Gonadotropin inhibitory hormone (GnIH) prevents the ‘priming’ effect of estradiol-17β

Gonadotropin inhibitory hormone (GnIH) mempengaruhi efek puncak estradiol-17β

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Abstract

GnIH inhibits GnRH-stimulated gonadotropin secretion in the sheep by action on the pituitary gonadotropes. Estradiol-17β (E₂) also acts at the level of the pituitary gonadotrope to exert negative and positive feedback effects on gonadotropin secretion. The positive effect facilitates the estrogen-induced surge in gonadotropin releasing hormone (GnRH) and LH. This study was undertaken to determine whether this ‘priming’ effect of E₂ is prevented by GnIH. Cultured pituitary cells were treated with GnRH or vehicle every 4 h for 24 h with and without GnIH and E₂ treatment. GnRH stimulated (LH) and follicle stimulating hormone (FSH) secretion was increased by E₂ treatment. The effect of E₂ was overcome by the inhibitory effect of GnIH. We conclude that GnIH may counteract the priming effect of E₂ on pituitary gonadotropes.

Key words: Gonadotropin, gonadotrope, gonadotropin inhibitory hormone, estradiol

Abstrak


Kata kunci: gonadotropin, pituitari, GnIH, estradiol

Introduction

Gonadotropin releasing hormone (GnRH) provides the primary stimulus for the reproductive axis, through action on the pituitary gonadotropes and gonadotropin inhibitory hormone (GnIH) counteracts this effect (Clarke, et al., 2008; Smith and Clarke, 2010). Our studies in sheep have presented convincing evidence that GnIH is an hypophysiotropic factor in this species. GnIH-expressing cells are located in the periventricular zone of the ventral paraventricular nucleus and the dorsomedial nucleus and these cells project to the external zone of the median eminence (Clarke, et al., 2008). Although there is no evidence to date that GnIH is secreted into hypophysial portal blood, the peptide has a potent effect on the gonadotropes through inhibition of the release of intracellular calcium caused by GnRH and it
also reduces amplitude of pulsatile LH secretion when administered intravenously to OVX ewes (Clarke, et al., 2008).

Prior to the preovulatory surge in LH the response of the gonadotropes to GnRH is increased. This is due to a ‘priming’ effect of GnRH as well as E2. GnRH self-priming was shown in a series of studies (de Koning, et al., 2001; Evans, et al., 1984; Waring and Turgeon, 1980; Veldhuis, et al., 1986). Increased GnRH pulse frequency during the follicular phase of the estrous cycle increases the number of gonadotropes expressing estrogen receptor α (ERα) (Clarke, 1997). The rapid non genomic effect of estrogen has been demonstrated in ovine gonadotropes (Cowley, et al., 1991; Clarke and Cummins, 1984; Yen et al., 1973). This sensitisation is seen in cultures of in sheep, bovine and rat pituitary cells (Drouin et al., 1976; Huang and Miller, 1980), at least part of which is due to upregulation of the GnRH receptor (GnRH-R) (Adams and Spies, 1981; Marian, et al., 1981; Nett, et al., 1984). The effect of E2 on GnRH-R expression and binding is seen in female gonadotropes in the follicular phase of the estrous cycle (Cowley, et al., 1998) and is also shown in models where E2 acts directly on the pituitary gland in vivo (Clarke, et al., 1988). E2 also acts on the gonadotropes to alter post-receptor GnRH signalling pathways, such as calcium flux (Heyward, et al., 1995), levels of diacylglycerol (DIG) (Chang, et al., 1988), protein kinase C (PKC) (Drouva, et al., 1990; Stojilkovic, et al., 1988) and cyclic adenosine monophosphate (cAMP) (Kamel and Krey, 1983; Tang, et al., 1982). E2 also increases the function of the inwardly rectifying potassium current in gonadotropes (Cowley, et al., 1999). E2 also causes migration of the LH-containing granules to the plasma membrane of the gonadotropes in preparation for the massive secretory event that occurs during the surge (Thomas and Clarke, 1997). The rapid non genomic effect of estrogen has been demonstrated in ovine pituitary gonadotropes. Both in vivo and in vitro effect of E2 are exhibited by phosphorylation of ERK-1/2 and cAMP-responsive element-binding protein (CREB) (Iqbal, et al., 2009).

