Role of LH pulse amplitude and the rate of LH increase on oestradiol-17 β secretion by immature rat ovaries in perfusion culture

J. J. Peluso and R. W. Steger*

*Department of Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, CT 06032, and *Department of Physiology, Southern Illinois University, Carbondale, IL 62901, U.S.A.*

Summary. Immature rat ovaries were placed in perfusion culture and exposed to LH in a variety of patterns, while tonic levels of FSH (200 ng/ml) were maintained. Perfusate was collected every 5 min and assayed for oestradiol-17 β . In Exp. I, LH was delivered at 50 ng/ml for 30 min then reduced to 0 ng/ml for an additional 30 min (50 ng/ml LH step) or as linear increasing concentrations reaching 50 ng/ml by 30 min of culture then linear decreasing concentrations reaching 0 ng/ml by 30 min of culture (50 ng/ml LH ramp). Although both delivery systems exposed the tissue to 250 ng LH over the 1-h culture period, only the 50 ng/ml step significantly increased oestradiol-17 β secretion rates ($P < 0.05$). In Exp. II, ovaries were not exposed to LH for the first 30 min of culture but then LH was administered at a concentration of 25 or 50 ng/ml for 30 min. The LH concentration was reduced to 0 ng/ml for an additional 30 min of culture. Oestradiol-17 β secretion rates were increased 50% by both treatments. These results suggest that the mechanism by which an LH pulse stimulates the immature ovary to secrete oestradiol-17 β is, in part (1) 'threshold'-dependent and not solely dependent on the amount of LH administered, and (2) dependent on the time required to achieve a 'threshold' level.

Introduction

As rats approach puberty, mean LH concentrations increase (Meijs-Roelofs *et al.*, 1983). During the transition from the juvenile to the peripubertal stage, LH is secreted in a pulsatile pattern (Andrews & Ojeda, 1981; Kimura & Kawakami, 1982; Urbanski & Ojeda, 1985b). Although the frequency of these pulses remains relatively constant at about one every 30 min, an increase in the amplitude of each LH pulse ultimately accounts for the increase in the overall mean LH values (Urbanski & Ojeda, 1985b).

LH (hCG) receptors are observed in the interstitial and theca cells of the prepubertal ovary by 10 days of age and in the granulosa cells of some antral follicles by 21 days of age (Peluso *et al.*, 1976; Uilenbroek & van der Linden, 1983). The increase in mean LH values, presumably acting through ovarian LH receptors, stimulates oestrogen secretion (Quattropani & Weisz, 1973; Lamprecht *et al.*, 1976) and follicular growth (Richards & Bogovich, 1982) within the immature ovary.

When immature rat ovaries are placed in perfusion culture, the secretion of both oestradiol-17 β and progesterone is increased by exposure to tonic levels of LH (Urbanski & Ojeda, 1985a). Discontinuous or pulsatile LH exposure also enhances steroid secretion from the immature ovary. The amount of steroid secreted in response to continuous or pulsatile LH is similar as long as the amplitude of the LH pulse is equal to the LH concentration of the continuous stimulation

(Urbanski & Ojeda, 1985a). Since the amount of LH in the pulsatile treatment is several times less than in the continuous treatment, this observation reinforces the concept that LH must reach a 'threshold' concentration in order to evoke a stimulatory response. A similar relationship between LH and oestradiol secretion has been observed for the adult rat ovary (Peluso *et al.*, 1984a, b) and isolated rabbit (D'Amato *et al.*, 1981) and preovulatory rat (Hedin *et al.*, 1983) follicles.

It is clear that exposure to LH pulses stimulate physiological responses from the ovaries of immature and mature rats. However, the characteristics of the LH pulse which convey its stimulatory action have not been defined. A single LH pulse can be broken down into two major components: amplitude and the time required to reach an amplitude. The present study was designed to evaluate the contributions of each of these pulse characteristics on the ability of an LH pulse to stimulate oestradiol secretion from the immature ovary.

