Elevated peripheral concentrations of LH block ovulation and increase thecal and serum progesterone in the hamster

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Summary. Insertion of osmotic minipumps containing 1 mg ovine LH on Day 1 (oestrus) elevated circulating serum concentrations of LH, progesterone and androstenedione when compared with values at pro-oestrus. Ovulation was blocked for at least 2 days at which time there were twice the normal numbers of preovulatory follicles. Follicular and thecal progesterone production in vitro was elevated when compared with that in pro-oestrous controls. Follicular and thecal androstenedione production in vitro was lower than in controls even though serum concentrations of androstenedione were elevated; the higher androstenedione values may be due to the increase in number of preovulatory follicles when compared with pro-oestrous controls. Follicles from LH-treated hamsters aromatized androstenedione to oestradiol and follicular production of oestradiol was similar to that in pro-oestrous follicles despite low follicular androstenedione production in the LH-treated group. Treatment with 20 i.u. hCG on Days 4 or 6 after insertion of an LH osmotic minipump on Day 1 induced ovulation of ~30 ova, indicating that the blockade of ovulation was not due to atresia of the preovulatory follicles. Serum progesterone concentrations on Days 2, 4 and 6 in LH-treated hamsters were >17 nmol/l, suggesting that the blockade of ovulation might have been due to prevention of the LH surge by high serum progesterone concentrations.

Introduction

Elevation of circulating concentrations of LH increases the number of ova shed by 2–3 times in the cyclic hamster (Garza, Shaban & Terranova, 1984); however, a few animals treated with human LH or hCG (in which numerous preovulatory follicles were observed) exhibited a blockade of ovulation. This blockade of ovulation was believed to be due to extremely elevated LH or hCG values which either altered the sensitivity of follicular gonadotrophin receptors to prevent ovulation or increased the serum concentration of progesterone resulting in a hypothalamic block to ovulation (Greenwald, 1978, 1982).

Previous studies have shown that hamsters actively immunized with hCG develop numerous large cystic follicles before developing high titres of antibodies to hCG (Knecht, 1980). It is well documented that hCG binds to the LH receptor of the ovary (Midgley, 1973) and therefore very high LH values might also induce development of cystic follicles in the hamster. It is known that LH stimulates thecal androgen production (Armstrong & Dorrington, 1977) and follicular aromatase activity (Wang, Hsueh & Erickson, 1981). The aim of the present study was to determine the effects of high peripheral LH concentrations on follicular and thecal steroidogenesis, aromatizing ability and the serum concentration of progesterone. Additional aims were to determine whether the high LH values altered follicular development and the ability of the follicles to ovulate, and the relationship between serum progesterone levels and the blockade of ovulation.
Materials and Methods

Treatments. Cyclic hamsters were maintained in rooms at 22°C on a 14 h light:10 h darkness schedule (lights on at 05:00 h). The day of ovulation (Day 1, oestrus) was ascertained by the characteristic mucous discharge from the vagina. After exhibiting 3 successive 4-day oestrous cycles, hamsters were used in the experiments.

To insert the osmotic minipumps on Day 1 at 09:00–10:00 h, hamsters were anaesthetized lightly with ether and a 1-cm incision was made through the skin on the back between the scapulae as described in detail by Garza et al. (1984). An Alzet osmotic minipump (No. 2001 from Alza Corporation, Palo Alto, California, U.S.A.) containing 1 mg ovine LH (NIH-S24 with less than 0·5% FSH contamination by weight) in 200 μl 0·01 M-phosphate-buffered saline, pH 7·0, supplemented with 0·1% bovine serum albumin was inserted subcutaneously. The pumps released 1 μl (5 μg LH) per hour. Control hamsters without pumps were subjected to the surgery on the day of oestrus and were decapitated on Day 4 (pro-oestrus) or Day 6 (dioestrus 1); blood was collected and serum saved for steroid and gonadotrophin radioimmunoassays. LH-treated hamsters were checked daily for a vaginal discharge indicating ovulation; they were killed on Day 6. At necropsy, the number of obvious preovulatory follicles was determined. In the LH-treatment group (N = 6–8/group), ~12 preovulatory follicles (antral follicles) were randomly selected and isolated from each animal by microdissection on Day 6. Preovulatory follicles were dissected only on the day of pro-oestrus from control hamsters that were sham operated on the day of oestrus. Each follicle was separately incubated for 3 h at 37°C in 1 ml of incubation medium (Medium-199; Gibco, Grand Island, NY, U.S.A.) supplemented with 0·1% bovine serum albumin. Three or 4 follicles/animal were stimulated in vitro in 1 ml incubation medium with 100 ng LH (NIH), or 0·1 or 1·0 μM-androstenedione. Thecae were also isolated from a separate group of follicles, cleaned (Terranova, Martin & Chien, 1982) and stimulated with 100 ng LH as described for follicles. After incubation for 3 h, media were frozen and saved for radioimmunoassay of progesterone, androstenedione and oestradiol.

