Role of LH pulse amplitude in controlling rat ovarian oestradiol-17β secretion in vitro

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Summary. Ovaries from di-oestrous rats were removed and placed in perfusion culture: 4–6 ovaries were cultured for 3 h with (1) no gonadotrophin; (2) tonic FSH (200 ng/ml); (3) tonic LH (30 ng/ml); (4) tonic FSH and tonic LH; (5) tonic FSH and hourly pulses of 40 ng LH/ml; or (6) tonic FSH and hourly pulses of 50 ng LH/ml. The total amount of LH administered was 3060 ng LH, regardless of mode of delivery. Perifusate was collected every 10 min and assayed for oestradiol-17β by RIA. The total amount of oestradiol-17β secreted was not altered by any treatment except when LH was administered in hourly pulses with an amplitude of 50 ng/ml; the total amount of oestradiol-17β secreted in 3 h was increased by 300% (P < 0.05). Without gonadotrophic stimulation, oestradiol-17β was secreted at a constant rate (4.48 ± 0.21 pg/mg 10 min−1). Tonic gonadotrophin stimulation did not alter this pattern. However, pulses of 50 ng LH/ml but not 40 ng LH/ml resulted in periodic increases in oestradiol-17β secretory rates. Thus, oestradiol-17β secretion is stimulated by LH pulses with the degree of stimulation dependent, in part, on the amplitude and/or the rate of change of the LH pulse.

Introduction

As small antral follicles grow, they develop the ability to synthesize and secrete oestradiol-17β (Bogovich & Richards, 1982). LH acts on developing follicles to induce thecal cell differentiation and subsequent thecal androgen synthesis (Carson, Richards & Kahn, 1981). FSH induces and maintains aromatase activity within the granulosa cells, thus allowing the thecal androgen to be converted into oestradiol-17β (Dorrington, 1977). In the rat rapid follicular growth (Peluso & Downey, 1982) and the associated enhancement of oestradiol-17β secretion (Butcher, Collins & Fugo, 1974) occur on the day of di-oestrus. Although LH levels are low during di-oestrus, they are thought to play an important role in stimulating follicular oestradiol-17β secretion. During di-oestrus, LH is secreted in hourly pulses (Gallo, 1981). In-vitro exposure to LH pulses which mimic those of the di-oestrous rat increase oestradiol-17β secretion by 20% over that of di-oestrous ovaries cultured with tonic levels of LH (Peluso, Gruenberg & Steger, 1983). The purpose of the present study was to assess the effect of LH pulse amplitude on in-vitro oestradiol-17β secretion from ovaries of di-oestrous rats.

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Materials and Methods

Preparation of media. Bovine luteinizing hormone (NIAMDD-bLH-10) with a biopotency of 1·06 NIH-LH-S1 units/mg (one unit of activity = 1 mg NIH-LH-S1) and bovine follicle-stimulating hormone (NIAMDD-bFSH-1) with a biopotency of 0·49 NIH-FSH-S1 units/mg (one unit of activity = 1 mg NIH-FSH-S1) were used in this experiment. LH and FSH concentrations of the solutions were expressed in NIH-RP1 units. Gonadotrophins were added to Medium 199 (Gibco, Detroit, MI, U.S.A.) which were supplemented with 2·2 g NaHCO<sub>3</sub>/l, 2 g bovine serum albumin (Fraction V)/l, 50 mg streptomycin sulphate/l, 62-9 mg penicillin-G/l, and 2·38 g Hepes/l. All media were subsequently equilibrated with 5% CO<sub>2</sub> in oxygen and the pH adjusted to 7.4. The medium was then transferred to holding flasks or culture chambers and stored at 5°C, for up to 4 days. All bottles were warmed to 25°C, injected with 200 i.u. regular insulin/l (Lente) and equilibrated with 5% CO<sub>2</sub> in oxygen 20 min before use. During the experiment, media in the holding flasks were gassed for 1 min in every 10 min with 5% CO<sub>2</sub> in oxygen.