Materials and Methods

Preparation of culture medium. Ovine luteinizing hormone (NIAMDD-oLH-23) and ovine follicle-stimulating hormone (NIAMDD-oFSH-14) were used in these experiments. LH contamination of the ovine FSH preparation was 0.04 × ovine LH-S-1. In a 200 ng FSH-RP-1 equivalent dose there were 27 ng LH-RP-1 activity. Stock solutions were prepared by dissolving 10 mg of each gonadotrophin in 10 ml Medium-199. Each gonadotrophin was mixed separately and frozen at -20°C for up to 3 months until added to culture media. LH and FSH concentrations in the final solutions were expressed in RP-1 units. The amount of LH within the medium referred to the amount of LH activity due to the ovine LH preparation and does not include the 27 ng LH-RP-1 units of activity of the ovine FSH preparation which was present in all the media.

Gonadotrophins were added to Medium 199 which was supplemented with 2.2 g NaHCO₃/l, 2 g BSA/l, 50 mg streptomycin sulphate/l, 62.9 mg K-penicillin-G/l, 2.38 g Hepes/l and insulin (200 i.u./l; Lente Iletin I, Eli Lilly, Indianapolis, IN). The pH of the medium was adjusted to 7.4.

Animals. Immature (21 days old) female Wistar rats were obtained from Charles Rivers Laboratory, and housed under controlled conditions of temperature, humidity, and photoperiod (12 h light/12 h dark; lights on at 07:00 h). The rats were used when they were 30–32 days of age. On the day of the experiment, they were decapitated between 09:00 and 12:00 h and both ovaries were removed. The ovaries were trimmed of fat and placed in oxygenated medium supplemented with 200 i.u. insulin/l and 400 i.u. heparin/l. After 10–15 min individual ovaries were placed in a 200 µl microchamber (Endotronics, Inc., Minneapolis, MN). The microchambers were placed in a water-jacketed chamber designed to maintain the microchambers at 37°C during perfusion. The medium flow rate through each of the microchambers was 10 ml/h. The medium was continuously gassed with 5% CO₂ in oxygen throughout the culture. Perfusate was collected every 5 min. The perfusate was frozen, stored at -20°C and subsequently assayed for oestradiol-17β.

The culture system. The Acusyst 1000 culture instrument (Endotronics, Inc.) used in these studies is a computer-controlled perfusion culture system which delivers regulatory agents to culture chambers by controlling various peristaltic pumps. The Acusyst software also performed all the mathematical modelling necessary to produce the desired delivery patterns of LH. The mathematical model has been previously described (Gruenberg *et al.*, 1983).

Experimental design. In Exp. I, immature ovaries were perfused with medium containing 200 ng FSH/ml and LH delivered (1) at a concentration of 50 ng/ml for the first 30 min then decreased to 0 ng/ml for the remaining 30 min (50 ng/ml LH step) or (2) as an ascending and descending ramp in which, for the first 30 min, LH concentrations increased linearly, reaching a peak of 50 ng/ml; LH concentration then decreased linearly to 0 ng/ml by 60 min of culture (50 ng/ml LH ramp). In both treatments, FSH (200 ng/ml) was always present and a total of 250 ng LH was delivered. A group of ovaries was perfused with FSH alone (200 ng/ml) and served as a control group.

For Exp. II, immature ovaries were perfused for 30 min with tonic FSH (200 ng/ml). At 30 min LH concentration was increased to 25 or 50 ng/ml. LH concentration remained at the respective elevated value until 30 min of culture when it was reduced to 0 ng/ml. The cultures were continued for an additional 30 min. FSH was present in the medium throughout the culture period.

Oestradiol-17β assay. Oestradiol-17β was assayed using a direct double-antibody radioimmunoassay (Radioassay Systems Laboratory, Carson, CA). The antiserum to oestradiol-17β cross-reacts 100% with oestradiol-17β, 15% with oestrone and <0.01% with several other steroids. The assay sensitivity was 30 pg/ml. All samples were assayed in duplicate. The intra- and interassay coefficients of variation were 8.3% and 12.9%, respectively.