To assess the ability of the blocked preovulatory follicles to ovulate, 2 groups of hamsters were given 1 mg LH osmotic minipumps on Day 1 (oestrus) and then treated with 20 i.u. hCG i.p. at 13:00 h on Day 4 or Day 6. Both groups (8 hamsters/group) were autopsied on the morning after the injection of hCG and the number of eggs shed was determined after irrigation of the oviducts. Control hamsters were sham operated on Day 1 and given saline (9 g NaCl/l) or 20 i.u. hCG at 13:00 h on Day 4 or Day 6. The numbers of tubal ova were assessed the following morning. Ova in a plug of granulosa cells requiring dispersion with hyaluronidase were recorded as fresh ovulations. No ova were observed free of the attached granulosa cells.

In another experiment cyclic hamsters were either sham-operated or given 1 mg LH osmotic minipumps at 09:00–10:00 h on Day 1 (as described above). Then hamsters were serially bled by cardiac puncture (0·1 ml) without anaesthesia as previously described (Bast, 1979) on Days 2, 4 and 6 at 09:00–10:00 h. Blood was allowed to clot at 4°C and the serum was saved for radioimmunoassay of progesterone.

Steroid assays. The antisera for the assays were raised to 11β-hydroxyprogesterone–bovine serum albumin (GDN-337; Gibori, Antczak & Rothchild, 1977), 11α-androstenedione succinate (No. 18; Terranova, 1981) and oestradiol-6-carboxy-methylxime–thyroglobulin (E₂-TG-K; Wright, Collins & Preedy, 1973). The procedures for assays of the serum values of progesterone, androstenedione and oestradiol were identical to those described previously (Terranova & Greenwald, 1978) with modification (Terranova, 1981). The sensitivities of the progesterone, androstenedione and oestradiol assays for sera were 1·6 nmol/l, 279 pmol/l and 24 pmol/l, respectively. The coefficients of variation for the intra-assay and inter-assay variances were, respectively, 2·6 and 6·6% for progesterone, 4·9 and 5·8% for androstenedione and 2·8 and 3·8% for oestradiol. These calculations were based on duplicate determinations for 6 assays of sera.

Incubation media were assayed without ether extraction as described earlier (Terranova &
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Garza, 1983). Antiserum, tritiated steroids and standards were diluted with 0.1 M-phosphate-buffered saline containing 0.1% gelatin, pH 7.0 and were added to 12 × 75 mm glass culture tubes in volumes of 100, 100 and 20 µl respectively. Parallelism was observed between known amounts of steroids in the media and the standard curves. Incubation media without steroids were the same as a ‘zero’ standard in each assay. In these assays, equilibrium was attained after 1 h at room temperature (22°C) or after overnight incubation (18 h) at 4°C. If the assays were at room temperature, the tubes were cooled to 4°C and then an ice-cold charcoal solution was added immediately. In both procedures, after a 20-min incubation at 4°C with the charcoal solution, the tubes were centrifuged at 4°C for 20 min at 1500 g. After centrifugation, the supernatants were collected, placed in liquid scintillation vials with cocktail and counted. Details of the charcoal separation and counting procedures have been published previously (Terranova & Greenwald, 1978). In the assays of unextracted media, the sensitivities for progesterone, androstenedione and oestradiol were 0.8 nmol/l, 0.35 nmol/l and 0.35 nmol/l, respectively. The coefficients of variation for the intra-assay and inter-assay variances were, respectively, 5.9 and 9.1% for progesterone, 5.7 and 8.3% for androstenedione and 4.2 and 7.1% for oestradiol. These calculations were based on duplicate determinations for 6 assays of media.

