PROSTAGLANDIN CHANGES INDUCED BY OVULATORY STIMULI
IN RABBIT GRAAFIAN FOLLICLES.
THE EFFECT OF INDOMETHACIN

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ABSTRACT

The levels of prostaglandin F (PGF) and prostaglandin E (PGE) were
determined by radioimmunoassay in follicles obtained from rabbits at
estrous and at 5 or 9 hours after an ovulatory injection of HCG, LH or
matting. The ovulatory stimuli produced a marked increase in follicular
prostaglandin levels (PGF and PGE). The increases produced by HCG and
LH, or mating were completely abolished by the intravenous injection of
indomethacin (20 mg per kg) 30 minutes prior to the gonadotropin treatment
or at the time of mating. These results further support the concept that
prostaglandin F and E play an important and obligatory role in the process
of ovulation.

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PROSTAGLANDINS

INTRODUCTION

The exact biochemical mechanism, by which ovulation occurs is not known at this time. Several investigators have recently demonstrated a role for prostaglandin in the ovulatory process (1-7). Using inhibitors of prostaglandin synthesis, spontaneous and LH induced ovulation was inhibited in the rat and rabbit, and this blockade could not be overcome by the administration of LH, suggesting that prostaglandins are involved in the mechanism of ovulation at the ovarian level itself (3-7). Subsequently, we have shown that both PGF and PGE increase markedly within the Graafian follicles of rabbits as ovulation approaches (8), thereby providing further proof for the involvement of these compounds in the rupture of the follicle. In the latter study, the ovulatory process in the rabbit was induced with an intravenous injection of HCG. In the present study, we report similar increases in follicular prostaglandins observed after the injection of LH, as the ovulatory stimulus and after mating. Furthermore, we report the effect of pretreatment with indomethacin, a potent inhibitor of prostaglandin synthesis (9), on follicular prostaglandin levels, after initiation of the ovulatory process by HCG, LH or mating.

EXPERIMENTAL PROCEDURE

Materials - Bovine luteinizing hormone (NIH-LH-B7) was obtained from the National Institutes of Health and the LH solution was freshly prepared in sterile 0.154 M NaCl at a concentration of 50 µg/ml. Human Chorionic Gonadotropin (HCG) was a gift of Doctor J. Jewell from the Ayerst Laboratory and a solution of 100 IU per ml was prepared in 0.154 M NaCl. Indomethacin was donated by the Merck Institute for Therapeutic Research. Immediately before use, it was suspended in 0.1 M phosphate buffer, pH 8.0, at a concentration of 100 mg per ml. PGE2 and PGF2α were gifts of Doctor J. Pike from the Upjohn Company. [5,6-3H] PGE1 and [5,6-3H] PGF1α were purchased from New England Nuclear. Rabbit antisera to PGE and PGF were kindly provided by Dr. H. R. Behrman from the Merck Institute for Therapeutic Research. All the reagents for the extraction and purification of prostaglandin, ethylacetate, isopropanol, methanol and benzene, were of spectrophotometric grade.

Animals - Non-pregnant, adult female, New Zealand rabbits between 6-8 months of age (about 3 kg body weight) were used in the present studies. They were housed in individual cages in air-conditioned quarters under controlled lighting conditions (9 hours dark, 15 hours light) and fed a standard rabbit chow and water at libitum.

The process leading to ovulation was initiated by the injection into the marginal ear vein of estrous rabbits of 100 IU of HCG or 50 µg of LH in 1 ml of 0.154 M NaCl. One-half hour prior to this injection, the animals received an injection into a marginal ear vein of either 0.6 ml of 0.1 M phosphate buffer or 0.6 ml of the same buffer containing 60 mg of indomethacin in suspension. The animals were sacrificed by a blow to the head at 5 or 9 hours after the injection of HCG or LH. In the mating experiments, estrous rabbits were mated with adult males and killed 10 hours later. Two animals received an intravenous injection of 60 mg of...
indomethacin immediately after mating. In all animals, the ovaries were removed after sacrifice and follicles dissected in the cold from the adjacent ovarian tissue as described by Mills et al. (10).

Methods - The determination of prostaglandin F and E was carried out in duplicate by the radioimmunoassay method of Orczyk and Behrman (2) and Jaffe et al. (11) with modifications described previously (8). All values were corrected for losses and expressed as pg of prostaglandin per follicle.

Statistical analysis of the data was carried out using a student t test for non-paired grouped data.

