaller follicles would have ovulated. An average of 1.3, 1.4, 2.0, 2.3 large follicles were aspirated at 0, 12, 24 and 36 h, respectively, and an oocyte was collected from 20% at 0 h rising to 73% of follicles at 36 h.

The results for the measurements of PGF-2 α , PGE, PGD-2 and PGEM are shown in Fig. 1. The concentration of PGF-2 α rose significantly between 0 and 36 h ($P < 0.01$). At 36 h after hCG, the concentration of PGF-2 α in fluid from follicles yielding eggs was significantly greater than in fluid from those without eggs ($P < 0.01$). The oestradiol concentration (mean $\pm$ s.e.m.) of follicles containing eggs was significantly greater than in follicles which did not (799 ± 105 ($n = 3$) and 462 ± 99 ng/ml ($n = 8$) respectively, $P < 0.05$). The concentration of PGF-2 α was significantly higher at 12 h than at 0 and 24 h ($P < 0.01$).

The pattern of concentration of PGE was similar to that of PGF-2 α with peaks at 12 and 36 h. However, there was no significant increase between 0 and 36 h although the concentration at 12 h was significantly greater than at 0 or 24 h after hCG ($P < 0.01$). The concentration at 24 h was also significantly less than at 36 h ($P < 0.01$). As with PGF-2 α , follicles which yielded eggs contained more PGE than those which did not (3384 ± 712 and 1162 ± 125 ng/ml respectively, $P < 0.01$). There was no such difference at 0, 12 h or 24 h after hCG.

Unlike those of PGF-2 α and PGE, the concentration of PGD-2 at 36 h after hCG was significantly less than the value at 12 h ($P < 0.01$), as was the concentration at 0 time ($P < 0.02$). Concentrations of the PGE metabolite did not change significantly after hCG administration.

The follicular fluid concentration of progesterone rose significantly after the injection of hCG to reach $11\,299 \pm 1698$ ng/ml at 36 h (Fig. 1). In contrast, there was a progressive fall in the concentration (mean $\pm$ s.e.m.) of oestradiol from 3099 ± 250 ng/ml at 0 h to 820 ± 126 ng/ml at 36 h after hCG.

Fig. 1. The concentrations of (a) oestradiol and progesterone, (b) PGE and PGF-2α and (c) PGD-2 and PGEM in follicular fluid. Values with a common letter are not significantly different ($P > 0.01$).

Discussion

The rise in the concentration of all the PGs occurring during the first 12 h after hCG probably reflects gonadotrophin stimulation of PG production from follicular cells as occurs from both human and animal tissues in culture (Plunkett, Moon, Zamecnik & Armstrong, 1975; Patwardhan

& Lanthier, 1981). There was no change in PGE metabolite concentration, indicating that this rise is most probably due to an increase in synthesis rather than a decrease in metabolism.

The nadir at 24 h has not been demonstrated in similar studies in animals (Ainsworth *et al.*, 1975; Downey & Ainsworth, 1980). However, a decrease in PGE-2 and 6-oxo-PGF-1 α (although apparently not statistically significant) has been reported at the metaphase I stage which corresponds to 30 h after the LH surge (Seibel *et al.*, 1984). In contrast, it was reported that the concentration of PGF-2 α increased with oocyte maturation although only 2 follicles corresponded to 0–8 h after the LH surge.

Both this study and that of Seibel *et al.* (1984) demonstrated an increase in the concentration of PGF-2 α as ovulation approaches. The concentration of PGE also tended to rise in both studies although the lack of a statistically significant rise between zero time and ovulation in our study probably resulted from a large between-subject variability. At the time of ovulation, PGF-2 α and PGE are synthesized in preference to PGD-2 which is found at very low levels. The fact that the concentration of PGF-2 α was higher in those preovulatory follicles yielding eggs than those from which eggs were not recovered makes it unlikely that the rise in PG concentration is due to cell death associated with follicular atresia. Of the eggs recovered, 6 cleaved and were therefore considered to be viable. Although failure to collect eggs may have been due to inadequate technique, it is also possible that the 3 'empty' follicles were less mature since cumulus cell dispersion is usually associated with increasing maturity (Testart, 1985) and it is likely that these eggs would aspirate more easily than those with a small, dense cumulus.

Ovulation is preceded by changes in the follicle wall which allows greater distensibility and also degradative changes allow loosening of the connective tissue matrix. It has been compared with inflammation (Espey, 1980) and it has been shown that PGs are involved in protease production and increased collagenolysis, both factors thought to be important in follicle rupture (Espey & Coons, 1976; Morales, Woessner, Howell, Marsh & LeMaire, 1978; Reich, Miskin & Tsafirri, 1985). PGF-2 α also increases ovarian contractility in man (Coutinho & Maia, 1972) and this, combined with the lower tensile strength of the follicular wall, could precipitate follicular rupture.

This is the first study to measure PGD-2 in follicular fluid since most antisera to native PGD-2 cross-react with PGF-2 α . However, our antiserum was raised to the methyloxime of the PGs and cross-reactivity between the two is <1%. PGD-2 is a potent anti-mitotic agent (Simmet & Jaffe, 1983), and it may be involved in the cessation of division of the granulosa cells which occurs as luteinization begins. This study suggests that hCG stimulates prostaglandin release from follicular tissues *in vivo* and does not appear to affect metabolism, at least of PGE. The concentrations of PGs do not correlate with those of either oestrogen or progesterone but the high preovulatory levels of PGF-2 α and PGE indicate a probable involvement in the mechanism of follicular rupture.

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