Statistical analysis. Oestradiol-17β values were expressed as a percentage of control values. The data generated in Exp. I were analysed by analysis of variance followed by Student–Newman–Keuls multiple range test. The data from Exp. II were evaluated using paired Student's *t* tests. Regardless of the mode of analysis only *P* values ≤0.05 were considered to be significant.

Fig. 1. The percentage change in oestradiol-17 β secretory rates (means $\pm$ s.e.m.) for ovaries cultured with a 50 ng/ml LH step (●—●) or a 50 ng/ml LH ramp (●---●) function. The oestradiol-17 β secretion rates for the controls are shown in the stippled area. The average oestradiol-17 β secretion rate for the control was 2.14 ± 0.02 pg/ml/mg ovarian wt.

Table 1. Effect of increasing LH step concentrations on the mean (pg/mg ovarian wt $\pm$ s.e.m.) and percentage increase in oestradiol-17 β secretion rate over the initial 30-min culture period

Treatment	Time in culture (min)	
	30–60	60–90
25 ng LH step	6.5 ± 2.4 ($165 \pm 23\%$)*	7.8 ± 3.8 ($175 \pm 32\%$)
50 ng LH step	2.6 ± 0.9 ($123 \pm 7\%$)*	3.8 ± 0.4 ($132 \pm 11\%$)*

*Values are significantly greater than oestradiol secretion rates of the first 30 min of culture ($P \leq 0.05$) ($n = 5$ /group). Mean oestradiol-17 β secretion rate for the first 30 min of culture was 16.2 ± 5.8 pg/mg ovarian wt.

Results

Immature ovaries, exposed to medium supplemented with FSH alone secreted 21.6 ± 2.2 pg oestradiol-17 β /mg ovarian weight/h. When immature ovaries were immediately exposed to a 50 ng/ml LH step, oestradiol-17 β secretion increased to 33.0 ± 4.7 pg/mg ovarian weight/h ($P < 0.05$). The 50 ng/ml LH step elevated the oestradiol-17 β secretion rate within 5 min (Fig. 1). Oestradiol-17 β secretion remained elevated throughout the 1-h culture period (Fig. 1). LH given as a linearly increasing and decreasing ramp did not stimulate oestradiol-17 β production over control levels (21.1 ± 1.7 pg/mg ovarian weight/h) ($P > 0.05$).

Regardless of whether LH was given as a 25 or 50 ng/ml LH step function, oestradiol-17 β secretion rates were elevated over control values during exposure to LH (i.e. 30–60 min of culture) and after LH was removed from the culture medium (i.e. 60–90 min of culture) ($P < 0.05$) (Table 1). Exposure to a 25 ng/ml LH step tended to result in higher oestradiol-17 β secretion rates than did the 50 ng/ml LH step but this increase was not significantly different.

Discussion

In peripubertal rats, LH is secreted in pulses ranging from a nadir of 20 to a peak of 60 ng/ml (Urbanski & Ojeda, 1985b). The present results demonstrate that the ovaries of 30–32-day-old rats respond to LH stimulation with an increase in oestradiol-17 β secretion if LH is at a concentration between 25 and 50 ng/ml. An increase in oestradiol-17 β secretion is not observed if LH concentration is increased from 25 ng/ml to 50 ng/ml, even though the ovary is exposed to twice as much LH. Urbanski & Ojeda (1985a) also failed to obtain a dose–response to LH stimulation at concentrations within the ‘physiological’ range. These observations clearly indicate that the immature ovary is not responding simply to the amount of LH. Rather, within ‘physiological’ levels of LH, the immature ovary appears to detect a ‘threshold’ concentration of LH which is required to enhance the secretion of oestradiol-17 β . Preliminary data indicate that this ‘threshold’ level is between 12 and 25 ng LH/ml (J. J. Peluso, unpublished observation).