RESULTS

Figure 1 shows the levels of prostaglandin F and E, in follicles obtained from rabbits sacrificed at estrous and 5 or 9 hours after an injection of an ovulatory dose of HCG, with and without pretreatment with indomethacin. The mean values ± SD for PGF and PGE at estrous were 56.8 ± 19.0 and 128 ± 59.4 pg per follicle respectively. At 5 hours after HCG injection the values were 304 ± 42.5 and 432 ± 47.4 pg per follicle and at 9 hours after HCG the corresponding values of PGF and PGE were 2124 ± 1560 and 2553 ± 827 pg per follicle. These prostaglandin levels at estrous and 5 and 9 hours after HCG injections are similar to the values reported earlier (8) and show a steady and significant increase as the time of ovulation approaches. This increase is completely abolished by the pretreatment of the animals with indomethacin. In these animals, the PGF and PGE levels at 5 hours were 25.9 ± 14.5 and 51.0 ± 62.0 pg per follicle and are significantly lower than the values for the animals at 5 hours not pretreated with indomethacin (p<0.001). These values are also lower than in the estrous controls (p = 0.05 for PGF and p = 0.1 for PGE). At 9 hours after HCG the values in the indomethacin pretreated animals were 36.9 ± 17.1 and <20 pg per follicle for PGF and PGE respectively. These levels are again significantly lower than in the animals not pretreated with indomethacin (PGF p = 0.05 and PGE p = 0.005) and are also lower than the estrous controls (p = 0.2 for PGF and p <0.02 for PGE).

Figure 2 shows the levels of prostaglandin in follicles obtained from rabbits at estrous and at 5 and 9 hours after the intravenous injection of an ovulatory dose of LH, with and without pretreatment with indomethacin. The estrous values are the same as in figure 1 and are represented here again for comparison. In the absence of indomethacin pretreatment, the mean levels ± SD of PGF and PGE at 5 hours after LH injection were 447 ± 173 and 301 ± 93.5 pg per follicle, respectively. At 9 hours the corresponding levels were 952 ± 388 and 1596 ± 946 pg per follicle. The prostaglandin levels at 5 and 9 hours after the ovulatory injection of LH again show a steady and significant rise above the estrous levels (p between 0.02 and 0.001). These increases are similar to the ones observed after the injection of HCG. At 9 hours, however, it appears that the increase observed after LH is less than after HCG for both PGF and PGE. This difference, however, is not statistically significant.
Preovulatory changes in the follicular levels of PGF (full bars) and PGE (cross hatched bars) induced by HCG and the effect of indomethacin. The height of the bars represents the mean. The number of experiments is shown in parenthesis, and the brackets indicate the standard deviation (SD). The ovulatory process was initiated by an intravenous injection of 100 IU of HCG and was preceded by 30 min with an intravenous injection of 0.6 ml of phosphate buffer or of a suspension of indomethacin (60 mg) in phosphate buffer. The animals were sacrificed at estrous and at 5 or 9 hours after the HCG injection and the prostaglandin levels determined by radioimmunoassay (2,11).
Figure 2 Preovulatory changes in the follicular levels of PGF (full bars) and PGE (cross hatched bars) induced by LH and the effect of indomethacin. These experiments were carried out in an identical fashion as in Figure 1, except that the intravenous injection of HCG was replaced by an intravenous injection of 50 μg of LH (NIH-LH-B7).
TABLE I

LEVELS OF PGF AND PGE IN RABBIT GRAAFIAN FOLLICLES

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of sacrifice</th>
<th># of rabbits</th>
<th>Level of PG (pg/follicle ± SD)¹</th>
<th>PGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>estrous</td>
<td>5</td>
<td>56.8 ± 19.0</td>
<td>128 ± 49.4</td>
</tr>
<tr>
<td>Buffer²</td>
<td>+ saline</td>
<td>5 hours</td>
<td>81.3 ± 9.8</td>
<td>241 ± 71.7</td>
</tr>
<tr>
<td>Indomethacin³</td>
<td>+ saline</td>
<td>5 hours</td>
<td>42.6 ± 29.9</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Mating⁴</td>
<td>10 hours</td>
<td>4</td>
<td>1669 ± 430</td>
<td>1648 ± 843</td>
</tr>
<tr>
<td>Mating +⁵</td>
<td>indomethacin</td>
<td>10 hours</td>
<td>29 (24-33)</td>
<td>67 (63-70)</td>
</tr>
</tbody>
</table>

¹In each experiment a single rabbit was sacrificed at the appropriate time, the follicles isolated and the levels of PGF and PGE measured in duplicate by radioimmunoassay (2,11). The values given are the mean ± SD of the total number of rabbits.

²Estrous rabbits received an intravenous injection of 1 ml of 0.154 M NaCl (the vehicle for HCG or LH) and were sacrificed 5 hours later. This injection was preceded by 30 min with an intravenous injection of 0.6 ml of phosphate buffer (the vehicle for indomethacin).

³Estrous rabbits treated as in 2 except that they received an intravenous injection of 60 mg of indomethacin suspended in 0.6 ml of phosphate buffer 30 min prior to the injection of 1 ml of 0.154 M NaCl.

⁴Estrous rabbits were allowed to mate with an adult male rabbit and then sacrificed 10 hours later.

