Regulation of 11β-hydroxyysteroid dehydrogenase type 1 gene expression by LH and interleukin-1β in cultured rat granulosa cells

M Tetsuka, L C Haines, M Milne, G E Simpson and S G Hillier

Reproductive Medicine Laboratory, Department of Reproductive and Developmental Sciences, University of Edinburgh Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK

(M Tetsuka is now at Laboratory of Animal Genetics and Reproduction, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan 080)

(Requests for offprints should be addressed to S G Hillier)

Abstract

Granulosa cells from preovulatory follicles show increased expression of 11β-hydroxyysteroid dehydrogenase type 1 (11βHSD1) at the time of ovulation. As ovulation may be an inflammatory process, this may be a mechanism of local enhancement of the activity of anti-inflammatory glucocorticoids. In this study, we examined direct effects of LH, the proinflammatory cytokine, interleukin-1β (IL-1β), and pharmacological activators of protein kinase A (PKA) (forskolin and dibutyryl (db) cAMP) and PKC (LH-releasing hormone and phorbol 12-myristate 13-acetate (PMA)) signalling on the expression of 11βHSD1 mRNA in vitro. Granulosa cells from immature female rat ovaries were cultured (pretreatment) in serum-free medium 199 containing recombinant human (rh) FSH (1 ng/ml) for 48 h to induce responsiveness to LH. Cell monolayers were then washed and cultured (test treatment) for a further 12 h in the presence of rhLH (0–100 ng/ml), IL-1β (0–50 ng/ml), or both. Total RNA was extracted from granulosa cell monolayers and taken for quantitative ribonuclease protection analysis of 11βHSD1 mRNA. The low level of 11βHSD1 mRNA detectable in unstimulated (control) cultures was increased approximately twofold by the 48-h pretreatment with rhFSH. Subsequent exposure to rhLH (1–100 ng/ml) for a further 12 h dose-dependently increased 11βHSD1 mRNA expression by an additional two- to threefold. Forskolin (10 µM), db-cAMP (2 mM), LH-releasing hormone (LHRH; 1 µM) and PMA (200 nM) were also stimulatory. IL-1β (0·05–50 ng/ml) stimulated 11βHSD1 mRNA expression in a dose-related manner, both in the absence and in the presence of rhLH (3 ng/ml). The interaction between IL-1β (5 ng/ml) and rhLH (3 ng/ml) was additive. Co-treatment with a 50-fold excess of IL-1 receptor antagonist fully reversed the action of IL-1β. We conclude that 11βHSD1 mRNA expression in functionally mature granulosa cells is directly stimulated by gonadotrophins and IL-1β in vitro, potentially involving post-receptor signalling via PKA- and PKC-mediated pathways. Thus both LH and IL-1β may serve physiological roles in the upregulation of 11βHSD1 gene expression by granulosa cells in ovulatory follicles.


Introduction

Ovulation induced by luteinising hormone (LH) or human chorionic gonadotrophin (hCG) is the consequence of a biochemical cascade involving locally produced proinflammatory cytokines such as interleukin-1β (IL-1β) (Espey 1980, 1994, Adashi 1998). At the time of ovulation, granulosa cells are highly active sites of glucocorticoid metabolism via 11β-hydroxyysteroid dehydrogenase (11βHSD) enzyme activity (Michael et al. 1997). 11βHSD enzymes are expressed in at least two isoforms that reversibly interconvert glucocorticoids (cortisol and corticosterone) and ‘inactive’ 11-oxosteroid precursors (cortisone and 11-dehydrocortisone) (Michael & Cooke 1994). 11βHSD type 1 (11βHSD1) is principally an 11-oxoreductase (converts cortisone to cortisol, and 11-dehydrocorticosterone to corticosterone), whereas 11βHSD type 2 (11βHSD2) is a strong 11-dehydrogenase (back-converts cortisol to cortisone, and corticosterone to 11-dehydrocorticosterone). We have previously shown that a switch occurs in 11βHSD gene expression during granulosa cell luteinisation, the pattern of which suggests an intraovarian role for anti-inflammatory glucocorticoids in limiting the inflammatory component of ovulation (Tetsuka et al. 1997, 1999, Thomas et al. 1998). Granulosa cells in developing follicles express mainly 11βHSD2 mRNA, but little or no 11βHSD1 mRNA. After exposure of preovulatory follicles to an ovulation-inducing
dose of LH or hCG, 11βHSD2 mRNA expression is suppressed, whereas 11βHSD1 mRNA is enhanced. This shift in potential for glucocorticoid inactivation (oxidation by 11βHSD2) to activation (reduction by 11βHSD1) is reflected in the predominantly reductive (cortisone to cortisol) mode of glucocorticoid metabolism undertaken by luteinising granulosa cells in vitro (Hillier & Tetsuka 1998, Thomas et al. 1998). Differential regulation of 11βHSD gene expression before follicular rupture may therefore be the means by which the intrafollicular concentration of cortisol is increased before ovulation (Harlow et al. 1997). To test the ability of gonadotrophins to act directly on granulosa cells to modulate gene 11βHSD expression, we have optimised a rat granulosa cell culture model in which follicle-stimulating hormone (FSH) treatment is first given to induce functional maturation (i.e. LH responsiveness), followed by LH to induce luteinisation. Here, we demonstrate that LH directly upregulates granulosa cell 11βHSD1 mRNA expression in vitro. We also show that this newly discovered action of LH is mimicked by IL-1β and various agonists of protein kinase A (PKA) and PKC signalling.