Experimental protocol. Female Wistar rats (5–6 months old) were housed under controlled conditions of humidity, temperature and photoperiod (12 h light/24 h). Oestrous cycles were monitored and only animals with at least 3 consecutive 4- or 5-day cycles were used. Between 10:00 and 10:15 h on the day of di-oestrous 1, rats were decapitated and the ovaries removed, placed in Medium 199 which was supplemented with 300 i.u. heparin sulphate/l and 200 i.u. insulin/l, trimmed of fat and incubated at room temperature for 2–4 min. At 10:30 h the ovaries were placed in a separate culture chamber to which 10 ml Medium 199 were added (Peluso et al., 1983). The ovaries were exposed to the following hormonal treatments: (1) no gonadotrophins; (2) tonic FSH (200 ng RP1/ml); (3) tonic LH (30 ng RP1/ml); (4) tonic FSH and tonic LH; (5) tonic FSH and pulsatile LH (amplitude = 50 ng RP1/ml). Regardless of the type of LH treatment, the total amount of LH to which each ovary was exposed was 3060 ng over the 3-h culture period (Text-fig. 1).

Text-fig. 1. Changes in LH concentrations within the culture chamber during the 3-h culture period. Ovaries were exposed to tonic LH or LH pulses of 40 or 50 ng/ml in amplitude.

The culture system and associated mathematical details have been published (Peluso & Gruenberg, 1983). LH pulses with an amplitude of 40 or 50 ng/ml were induced by connecting two peristaltic pumps to a culture chamber (a 20-ml syringe) which contained 10-ml medium in which the LH concentration was 20 or 10 ng RP1/ml. For the first 30 min of each hour a pump delivered medium which contained either 44-47 or 58-94 ng LH RP1/ml, thereby increasing the LH levels within the culture chamber to 40 or 50 ng RP1/ml, respectively. After 30 min the first pump was
turned off and the second pump switched on for the remaining 30 min of the hour. This second pump delivered medium that contained 15-53 or 1.06 ng LH RP1/ml, thus decreasing the LH concentration to 20 or 10 ng/ml, respectively. Both pumps delivered medium at a rate of 34 ml/h.

Perifusate was collected every 10 min and assayed for oestradiol-17β by radioimmunoassay. Oestradiol-17β levels were determined on 1 ml unextracted medium. The reagents for the assay were purchased from Wein Laboratories (Succasunna, NJ, U.S.A.). The antiserum to oestradiol-17β cross-reacted 100% with oestradiol-17β, <0.3% with progesterone, <0.3% with dehydroepiandrosterone, <0.3% with testosterone and <0.3% with several other steroids. The assay sensitivity was 20 pg/ml and the intra- and inter-assay coefficients of variation were 8.1% and 5.3% respectively. All samples were assayed in duplicate with all the samples collected from an individual ovary run in a single assay. Data were analysed by one- and two-way analysis of variance followed by the Newman–Keuls procedure. Differences between values were considered significant at *P* < 0.05.

**Results**

The total amount of oestradiol-17β secreted by ovaries, from di-oestrous rats, cultured without gonadotrophins averaged 80 ± 19 pg/mg 3 h⁻¹. Neither tonic levels of LH and/or FSH nor tonic FSH plus LH pulses of 40 ng/ml increased total oestradiol-17β secretion. Hourly pulses of 50 ng LH/ml in the presence of FSH increased total oestradiol-17β secretion by 300% (*P* < 0.05) (Text-fig. 2).

**Text-fig. 2.** The effects of various gonadotrophin treatments on total amounts of oestradiol-17β secreted by rat ovaries during the 3-h culture period. Values are means ± s.e.m. of 4–6 ovaries per group.