Radioimmunoassays for gonadotrophins. Serum FSH and LH concentrations were determined by radioimmunoassay kits from NIAMDD, Bethesda, Maryland, U.S.A. The methods were similar to those of Bast & Greenwald (1974). Antiserum were anti-rat FSH, serum 10 and anti-rat LH, serum 3. Reference preparations used for standards were rat FSH-RP-1 (2.1 × NIH-FSH-S1) and LH-RP-1 (0.03 × NIH-LH-S1). In the FSH assays 100 µl sera were used and in the LH assays 25–100 µl were assayed. Duplicate serum samples were assayed. The lower limits of assay sensitivity for FSH and LH were 6.3 and 0.4 ng/tube, respectively. The coefficients of variation for intra-assay and inter-assay variances were 4.1 and 10.1% for FSH and 5.4 and 9.8% for LH.

Statistics. When appropriate, analysis of variance or Student’s t test was used to determine the level of significance of differences. Differences were considered to be significant at P < 0.05.

Results

Hamsters with LH pumps installed on Day 1 (oestrus) exhibited a blockage of ovulation; on Day 6, there were 29.6 ± 4 (mean ± s.e.m., N = 6) large (> 700 µm diameter) preovulatory follicles per pair of ovaries. Control hamsters exhibited 11.4 ± 1.1 (N = 6) preovulatory follicles (diameter 490–610 µm) per animal. Serum concentrations of progesterone, androstenedione and oestradiol were significantly higher in LH-treated hamsters than in controls (Table 1). Levels of LH (610 ± 147 ng/ml) in LH-treated hamsters were significantly higher (t test) than in controls (14 ± 2 ng/ml). Concentrations of FSH were not statistically different in the sera of the 2 groups (LH treated, 142 ± 19 ng/ml; controls, 121 ± 18 ng/ml). Follicular and thecal progesterone production in vitro was significantly higher in LH-treated hamsters when compared with pro-oestrous controls (Table 1), but androstenedione production was less (P < 0.05) than control values. Follicular oestradiol production was not significantly different in LH-treated and control hamsters. Thecal oestradiol production was less than the sensitivity of the assay (< 0.36 pmol/theca/3 h).

Oestradiol accumulation in media with follicles from LH-treated hamsters was greater than that of controls when 0.1 µM-androstenedione was added to the incubation media but not when 1 µM-androstenedione was added (Table 1).

In control hamsters the numbers of ova were 11.7 ± 1.1 (N = 7) after hCG treatment on Day 4 and 4.8 ± 2.1 (N = 6) after hCG treatment on Day 6 (control hamsters not treated with hCG ovulated 12.3 ± 0.9 ova (N = 5) on Day 4). Administration of hCG to LH-treated animals induced ovulation of 27.9 ± 1.1 (N = 8) ova on Day 4 and 32.7 ± 1.9 (N = 8) ova on Day 6 (P < 0.01 for both, t test).
Table 1. Serum concentrations of steroids, follicular and thecal steroidogenesis and follicular aromatizing ability of androstenedione to oestradiol in hamsters on Day 6 after installation on Day 1 (oestrus) of an Alzet osmotic minipump containing 1 mg ovine LH (control hamsters received osmotic minipumps containing 0.1% bovine serum albumin in phosphate-buffered saline on Day 1 and were autopsied on Day 4 (pro-oestrus) or Day 6 (dioestrus I)).

