Gonadotrophin releasing hormone causes a biphasic secretion of luteinizing hormone and follicle stimulating hormone by cultures of rat anterior pituitary cells


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Abstract. Primary monolayer cultures of adult male rat anterior pituitary cells secreted both LH and FSH in a biphasic manner when incubated with gonadotrophin releasing hormone (GnRH). The peaks of secretion of LH and FSH were coincident; the first occurred between 15 and 30 min and the second between 1 and 3 h after the addition of GnRH. Approximately 20% of the total amount of gonadotrophins secreted in the 6 h treatment with GnRH was contained in the first peak. Inhibitors of the secretion of gonadotrophins affected LH and FSH secretion differently. Inhibin suppressed the secretion of FSH to a greater extent than that of LH, whereas testosterone and cycloheximide had a greater effect on LH. Neither phase of secretion of LH or FSH was reduced preferentially by inhibin or testosterone but the greater effect of cycloheximide was on the second phase of secretion.

The levels of luteinizing hormone (LH) in the sera of men, pubertal children, women in certain phases of the menstrual cycle and rams increase in a biphasic manner during constant iv infusions of gonadotrophin releasing hormone (GnRH; Bremner & Paulsen 1974, 1977; de Kretser et al. 1975, 1976, 1978; Happ et al. 1976; Koninckx et al. 1976; Wang et al. 1976). Generally levels of LH rise rapidly over the first 30–40 min of infusion and then either remain constant or fall slightly until a second rise commences after 90 min. In man the second rise continues until the 4th h of infusion and thereafter the levels remain constant, whereas in rams there is a decline in the levels of LH after 3 h despite continuation of the infusion (Bremner et al. 1976; Bremner & Paulsen 1977). The levels of follicle stimulating hormone (FSH) rise more gradually and distinct phases of secretion are not as obvious. In studies with rats separate phases in the secretion of LH during infusion of GnRH are less distinct. However, it appears that the increment in LH levels during the first 30 min of infusion is less than that seen between 60 and 90 min (Blake 1976; Fink et al. 1976). As yet, no clear explanation exists for the biphasic pattern of the secretion of LH and the discrepancy between the patterns of LH and FSH in vivo.

The pattern of secretion of gonadotrophins by pituitary cells in vitro is less clear since there are few detailed studies of the early effects of exposure to GnRH. By perfusing columns of rat pituitary cells attached to beads, Hopkins (1977) demonstrated biphasic secretion of LH with peaks at 2 and 90 min after the addition of GnRH to the perfusion medium. A similar pattern in the secretion of LH by monolayer cultures of porcine pituitary cells has also been reported with peaks at 2 and 10–20 min (Walker & Hopkins 1978). The present study was undertaken to determine if there is biphasic secretion of both LH and FSH by cultures of rat pituitary cells. Such cultures might provide a useful method to investigate mechanisms of biphasic secretion and the action of inhibitors of the secretion of gonadotrophins.
Primary monolayer cultures of anterior pituitary cells from adult male Sprague-Dawley rats were prepared by distributing 10^6 cells to each 10 mm × 35 mm plastic culture dish (Eddie et al. 1979). The cells were cultured in 2 ml Dulbecco modified Eagle’s medium containing 1% non-essential amino acids, 1% fresh glutamine, 100 U penicillin/ml (DMEM), supplemented with 5% horse serum and 2.5% foetal calf serum. Radioimmunoassays of rat FSH and LH in the culture media were performed using reagents donated by the NIAIDDD (Eddie et al. 1978). The standards were FSH-RP-1 and LH-RP-1. Testosterone was measured by radioimmunoassay (Baker et al. 1976) and protein by absorbance at 280 nm or the method of Lowry et al. (1951).

**Inhibin preparations**

Ovine inhibin was prepared from testicular lymph by fractionation with ammonium sulphate (25–55% saturation) and ethanol (50–80% saturation) as previously described (Eddie et al. 1979). Rat inhibin was prepared from the media of cultures of adult seminiferous tubules by gel filtration on Sephadex G-100. Material eluting in the 10 000–30 000 molecular weight zone was dialyzed and lyophilized (Eddie et al. 1978). The specific activities of ovine testicular lymph (OTL) protein and the rat inhibin, as determined by bioassay (Eddie et al. 1979) were 2.9 (2.6–3.3) and 38 (32–44) U/mg protein respectively (mean and 95% confidence limits).

**Culture procedure**

The pituitary cell cultures were incubated for 48 h to allow cells to attach to the dishes. Each test substance, diluted in Dulbecco phosphate buffered saline (DPBS; Dulbecco & Vogt 1954) was added to three or four dishes and culture continued for a further 3 days. Testosterone was dissolved in ethanol (1 mg/ml) diluted with DPBS and added to the media such that the final concentration of ethanol was < 0.01%. Control dishes received DPBS alone. On the fifth day the media were removed and the cells washed with DMEM. The cultures were then exposed to repeated changes of fresh media (1.2 ml) without GnRH for 1 h and then with maximally stimulating concentration of GnRH (10 nM).

