Preovulatory changes in ovarian cyclic AMP and prostaglandins in immature rats injected with PMSG

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Summary. In 28-day-old rats injected with PMSG, ovarian cyclic AMP and PGE and PGF increased 2 and 3–4 h respectively after the LH peak.

Cyclic AMP and prostaglandins are believed to be involved in the action of LH on ovarian follicles in mammals. Cyclic AMP has been implicated as a mediator of the LH effect on luteinization (Kolena & Channing, 1972) and on ovum maturation (Tsafriri, Lindner, Zor & Lamprecht, 1972a), whereas prostaglandins (PGs) have been implicated in follicular rupture. Thus, indomethacin, an inhibitor of prostaglandin synthetase, blocks the LH-induced ovulation in rats and rabbits and this blockage can be overcome by PGE-2 (Armstrong & Grinwich, 1972; Tsafriri *et al.*, 1972b). Moreover, follicular levels of PGE and PGF increase around the time of ovulation in the rabbit (Armstrong, Moon & Grinwich, 1972; Marsh, Yang & LeMaire, 1974) and rat (Bauminger & Lindner, 1975).

In a previous report (Nilsson, Rosberg, Hillensjö & Ahrén, 1975) we have shown that in immature rats injected with PMSG there is a transient elevation of ovarian cyclic AMP on the day before ovulation. The aim of the present study was to clarify the relationship between the levels of serum LH, ovarian cyclic AMP, PGE and PGF during the periovulatory period in immature rats injected with PMSG.

Methods

Immature Sprague–Dawley rats maintained under standardized environmental conditions (light 05:00–19:00 h; $22 \pm 1^\circ\text{C}$) were injected s.c. with 8 i.u. PMSG (NIAMDD-PMSG-2) on the morning of Day 28 of age. This treatment results in the ovulation of 12 ± 2 (s.e.m.) ova between 02:00 and 05:00 h on Day 31 (Herlitz, Koch, Khan & Ahrén, 1976). At different times on Days 30 and 31 (see Table 1) the animals were killed by decapitation and the trunk blood was collected for LH determination. The limits of the time intervals were chosen to give at least 5 animals in each group. The ovaries were placed in ice-chilled NaCl and trimmed of extraneous tissue. The tissue was then weighed, frozen in Frigen II and stored at -20°C until analysed. Ovarian cyclic AMP in one ovary was determined by the protein-binding technique of Gilman (1970) and protein content by the method of Lowry, Rosebrough, Farr & Randall (1951). PGE and PGF in the other ovary of each animal were analysed by radioimmunoassay as described previously (Bauminger, Lieberman & Lindner, 1975). Serum LH was determined by radioimmunoassay with a kit obtained from the Rat Pituitary Program of the National Institute of Arthritis Metabolism and Digestive Diseases (NIAMDD). The values are expressed in terms of ng NIAMDD-RP-1/ml and the detection limit was 100 ng/ml. Statistical analysis was performed with Student's *t* test or with analysis of variance (with one criterion of classification) followed by the Student–Newman–Kuel's test. A *P* value less than 0.05 was considered significant.

Results

The results are summarized in Table 1. Administration of PMSG to 28-day-old rats elicited an LH surge on the afternoon of Day 30, which was soon followed by an increase in ovarian cyclic AMP. The rise in ovarian PGE and PGF content occurred 3–4 h after the initial increase in serum LH levels and reached peak levels shortly before ovulation.

Table 1. Perioviulatory changes (mean $\pm$ s.e.m., no. of animals in parentheses) in serum LH, ovarian cyclic AMP levels, and ovarian PGE and PGF levels in immature rats treated with PMSG

Time (h)	LH (ng/ml)	cAMP (pmol/mg protein)	PGE (pg/mg ovary)	PGF (pg/mg ovary)
Day 30				
12:00–14:00	(11) 108 ± 4	(10) 31.5 ± 1.4	(9) 79 ± 5	(9) 122 ± 16
14:00–16:00	(15) 171 ± 42	(14) 33.6 ± 1.7	(5) 82 ± 13	
16:00–17:00	(16) $338 \pm 72^{*\dagger}$	(15) 37.9 ± 2.8	(6) 66 ± 6	(8) 120 ± 12
17:00–18:00	(10) $305 \pm 53^*$	(16) $39.8 \pm 2.6^{*\dagger}$	(6) 108 ± 18	(10) 137 ± 20
18:00–19:00	(16) $484 \pm 97^*$	(16) $43.9 \pm 4.5^*$	(16) $159 \pm 22^{*\dagger}$	(9) $195 \pm 22^{*\dagger}$
19:00–20:00	—	(6) $42.7 \pm 4.0^*$		
21:00–23:00	(11) $216 \pm 35^*$	(11) 36.0 ± 2.8	(6) $200 \pm 14^*$	—
23:00–01:00		(6) 36.9 ± 1.6	(10) $304 \pm 48^*$	(10) $210 \pm 29^*$
Day 31				
02:00–04:00	(6) 111 ± 5	(5) 33.3 ± 2.4	(11) $147 \pm 20^*$	(11) $173 \pm 18^*$
04:00–07:00	(6) 106 ± 5	(12) 29.7 ± 1.2	(11) 101 ± 14	(11) 138 ± 14
07:00–09:00	below 100	—	(11) 75 ± 7	(10) 146 ± 11

* These values were significantly different ($P < 0.05$) from the corresponding ones at 12:00–14:00 h (analysis of variance).

† These values represent the first significant ($P < 0.02$) elevation from the 12:00–14:00 h value (Student's *t* test).

Discussion

Changes in ovarian PG content around the time of ovulation have previously been reported for the rabbit (LeMaire & Marsh, 1975) and the adult rat (Bauminger & Lindner, 1975). In the rat, the preovulatory increase in ovarian cyclic AMP is moderate (less than 40%), but it reflects changes in the cyclic AMP content of the whole ovarian tissue, and it is reasonable to assume, therefore, that the increase in cyclic AMP levels of the ovarian cells that contain LH-receptors is more pronounced. Cyclic AMP levels had declined by the time of ovulation and there was no evidence for a second peak, as observed in the rabbit ovary after hCG administration (Goff & Major, 1975).

Our observation that the rise in ovarian cyclic AMP seems to precede that of prostaglandins is in accordance with data obtained from in-vitro experiments on preovulatory follicles in which it was shown that LH stimulates cyclic AMP formation within minutes (Lamprecht, Zor, Tsafiri & Lindner, 1973; Marsh *et al.*, 1974; Nilsson, Rosberg & Åhrén, 1974) whereas PG synthesis is elevated only after several hours (Marsh *et al.*, 1974; Yang, Marsh & LeMaire, 1974; Bauminger & Lindner, 1975). This is, however, the first study in which the levels of LH, cyclic AMP and PG *in vivo* have been simultaneously examined. The present results and those of others makes it unlikely that PGs are mediators of LH action on follicular cyclic AMP production, as suggested by Kuehl, Humes, Tarnoff, Cirillo & Ham (1970), although prostaglandins are capable of stimulating ovarian cyclic AMP formation *in vitro* (Zor *et al.*, 1973; Nilsson *et al.*, 1974). Marsh *et al.* (1974) have shown that cyclic AMP added to slices of rabbit ovaries *in vitro* can stimulate PG formation, and our results support their conclusions that the increase in ovarian cyclic AMP levels caused by LH triggers the synthesis of PGs by the ovary.

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