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Control (Day 4)</th>
<th>Control (Day 6)</th>
<th>LH (Day 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum steroid conc. (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>&lt;1.6*</td>
<td>15.9 ± 3.2</td>
<td>17.8 ± 4.1</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>1.13 ± 0.14</td>
<td>1.06 ± 0.23</td>
<td>2.46 ± 0.64*†</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>0.30 ± 0.06</td>
<td>0.10 ± 0.01</td>
<td>0.29 ± 0.08*</td>
</tr>
<tr>
<td>Follicular and thecal steroids (pmol/follicle/3 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle</td>
<td>15.3 ± 3.1</td>
<td>—</td>
<td>62.9 ± 8.7*</td>
</tr>
<tr>
<td>Theca</td>
<td>2.85 ± 0.46</td>
<td>—</td>
<td>68.2 ± 9.2*</td>
</tr>
<tr>
<td>Androstenedione</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle</td>
<td>5.23 ± 1.03</td>
<td>—</td>
<td>2.43 ± 0.53*</td>
</tr>
<tr>
<td>Theca</td>
<td>15.9 ± 1.4</td>
<td>—</td>
<td>5.58 ± 1.42*</td>
</tr>
<tr>
<td>Oestradiol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle</td>
<td>13.1 ± 2.7</td>
<td>—</td>
<td>12.1 ± 2.1</td>
</tr>
<tr>
<td>Theca</td>
<td>&lt;0.36</td>
<td>—</td>
<td>&lt;0.36</td>
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<tr>
<td>Aromatizing ability (pmol/follicle)</td>
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<td></td>
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<tr>
<td>0 µM-Androstenedione</td>
<td>0.51 ± 0.06</td>
<td>—</td>
<td>1.09 ± 0.06</td>
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<tr>
<td>0.1 µM-Androstenedione</td>
<td>3.19 ± 0.24</td>
<td>—</td>
<td>22.4 ± 4.9*</td>
</tr>
<tr>
<td>1.0 µM-Androstenedione</td>
<td>11.9 ± 1.9</td>
<td>—</td>
<td>15.1 ± 4.6</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. 
* P < 0.05 when compared with control values on Day 4 (*) or Day 6 (†) (Student's t test).

Table 2. Effects of installation of an LH (1 mg ovine LH-S24) osmotic minipump on Day 1 (oestrus) of the cycle on the serum concentration of progesterone (nmol/l) on Days 2 (dioestrus I), 4 (pro-oestrus) and 6 (dioestrus I).

<table>
<thead>
<tr>
<th>No. of hamsters</th>
<th>LH</th>
<th>Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>28.3 ± 4.5</td>
<td>20.7 ± 3.8</td>
</tr>
<tr>
<td>Day 4</td>
<td>30.5 ± 3.5</td>
<td>&lt;1.58</td>
</tr>
<tr>
<td>Day 6</td>
<td>22.9 ± 2.5</td>
<td>20.0 ± 3.2</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. Analysis of variance with 2-way design using repeated measures revealed a significant effect of treatment (P < 0.05).
* Sham-operated on Day 1.

Progesterone concentrations in LH-treated hamsters remained elevated throughout the treatment period (Table 2).

Discussion

Extremely elevated concentrations of LH throughout the oestrous cycle disrupted the normal 4-day cycle. This was indicated by the presence of numerous (~30) large preovulatory follicles in the ovaries on the day of expected dioestrus I; at this time, small follicles and large corpora lutea are usually present in the ovaries (Greenwald, 1974). The supernumerary follicles observed in the ovaries correlate well with the previously reported LH-induced superovulation in the hamster (Garza et al., 1984). In that study, 400 µg ovine LH (NIH-S24) in the osmotic minipump induced the ovulation of ~30 ova without disrupting the 4-day cycle. However, in the present study a larger
dose of LH (1 mg ovine LH) in the osmotic minipump disrupted the cycle but still led to the development of ~30 preovulatory follicles. Presumably, the mechanism by which LH blocked ovulation was by increasing the circulating concentration of progesterone (Table 2). Administration of progesterone to hamsters on the day of dioestrus II effectively blocks ovulation (Greenwald, 1978, 1982) by preventing the pulsatile release of LH and FSH on the expected day of pro-oestrus. Although in the present study the LH and FSH concentrations were not determined at the time of the expected pro-oestrous surges of gonadotrophin, it is likely that the surges were prevented since the preovulatory follicles from the LH-treated hamsters were able to produce large amounts of oestradiol in vitro (Table 1). If the LH surge had occurred, oestradiol production would have been expected to be substantially reduced as previously reported for the hamster (Saidapur & Greenwald, 1979). However, this must be interpreted cautiously because oestradiol production decreases after phenobarbital blockade of ovulation (Terranova, 1981).