Without gonadotrophin stimulation, ovaries secreted oestradiol-17β at a rate of 4.48 ± 0.21 pg/mg 10 min⁻¹ throughout the culture period. Assuming that the secretory rates are distributed normally, a 95% confidence interval for basal rates can be set by calculating the mean ± two standard deviations (Brinkley, Wilfinger & Young, 1973). Thus, basal oestradiol-17β secretory rates ranged from 0.88 to 8.08 pg/mg 10 min⁻¹. Oestradiol-17β secretion rates remained within this basal range throughout the culture period when ovaries were exposed to tonic LH and/or FSH (Text-fig. 3). In 1 of 6 ovaries cultured with tonic FSH and LH pulses of 40 ng/ml, oestradiol-17β
**Text-fig. 3.** Oestradiol-17β secretory rates in response to various gonadotrophin treatments. Each point represents the mean of 4 replicates. The slope of each line is shown at the right of each panel. The slopes are not different from each other or from a slope of zero.

**Text-fig. 4.** Oestradiol-17β secretory rates of individual ovaries cultured with hourly pulses of 50 ng LH/ml in the presence of FSH. The range of basal oestradiol-17β secretory rates is shown by the shaded areas.

The secretory rate was elevated over basal rates once during each hour of culture, with this increased secretion corresponding temporally to the LH pulse (data not shown). Hourly LH pulses of 50 ng/ml in the presence of tonic FSH induced periodic increases in oestradiol-17β secretory rates in all 4 ovaries tested (Text-fig. 4).

**Discussion**

Of all the treatments tested in this study, only tonic FSH and hourly LH pulses of 50 ng/ml stimulated oestradiol-17β secretion. The mechanism(s) by which these pulses enhance oestradiol-17β secretion remains obscure. However, hourly LH pulses of 40 ng/ml in the presence of FSH were ineffective in promoting oestradiol-17β secretion. These results suggest that the responsiveness of
the ovary may be due to an amplitude-sensitive mechanism with the ovary being able to discern a difference in amplitude as small as 10 ng LH/ml. The ovary may also possess a rate-sensitive mechanism, with the rate at which the LH concentration changes being significant, because the non-stimulatory pulse of 40 ng LH/ml has both a lower amplitude and a slower rate of change than the stimulatory pulse of 50 ng/ml. In studying the potassium-induced secretion of MSH and endorphin from perfused neurointermediate lobes of the pituitary, Keith, Allen, Stack, Robertson & Kendall (1983) have shown that the rate at which the potassium concentration increases to a specific amplitude is critical in determining the amount of hormone secreted. Therefore, both amplitude and rate of change in the LH stimuli may be important factors in controlling ovarian secretory responses. This possibility is currently being investigated.

Hourly pulses of 50 ng LH/ml increased total oestradiol-17β secretion by intermittently increasing oestradiol-17β secretory rates over basal levels. LH initially stimulates ovarian oestradiol-17β secretion (Hillensjö, Ekholm & Hedin, 1983) but then decreases ovarian adenylate cyclase activity, cAMP production and the number of LH receptors within 30 min (Jaaskelainen, Hyvonen & Rajaniemi, 1980), thereby desensitizing the ovary to further LH stimulation. Further, exposure to smaller doses of LH has a priming effect on ovarian tissue which results in a potentiation of progesterone, 20α-dihydroprogesterone and cAMP secretion in response to a second LH stimulus (Hedin, Ekholm & Hillensjö, 1983). Similarly, a pulse of LH can increase rather than decrease the number of testicular LH receptors and subsequently increase the responsiveness of the testicular tissue to LH (Powell, Rajan, Cohn & Bhalla, 1981). A putative up-regulation of ovarian LH receptors could account for the ovary remaining responsive to the second and third pulses of 50 ng LH/ml. Since LH is secreted in discrete hourly pulses (Gallo, 1981), the ability of the ovary to remain responsive to repeated LH stimuli could provide a mechanism to account for the gradual increase in plasma oestradiol-17β levels that are observed throughout diestrus (Butcher et al., 1974).

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References


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