The question arises as to whether the negative effect of GnIH may counter the priming effects of E2. In order to address this, we have developed an in vitro model that allows study of the response to GnRH over 16h, which is the time-frame over which E2 acts to cause a positive feedback effect (Sari, et al., 2009). Herein, we describe studies to dissect the effects of GnRH in combination with E2 and/or GnIH and we show that GnIH may counteract the priming effect of E2.

Methodology

Animals

Adult ovariectomised (OVX) ewes of the Corriedale breed were of similar age (5 years) and body weight and were sourced from the Monash University Sheep Facility, Werribee, Victoria. All experiments were conducted according to the guidelines established by the Australian Prevention of Cruelty to Animals Act 1986 and the study was approved in advance by the Monash University Animal Ethics Committee.

Pituitary collection and preparation

Animals were euthanized by an overdose of sodium pentobarbital (Lethabarb; Virbac, Peakhurst, NSW, Australia) and pituitaries were collected into Dulbecco’s modified Eagle’s medium (DMEM) (Thermo Electron Corp., Melbourne, Australia) containing 0.1% bovine serum albumin (BSA) (Sigma-Aldrich Inc, St. Louis, USA). The cells were passed through fine mesh and centrifuged, then resuspended in 0.1% BSA/PBS. Yield was approximately 80-90 x 10^6 cells/well. Animals were mixed. Cultures of 1x10^6 cells/pituitary with >95% viability (trypan blue exclusion test). For culture, the pelleted cells were resuspended in DMEM containing 10% fetal calf serum, streptomycin and antimycotic (all from Gibco Invitrogen Corp., Auckland, NZ).

Effect of GnIH and E2 on gonadotropin secretion

For each experiment, pituitaries from 2 OVX ewes were collected and the cells from both animals were mixed. Cultures of 1x10^6 cells/well were mixed and

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The samples were stored at -20°C until assayed for FSH assays was 0.1 ng/mL and the intra-assay coefficient of variation (CV) was less than 10% over the range of 0.2-15.2 ng/mL. Inter-assay CV was 5.4%.

On day 3 the following treatments were applied:-
- GnRH (Auspep, Parkville, Vic., Australia) at doses of 10⁻⁸, 10⁻⁹ and 10⁻¹² M or vehicle;
- E₂ (Intervet, Artarmon, NSW, Australia) at doses of 10⁻⁸, 10⁻¹² M or vehicle;
- GnRH (10⁻¹² or 10⁻¹⁰ M) plus E₂ (10⁻¹⁰ or 10⁻⁸ M) or vehicle;
- Treatments that were the same as above with and without treatment GnIH (Auspep, Parkville, Vic., Australia) at a dose of 10⁻⁹ M.

At each timepoint (0, 4, 8, 12 and 16h) after commencement of GnRH/ E₂/GnIH/vehicle treatment, medium was removed and replaced with fresh medium containing the relevant treatment. The samples were stored at -20°C until assayed for LH and FSH by radioimmunoassay. The experiments were replicated in 3 separate cultures.

Radioimmunoassays
Samples were diluted 1:20 for assay. LH concentrations were measured by radioimmunoassay as described by Lee, et al., (1976) using ovine NIH-OH-S18 as the standard. The average sensitivity of the LH assays was 0.1 ng/mL and the intra-assay coefficient of variation (CV) was less than 10% over the range of 0.5-9.4 ng/mL. Inter-assay CV was 10.4%. The method of Bremner, et al., (1980) was used for FSH measurement using ovine NIH-OVAMDD-RP-1 as the standard. The average sensitivity of the FSH assays was 0.1 ng/mL and the intra-assay coefficient of variation (CV) was less than 10% over the range of 0.2-15.2 ng/mL. Inter-assay CV was 5.4%.