Matson, Gledhill & Collins (1984), using PMSG-primed immature hamsters (25 days old), showed that preovulatory follicles produced large amounts of oestradiol 72–78 h after 40 i.u. PMSG, but not 96–102 h after PMSG; in addition, a large increase in the production of follicular progesterone was evident at 96 h when compared with the 72-h group and the 96-h group therefore represents a 24-h blockage of ovulation when compared with the 72-h control. In the present study, 2 days of ovulatory delay significantly increased the progesterone production by preovulatory follicles (Table 1) and oestradiol synthesis was maintained. The difference in oestradiol changes between the 2 studies might be explained by the elevated serum LH (610 ng/ml) in our study when compared with the LH level (~4 ng/ml) in the PMSG model (Matson et al., 1984). It is well known that LH maintains androgen and oestradiol synthesis and the exogenous LH perhaps prevented the decrease in oestradiol production even though the increase in follicular progesterone production and decrease in androstenedione was not prevented by LH. An oestrogen–progesterone shift similar to that described by Matson et al. (1984) has been observed in phenobarbital-treated hamsters in which serum LH levels were quite low (Terranova, 1980, 1981).

Since LH can maintain and increase aromatase activity of FSH-primed granulosa cells (Wang et al., 1981), LH might have been causal in maintaining oestradiol production by the delayed follicles of LH-treated hamsters. The accumulation of oestradiol in the media containing preovulatory follicles from LH-treated hamsters was greater than that from controls at the lower dose of androstenedione (0·1 µM) but not at the higher dose (1 µM) when compared with controls (Table 1); the reason for the differences is unknown. The amount of androstenedione used (0·1–1·0 µM) might have saturated the system so that oestradiol synthesis plateaued. The fact that the 2-day delayed follicles were able to produce oestradiol in vitro at a level similar to controls (Table 1) contrasts with previous models using barbiturates (Uilenbroek, Woutersen & van der Schoot, 1980; Braw & Tsafiri, 1980; Terranova, 1981) in which oestradiol secretion by 2-day delayed follicles was reduced. The exogenous LH may maintain oestradiol secretion by the follicles through enhancement of aromatase; however, the number of granulosa cells may be greater than in controls since the LH-treated follicles are larger.

Whether the increased follicular progesterone production and decreased androstenedione production in delayed follicles are causally related to the exogenous LH is questionable since a similar androstenedione–progesterone shift has been described for 2 other systems when serum LH is quite low. An increase in the sensitivity of the delayed follicles to circulating levels of LH might be a possible explanation since an increase in hCG receptor has been found on the granulosa cells (Uilenbroek et al., 1980) and follicles (P. F. Terranova, unpublished data) of animals in which ovulation is delayed by barbiturates. Therefore, either elevated serum concentrations of LH in the presence of 'normal' concentrations of hCG (LH) receptor or increased levels of hCG (LH) receptors in the face of 'normal' serum concentrations of LH may induce a similar androstenedione (oestradiol)–progesterone shift.

Increased thecal progesterone production and decreased androstenedione production was apparent in LH-treated hamsters when compared with controls (Table 1). Qualitatively similar
changes in thecal progesterone and androstenedione were observed when preovulatory follicles were exposed to an LH surge (Terranova et al., 1982). In LH-treated hamsters the quantity of progesterone produced by theca in vitro accounts for all the progesterone produced by the follicles in vitro (Table 2), but in controls thecal progesterone production was less than follicular progesterone, indicating that the granulosa cells provide progesterone in the intact follicle, that the granulosa cells enhance thecal progesterone synthesis and/or that theca enhances progesterone synthesis by granulosa cells. Makris & Ryan (1977) have reported an interaction between theca and granulosa in progesterone synthesis by hamster follicle cells, but the interaction between theca and granulosa appears to be lacking in LH-treated hamsters.

The observation that exogenous hCG induced superovulation of ~30 ova on Days 4 and 6 after continuous treatment with 1 mg LH osmotic minipumps on Day 1 coincides closely with the number of preovulatory follicles grossly determined on Day 6. Since ~30 fresh ova were recovered it is likely that the large preovulatory follicles were not atretic and therefore the blockade of ovulation was not due to an inability of the follicles to respond to an ‘LH surge-like’ stimulus. We suggest that LH increases the blood flow to the ovary and thus more FSH (and LH) reaches the ovary resulting in enhanced follicular development. It is well known that LH increases ovarian blood flow (Niswender, Reimers, Diekman & Nett, 1976). However, the mechanism by which LH increases blood flow is unknown although histamine may be involved (Piacsek & Huth, 1971).

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