Two experimental procedures were used. In the first experiment media were changed every 15 min before and for 75 min after the addition of GnRH, and then every 60 min. In the second experiment media were changed every 30 min before and for 60 min after the addition of GnRH, and then every 60 min. In both experiments control dishes previously exposed to DPBS alone were used to study basal and GnRH-stimulated secretion. With the first procedure, ovine inhibin (2 U/ml) or testosterone (25 nM) were included in the media for the three days of pre-incubation and throughout the frequent changes of media before and during treatment with GnRH. In the second, rat inhibin (0.5 U/ml) was used with the same procedure, but cycloheximide (7 μM) was added only 30 min before and during addition of GnRH to cultures that have been pre-incubated for 3 days with DPBS alone. In both experiments, the media were collected, diluted with bovine serum albumin (10 mg/ml) in 0.05 m phosphate buffered saline and stored frozen until assayed for LH and FSH. The rate of secretion of the gonadotrophins was calculated for each change of media and expressed as μg/h/culture dish.

Frequent changes of culture media did not appear to affect the secretory response to GnRH because when the amounts of LH and FSH in the media from the second experiment were compared with those of cultures prepared from the same cell suspension pre-incubated with DPBS for 3 days and exposed to 10 nM GnRH for various intervals up to 6 h without changes of media, there was no significant difference for either hormone.

**Results**

**Experiment 1**

**Basal secretion**

The rate of secretion of FSH and LH fell gradually during the first 75 min of incubation while media changes were made at intervals of 15 min (Fig. 1a). When the intervals were increased to 60 min there was a further reduction in the rate of secretion. Thereafter the rate remained constant.

**Response to GnRH**

When GnRH was added to the media two peaks of secretion of FSH occurred (Fig. 1b). The first reached a rate of secretion of 0.28 μg/h at 15 min and was followed by a second peak of between 0.22 and 0.27 μg/h at 1 to 2 h. Of the total FSH secreted during the 6 h exposure to GnRH 26.4% was in the first peak.

The GnRH-stimulated secretion of LH was also biphasic. The first peak reached 0.93 μg/h and a second peak of 0.82 to 0.92 μg/h was coincident with the peak of FSH secretion. The first peak of LH represented 19.7% of the total LH secreted during exposure to GnRH.

**Effect of ovine inhibin and testosterone**

Treatment of the pituitary cells for 3 days with ovine inhibin (2 U/ml) resulted in a marked suppression in the rate of GnRH-stimulated secretion of FSH (Fig. 1c). Levels of FSH in most samples of media were not detectable (< 6.4 ng/ml). The rate of secretion of LH was also reduced but to a lesser
Biphasic secretion of FSH and LH: effect of inhibin and testosterone. Pituitary cells were treated with (a) and (b) Dulbecco phosphate buffered saline, (c) ovine inhibin (2 U/ml) or (d) testosterone (25 nm) for 3 days, washed, then exposed to fresh test substance for 3 intervals of 15 min. At 0 h (b), (c) and (d) 10 nM GnRH (shaded bar) was added and was replaced at 15 min intervals to 75 min, then hourly to 6 h. The concentration of FSH and LH in the media was measured by radioimmunoassay. Values are means ± SEM of the rates of hormone secretion in four culture dishes per treatment.

Table 1.
Effect of inhibin, testosterone and cycloheximide on the two peaks of GnRH-stimulated secretion of gonadotrophins.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
<td>Peak 2</td>
</tr>
<tr>
<td>Rat inhibin (0.5 U/ml)</td>
<td>80.1</td>
<td>64.5</td>
</tr>
<tr>
<td>Ovine inhibin (2.0 U/ml)</td>
<td>&lt; 29.5</td>
<td>&lt; 11.2</td>
</tr>
<tr>
<td>Testosterone (25 nmol/l)</td>
<td>65.5</td>
<td>92.4</td>
</tr>
<tr>
<td>Cycloheximide (7 µmol/l)</td>
<td>91.1</td>
<td>45.3</td>
</tr>
</tbody>
</table>

Pituitary cells were exposed to repeated changes of media for 1 h without GnRH, then with 10 nM GnRH for 6 h. The cultures were pre-treated with inhibin or testosterone for 3 days, or with cycloheximide for 30 min before exposure to GnRH. Figures represent per cent of FSH and LH secreted by pre-treated cells compared to that secreted by cells exposed to GnRH alone. Peak 1 is from 0 to 45 or 60 min and Peak 2 is the remaining time up to 6 h exposure to GnRH.
Biphasic secretion of FSH and LH: effect of inhibin and cycloheximide. Pituitary cells were treated with (a) and (b) Dulbecco phosphate buffered saline, (c) rat inhibin (0.5 U/ml) for 3 days or (d) cycloheximide (7 µM) for 30 min, washed, then exposed to fresh test substance for 2 intervals of 30 min. At 0 h (b), (c) and (d) 10 nM GnRH (shaded bar) was added and was replaced at 30 min intervals to 1 h, then hourly to 6 h. The concentration of FSH and LH in the media was measured by radioimmunoassay. Values are means ± SEM of the rate of hormone secretion in three culture dishes per treatment.