Statistical Analysis
Data are presented as mean ± SEM. Statistical analysis was performed using SPSS (version 15.0, SPSS Inc, Chicago, IL, USA) using repeated measures ANOVA and Scheffe’s method as a post-hoc test.

Results and Discussion
Repetitive treatment with GnRH at 4h intervals at the doses 10⁻⁸ M and 10⁻¹⁰ M (but not 10⁻¹² M) stimulated LH and FSH secretion (Fig 1, panels A and B). Stimulation of LH (Fig 1, panel A) was more marked than that of FSH (Fig 1, panel B). At doses 10⁻⁹ and 10⁻⁸ M (but not at lower doses), treatment with E₂ alone increased LH secretion (Fig 1, panel C, P<0.05; P<0.01).

At these doses, E₂ reduced FSH secretion (Fig 1, panel D, P<0.05). Based on the results of the above treatments, doses of GnRH and E₂ were chosen that might reveal the priming effect of E₂ on GnRH action. We used 10⁻¹⁰ and 10⁻⁹ M doses of E₂ with 10⁻¹⁰ M and 10⁻¹² doses of GnRH alone or in combination. At a dose of 10⁻¹⁰ M GnRH, the priming effect of E₂ on LH response was seen at the 8h time point only and only with 10⁻⁸ M E₂ (Fig 2A; P<0.01). Interestingly, a priming effect on FSH secretion was seen at both doses of E₂ and at all time points (Fig 2B; P<0.05). At a dose of 10⁻¹² M GnRH, there was no overt priming effect of E₂, since the combined effect of GnRH plus E₂ was not greater than that of E₂ alone (Fig 3, panels A and B). With a dose of 10⁻¹⁰ M GnRH, GnIH (10⁻⁸ M) reduced the secretion of LH and FSH (Fig 2, panels A and B). With combined GnRH and E₂ (10⁻⁸ M), GnIH prevented the priming effect of E₂ on the secretion of both LH and FSH. This effect was seen at all time points.

Using an in vitro model, we have shown E₂ priming of the response of pituitary gonadotropes to GnRH and this is apparent over the time course that is relevant to the LH-surge generation obtained by E₂ treatment of OVX ewes (Clarke, et al., 2005). This provides a means by which we can examine factors that might influence this priming effect and will also permit future studies on the sub-cellular signalling events underlying this phenomenon.

GnRH at the doses 10⁻⁷ M and 10⁻¹⁰ M (but not lower doses) stimulated LH and FSH secretion. As shown in other culture systems (Huang and Miller, 1980) and in vivo (Clarke, et al., 1984), GnRH elicits a greater incremental rise in secretion of LH than FSH, but the effect on both gonadotropins is sustained throughout 16h, with 4 hourly replacement of the medium with GnRH. Interestingly, a dose-response effect on LH secretion is seen at 8h, but this is lost at later time points, where effects of 10⁻¹⁰ M GnRH are as effective as 10⁻⁸ M GnRH. The priming effect of E₂ on GnRH stimulated LH and FSH secretion is apparent only at a sub-maximal (10⁻⁸ M) dose of GnRH and only with relatively high E₂. Further studies may allow us to determine the relative effects of GnRH and E₂ on sub-cellular signalling in this system.
using primary pituitary cells. The priming effect of E\textsubscript{2} is most readily apparent at the 8h timepoint in relation to LH secretion, but is seen at all time points for FSH secretion.