⁵In this experiments, 2 animals received an intravenous injection of 60 mg of indomethacin in 0.6 ml phosphate buffer immediately after mating. The numbers indicate the average of the 2 animals, with the individual values between parenthesis.
Pretreatment of the rabbits with indomethacin abolishes completely
the increase in prostaglandin produced by LH as it did for HCG. The
values at 5 hours in the pretreated animals were 63.1 \pm 26.9 and 38.2
\pm 12.7 picogram per follicle for PGF and PGE respectively and at 9 hours
48.5 \pm 18.7 and <20 picogram per follicle. The values for PGF at 5 hours
and 9 hours in the pretreated animals are significantly lower than those
in the animals not given indomethacin (p = 0.02, p = 0.01) but not
significantly different from the estrous values. The PGE levels at 5
and 9 hours are again significantly lower in the indomethacin pretreated
as compared to the not pretreated animals, (p = 0.01, p = 0.05) and are
also significantly lower than the estrous levels (p = 0.05, p = 0.01).

In order to rule out any effect of the injection vehicles or animal
handling on prostaglandin levels, two additional experiments were carried
out. In the first of these experiments, 3 estrous rabbits were given an
intravenous injection of 0.6 ml of phosphate buffer (the vehicle for the
indomethacin injection) and 30 min later an intravenous injection of
1 ml of 0.154 M NaCl (the vehicle for LH or HCG) and then sacrificed
5 hours later. The concentrations of PGF and PGE in these animals were
not significantly different from the estrous controls (Table I). In the
other experiment, three animals received an intravenous injection of
indomethacin followed 30 min later by an intravenous injection of 1 ml
of 0.154 M NaCl. Again, the animals were sacrificed at 5 hours. The
concentrations of PGF (Table I) was similar to the PGF level at estrous
but the PGE value was significantly lower than at estrous (p = 0.01).

In order to assess the effect of mating on prostaglandin levels,
four animals were mated and sacrificed 10 hours later. The large pre-
ovulatory follicles were dissected and the PCF and PGE values measured.
The results listed in Table I show a significant increase over the estrous
levels (p = 0.001), similar to the increase in prostaglandin levels
observed at 9 hours after either HCG or LH injection. Two animals were
mated and received immediately thereafter an intravenous injection of
60 mg of indomethacin in 0.6 ml of phosphate buffer. They were also
sacrificed 10 hours later and the results listed in Table I, show that
their follicular levels of PGF and PGE are far below the levels of the
mated animals without pretreatment and similar to the follicular prosta-
glandin levels of estrous animals.

DISCUSSION

In a previous study, we have shown that in the rabbit Graafian
follicle, the levels of PGF and PGE increase many fold as ovulation
approaches. In that study, the ovulatory process was initiated by an
intravenous injection of HCG (8). In the present study, we have
demonstrated that the intravenous injection of an ovulatory dose of LH,
initiates similar increases in prostaglandin levels, although minor
difference between the HCG and LH effects are observed. The lower
values of both prostaglandin observed at 9 hours after LH injection,
compared to those observed 9 hours after HCG, might be due to differences
in the half life of these two gonadotropins. HCG is known to have a
much longer half life than LH in the human (12). Mating itself, which
is of course the physiologic stimulus for ovulation in the rabbit, also
produced marked increases of PGF and PGE within the follicles similar
to the increases seen following HCG or LH. These experiments therefore, further indicate that indeed increasing levels of prostaglandin within the Graafian follicle are associated with the normal physiological process of ovulation.

That these increasing levels of prostaglandin in the preovulatory follicle play an important role in ovulation itself is further suggested by the experiments using indomethacin. This compound is a well known potent blocker of prostaglandin synthesis and was shown by several investigators to block ovulation, probably by acting at the ovarian level (3-7, 13). In our experiments, indomethacin administered prior to the injection of an ovulatory dose of HCG or LH completely abolished the expected increases in PGF and PGE 5 and 9 hours later. Similarly, indomethacin administration abolished also the increase seen 10 hours after mating. These experiments support the concept that prostaglandins play an important local role within the follicle in the process of ovulation. The experiments with indomethacin indicate, furthermore, that the preovulatory increase observed after HCG, LH or mating is due to an increase in synthesis, rather than an increase in storage or a decrease in degradation of the prostaglandins. What the exact role is played by prostaglandins in the ovulatory process is uncertain at this time, but it is quite clear that any theory attempting to explain the process of ovulation should take into consideration the changes observed in follicular prostaglandins.

How ovulatory stimuli such as HCG, LH or mating produce an increase in follicular prostaglandin levels remains to be elucidated. It is possible that, similar to TSH in the thyroid, LH produces its effect on prostaglandin synthesis in follicles via cyclic AMP (14), and a stimulation of a phospholipase A2 activity (15). We have previously shown that LH stimulates the synthesis of cyclic AMP in vitro from 3H adenine in isolated rabbit Graafian follicles (16). The capability of these follicles to synthesize cyclic AMP and to respond to LH decreases, however, as ovulation approaches (17). This decrease takes place while prostaglandin levels within the follicles increase. It is possible, therefore, that the increase in follicular prostaglandin synthesis leads to the rupture of the follicle in some yet unknown fashion and also leads to an inhibition of the stimulation of adenyl cyclase by LH. This later "negative feedback" mechanism has been proposed for the stimulation of the thyroid by TSH (14). Further experiments to elucidate these mechanisms in the ovarian follicle are in progress.
REFERENCES