Extent (Fig. 1c and Table 1). In contrast to the effect of bovine inhibin, treatment of the cells with 25 nm testosterone suppressed the GnRH-stimulated secretion of LH to a greater extent than that of FSH (Fig. 1d and Table 1). Neither inhibitor of the secretion of gonadotrophins appeared to affect one or other phase of secretion preferentially.

Experiment 2
Basal secretion and response to GnRH
As was seen in Experiment 1, basal secretion decreased to a constant rate after 2 h, and the secretion of FSH and LH in response to GnRH was biphasic (Figs. 2a and b). The peak secretion rate of FSH, 0.78 µg/h at 30 min, was followed by a second peak of 0.80 to 0.97 µg/h at between 2 to 3 h, with 20% of the total FSH secreted during exposure to GnRH in the first peak. For LH, the first peak of 1.14 µg/h was followed by a peak of 1.80 to 2.18 µg/h coincident with the peaks of FSH secretion (Fig. 2b). The first peak of LH secretion contained 12.6% of the total LH.

Effect of rat inhibin and cycloheximide
Pre-treatment of cells with rat inhibin (0.5 U/ml) caused equal suppression of the rate of GnRH-stimulated secretion of FSH and LH (Fig. 2c and Table 1). Cycloheximide (7 µM) also reduced the rate of secretion of gonadotrophins, with the greatest effect being on the secretion of LH (Fig. 2d and Table 1). The two peaks of GnRH-stimulated secretion were no longer apparent following cycloheximide treatment, the main inhibitory effect being on the second peak.
Discussion

These experiments show that GnRH causes a biphasic secretion of both gonadotrophins by rat pituitary cells in monolayer culture. Similar phases of secretion of LH have been observed in vivo during infusion of GnRH into sheep and man (Bremner et al. 1976; de Kretser et al. 1975, 1976, 1978; Wang et al. 1976). However, biphasic secretion of FSH is not obvious from the changes in the levels in serum. This difference in the patterns of gonadotrophin secretion was attributed to the longer half-life of FSH compared to that of LH (de Kretser et al. 1975; Konincx et al. 1976). In vitro, where the half-lives of the gonadotrophins do not apply, the pattern of secretion of FSH is also biphasic.

The pattern of LH release seen during infusion of GnRH into rats differs from that seen in sheep and man; a rapid initial increase in LH levels does not occur. Instead, LH levels rise slowly early in the period of infusion, then increase at a more rapid rate (Blake 1976; Fink et al. 1976). This is in contrast to the pattern of LH secretion by rat pituitary tissue in vitro where a biphasic pattern commences with a rapid initial peak in secretion (Kercret & Duval 1978). This discrepancy between patterns seen in vivo and in vitro may be related to dissimilar steroid levels in different systems. Sensitivity of the pituitary to stimulation by GnRH changes during the oestrous cycle and following treatment of male rats with exogenous steroids (Blake 1976; Fink et al. 1976). Similarly, pituitary sensitivity to GnRH alters during the menstrual cycle and may explain why biphasic secretion is not apparent in all stages of the cycle (Wang et al. 1976; Hoff et al. 1977; de Kretser et al. 1978). The secretion of LH is also biphasic during stimulation of pituitary cells in culture but no descriptions have been made of the secretion of FSH (Hopkins 1977; Hopkins & Walker 1978; Walker & Hopkins 1978).

The secretion of FSH was reduced by exposure of the cells to preparations of ovine and rat inhibin. The secretion of LH was also reduced but to a lesser extent. In contrast, treatment of the cells with testosterone at a physiological concentration suppressed the secretion of LH to a greater extent than that of FSH. These results agree with reports that the predominant effect of inhibin is on the secretion of FSH (Baker et al. 1976; Steinberger & Steinberger 1976; Eddie et al. 1978; Franchimont et al. 1978; de Jong et al. 1978). Also, the greatest effect of testosterone on pituitary cells in culture is to suppress the GnRH stimulated secretion of LH, while the secretion of FSH is either slightly lowered or augmented (Drouin & Labrie 1976; Labrie et al. 1978; Eddie et al. 1979).

Neither inhibin nor testosterone appear to reduce either phase of the secretion of LH or FSH preferentially. In contrast, the greatest effect of cycloheximide is on the second phase of gonadotrophin secretion, suggesting that synthesis of protein is required for the second phase of gonadotrophin secretion in response to stimulation by GnRH.

Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia and by grants from the World Health Organization and The Ford Foundation.

References


Received on June 9th, 1981.