The pulsatile pattern of LH secretion appears to be important in stimulating oestradiol-17 β secretion from immature ovaries both *in vivo* (Advis *et al.*, 1979; Andrews & Ojeda, 1981) and *in vitro* (Urbanski & Ojeda, 1985a). Previous studies indicate that the amplitude is a major factor in determining the amount of oestradiol-17 β secreted (Urbanski & Ojeda, 1985a). While the amplitude or ‘threshold’ is an important factor, the time required to achieve the stimulatory amplitude may play a key role in determining the stimulatory potential of an LH pulse. Support for this hypothesis can be found from an analysis of Exps I and II. In Exp. I, ovarian oestradiol-17 β secretion was not stimulated by a 50 ng/ml LH ramp, while a 50 ng/ml LH step promotes a 50% increase in oestradiol-17 β secretion. Since both the 50 ng/ml step and ramp possess the same amount of LH (250 ng) and the same amplitude (i.e. 50 ng/ml), some other factor(s) must account for the different responses obtained from these two types of LH pulses.

The 50 ng/ml LH ramp could have failed to stimulate oestradiol-17 β secretion because the amplitude of 50 ng/ml was only maintained for about 5 min compared to 30 min in the 50 ng/ml LH step. However, LH does not have to be present continuously in order to evoke a stimulatory response. YoungLai (1975) has shown that exposure of isolated rabbit follicles to LH for just 1 sec is sufficient to stimulate and maintain androgen secretion for up to 6 h. Similarly, hCG need only be present for 1 min to increase carbohydrate metabolism of the prepubertal ovary to the same extent as a 2-h exposure to hCG (Ahren *et al.*, 1971). The observation that the 50 ng/ml LH step increases oestradiol-17 β secretion rates within 5 min confirms the fact that in the prepubertal ovary LH acts very rapidly to evoke biological responses. Moreover, the data from Exp. II demonstrate that a 30-min exposure to a 25 ng LH/ml is sufficient to stimulate oestradiol-17 β secretion rates. Since LH concentrations are at 25 ng/ml or greater for 30 min in the 50 ng/ml ramp function, a limited exposure to LH cannot be used to explain the inability of the 50 ng/ml LH ramp to promote oestradiol-17 β secretion over control levels.

It could be argued that in the ramp function the 50 ng LH/ml concentration is not reached until 30 min into the perfusion culture, while a 50 ng LH/ml concentration is achieved immediately in the 50 ng/ml LH step function. During this 30 min delay, the ovarian tissue could perhaps become less responsive to LH. In Exp. II, LH stimulation was delayed for 30 min then increased to 25 or 50 ng/ml. These elevated concentrations were maintained for 30 min then rapidly decreased to 0 ng/ml. Regardless of whether LH was administered as a 25 or 50 ng/ml LH step, oestradiol-17 β secretion is elevated to the same degree. This observation negates the possibility that the tissue becomes less responsive during perfusion culture without exposure to LH.

The only remaining difference between the 50 ng/ml LH step function and the 50 ng/ml LH ramp function is the rate at which LH concentrations increase to a stimulatory amplitude. Both amplitude and the rate at which a secretagogue increases to a stimulatory level are important factors in controlling MSH secretion from pituitary fragments *in vitro* (Keith *et al.*, 1983). The time required to reach ‘threshold’ amplitude therefore appears to be a second component of the LH pulse that is necessary to convey a stimulatory action.

In summary, the data presented in this paper support the concept that it is the pulse characteristics of 'threshold' amplitude and the rate at which the 'threshold' level is reached that are important in controlling oestradiol-17 β secretion from immature rat ovaries *in vitro* and potentially *in vivo*. Further, since most hormones are secreted in an episodic or pulsatile manner, 'threshold' and the time required to reach a 'threshold' may be universal elements in the mechanism by which hormonal pulses evoke physiological responses from their target tissues *in vivo*.

The LH and FSH were gifts from the National Pituitary Agency.