In a physiological setting, the gonadotropes would not be exposed to E\textsubscript{2} alone, but a response to the steroid hormone will be useful for the study of signalling, especially rapid E\textsubscript{2} effects. Earlier studies have shown that chronic treatment bovine and rat pituitary cell culture with E\textsubscript{2} stimulates basal LH release (Kamel and Krey, 1983). In the present study, we found that doses of 10\textsuperscript{-8}M or 10\textsuperscript{-9}M increases basal LH secretion from ovine gonadotropes. On the other hand, treatment with E\textsubscript{2} alone reduces FSH secretion, which is consistent with other studies (Huang and Miller, 1980; Miller and Huang, 1985). Whereas the effect of E\textsubscript{2} in the follicular phase of the estrous cycle is to stimulate LH secretion, FSH levels fall (Goodman, et al., 1982), but this could be due to the effect of inhibin (Clarke, et al., 1986). Our culture system will allow us to determine signalling effects of GnRH and E\textsubscript{2} but it will be difficult to discriminate between those relevant to LH secretion vs FSH secretion. Treatment of ovine pituitary cell culture with an antibody to activin B reduced FSH secretion without affecting LH secretion. This effect was accompanied by the reduction in activin β\textsubscript{B} mRNA expression, suggesting that the inhibitory effect of E\textsubscript{2} on FSH secretion from the pituitary gonadotropes may be mediated by activin (Baratta, et al., 2001).

Figure 1. Effects of either GnRH or E2 across 4-16h on LH and FSH secretion from OVX ewe pituitary cells in primary culture. Medium was removed every 4h and replaced with medium containing the appropriate dose of GnRH or E2. Effects of dose of GnRH are shown in panels A and B. For panels A and B, a vs. b, P<0.05; b vs. c, P<0.05; a vs. c, P<0.01. Panels C and D show the effect of treatment with E2 alone on LH and FSH secretion. The data are means (±SEM) of 3 cultures. For Panels C and D, a vs. b, P<0.05; b vs. c, P<0.05; a vs. c, P<0.01.
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The model we used indicates a time when positive feedback mechanism is operative, showing the effect of E2 to sensitize cells to respond to GnRH (Clarke and Cummins, 1984). This model allows examination of the effect of GnIH in the positive feedback effect of E2. As mentioned in the introduction, E2 affects a number of signalling pathways, in gonadotropes. One or more of these may relate to priming by E2 and this is unmasked with a submaximal dose of GnRH (10^{-10}M) and a dose of 10^{-8}M E2. At these doses, an effect is seen on LH secretion and the effect of E2 on FSH secretion is the opposite of that seen with E2 alone. In this setting, GnIH reduces the stimulatory effect of GnRH, consistent with our earlier studies (Clarke, et al., 2008) and also prevents the priming effect of E2. In ovine brain, the expression of GnIH mRNA is also reduced in the late-follicular phase of the ewe estrous cycle. GnIH treatment also eliminates pulsatile LH secretion in the mid-follicular phase and blocks LH surge (Sari, et al., 2009). These results indicate that the reduction of GnIH expression in the follicular phase of the ewe is permissive for LH surge.

Figure 2. Inhibitory effects of GnIH (10-9M) on either GnRH (10-10M) alone or in combination with E2 (10-8 and 10-10M) across 4-16h on LH and FSH secretion (panel A and B, respectively) from OVX ewe pituitary cells in primary culture. Medium was removed every 4h and replaced with medium containing the appropriate dose of GnRH or E2. For panels A and B, a vs. b, P<0.05; c vs. d, P<0.01. The data are means (±SEM) of 3 cultures.

Treatment effects of E2 alone are the same as those shown in Fig 1 and are presented here for comparison.
The combination of $E_2$ and GnRH at the time of positive feedback involves a range of priming events at the sub-cellular level, as indicated in the Introduction. As to which of these events is compromised by the inhibitory effect of GnIH remains to be determined and is the focus of ongoing studies.

**Conclusion**

GnIH is an hypophysiotropic hormone which is able to prevent the priming effect of $E_2$ on gonadotropes in the period leading up to a surge in LH secretion.

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