References

- Advis, J.P., Andrews, W.W. & Ojeda, S.R. (1979) Changes in ovarian steroidal and prostaglandin E responsiveness to gonadotropins during the onset of puberty in the female rat. *Endocrinology* **104**, 653–658.
- Ahren, K., Hamberger, L. & Perklev, T. (1971) Rapid interaction between gonadotrophic hormones and the prepubertal rat ovary. *Acta physiol. scand.* **82**, 191–201.
- Andrews, W.W. & Ojeda, S.R. (1981) A detailed analysis of the serum luteinizing hormone secretory profile in conscious, free-moving female rats during the time of puberty. *Endocrinology* **109**, 2032–2039.
- D'Amato, C., Calvo, F.O., Stockert, B. & Bahr, J.M. (1981) Steroid secretion by perfused rabbit follicles: effect of repeated gonadotropin challenges. *Biol. Reprod.* **25**, 843–850.
- Gruenberg, M.L., Steger, R.W. & Peluso, J.J. (1983) Follicular development, steroidogenesis and ovulation within ovaries exposed *in vitro* to hormone levels which mimic those of the rat estrous cycle. *Biol. Reprod.* **29**, 1265–1275.
- Hedin, L., Ekholm, C. & Hillensjo, T. (1983) Dose-related effects of luteinizing hormone on the pattern of steroidogenesis and cyclic adenosine monophosphate release in superfused preovulatory rat follicles. *Biol. Reprod.* **29**, 895–904.
- Keith, D.L., Allen, R.G., Stack, J., Robertson, L.M. & Kendall, J.W. (1983) Potassium-modulated secretion of immunoreactive melanocyte-stimulating hormone and endorphin from mouse neuro-intermediate lobes. *Endocrinology* **112**, 1886–1889.
- Kimura, F. & Kawakami, M. (1982) Episodic LH secretion in immature male and female rats as assessed by sequential blood sampling. *Neuroendocrinology* **35**, 128–132.
- Lamprecht, S.A., Kohen, F., Ausher, J., Zor, U. & Lindner, H.R. (1976) Hormonal stimulation of oestradiol-17 β release from the rat ovary during early postnatal development. *J. Endocr.* **68**, 343–344.
- Meijs-Roelofs, H.M.A., Kramer, P. & Sander, H.J. (1983) Changes in serum concentration of luteinizing hormone in the female rat approaching puberty. *J. Endocr.* **98**, 241–249.
- Peluso, J.J., Steger, R.W. & Hafez, E. (1976) Development of gonadotrophin-binding sites in the immature rat ovary. *J. Reprod. Fert.* **47**, 55–58.
- Peluso, J.J., Downey, M.C. & Gruenberg, M.L. (1984a) Role of LH pulse amplitude in controlling rat ovarian oestradiol-17 β secretion *in vitro*. *J. Reprod. Fert.* **71**, 107–112.
- Peluso, J.J., Gruenberg, M.L. & Steger, R.W. (1984b) Regulation of ovarian follicular growth and steroidogenesis by low-amplitude LH pulses. *Am. J. Physiol.* **246**, R184–R189.
- Quattropiani, S.L. & Weisz, J. (1973) Conversion of progesterone to estrone and estradiol *in vitro* by the ovary of the infantile rat in relation to the development of its interstitial tissue. *Endocrinology* **93**, 1269–1276.
- Richards, J.S. & Bogovich, K. (1982) Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. *Endocrinology* **111**, 1429–1438.
- Uilenbroek, J.Th.J. & van der Linden, R. (1983) Changes in gonadotropin binding to rat ovaries during sexual maturation. *Acta endocr., Copenh.* **103**, 413–419.
- Urbanski, H.F. & Ojeda, S.R. (1985a) *In vitro* simulation of prepubertal changes in pulsatile luteinizing hormone release enhances progesterone and 17 β -estradiol secretion from immature rat ovaries. *Endocrinology* **117**, 638–643.
- Urbanski, H.F. & Ojeda, S.R. (1985b) The juvenile-peripubertal transition period in the female rat: establishment of a diurnal pattern of pulsatile luteinizing hormone secretion. *Endocrinology* **117**, 644–649.
- YoungLai, E.V. (1975) Rapid interaction between luteinizing hormone and isolated rabbit follicles. *J. Endocr.* **67**, 289–296.

Received 10 December 1986