Materials and Methods

Hormones and reagents

Recombinant human (rh) FSH (3860 IU/mg) and rhLH (6350 IU/mg) were generously donated by Dr C Howles (Serono Laboratories UK Ltd, Welwyn Garden City, Hertfordshire, UK). rhIL-1β and rhIL-1 receptor antagonist (rhIL-1ra) were obtained from R&D System Hertfordshire, UK). rhIL-1 (Serono Laboratories UK Ltd, Welwyn Garden City, Hertfordshire, UK) were precoated with DCS and washed twice with DPBS before inoculation with 0.5 ml culture medium containing approximately 2 x 10^5 viable cells (Hillier & de Zwart 1982) All incubations were at 37 °C in a humidified incubator gassed with 5% CO2 in air. To induce granulosa cell maturation and responsiveness to LH, the cells were pretreated for 48 h in the presence of rhFSH (1 ng/ml) and testosterone (1 μM). Cell monolayers were then washed once with DPBS before incubation for 12 h in fresh medium containing the test substance(s) under investigation, this being the approximate time required for hCG to induce ovulation in vivo (Tetsuka et al. 1999).

RNA preparation

Granulosa cells were lysed in ice-cold 4 M guanidinium thiocyanate solution containing 25 mM sodium citrate, 0.5% (wt/vol) sarcosyl, and 0.1 M β-mercaptoethanol (all from Sigma). Total RNA was extracted with phenol–chloroform (Chomczynski & Sacchi 1987).

32P-Labelled probes

The 11βHSD1 RNA probe was synthesised from cDNA generated by reverse-transcription (RT)–PCR. Oligonucleotide primer pairs were obtained from Cruachem Ltd (Glasgow, UK). The length of resultant DNA was 620 bp (nucleotides (nt) 109–728 (Agarwal et al. 1989), GenBank accession No. J05107).

Single-strand cDNA was reverse-transcribed from total RNA (1 μg) obtained from immature rat liver (11βHSD1) using Moloney’s murine lymphoma virus reverse transcriptase (Stratagene Cloning Systems, La Jolla, CA, USA) at 37 °C for 60 min. The resultant cDNA was used for PCR amplification (30 cycles) using Pyrococcus furiosis U DNA polymerase (Stratagene Cloning Systems). Each PCR cycle consisted of: 45 s denaturing at 94 °C, 45 s annealing at 60 °C and 2 min extension at 72 °C, with the final extension for 10 min. The resultant PCR product was cloned using pCR–Script Amp SK(+) Cloning Kit (Stratagene Cloning Systems) and sequenced to verify the authenticity of the products. The 18S ribosomal RNA (18S rRNA) was synthesised from cDNA containing DNA encoding a highly conserved region of human 18S rRNA (pt7 RNA 18S; Ambion, Austin, TX, USA). RT–PCR generated 11βHSD1 cDNA was linearised with EcoR V. The RNA probes were labelled with [32P]UTP via free access

(800 Ci/mmol; Amersham International, Aylesbury, Buckinghamshire, UK) using reagents supplied by Promega (Madison, WI, USA). The resultant RNA probes protect 257 (corresponding to nt 472–728 (Agarwal et al. 1989)) and 80 nt RNAs for 11βHSD1 and 18S rRNA, respectively. The specific activities of these probes were 1·3 × 10⁸ c.p.m./µg for 11βHSD1 and 8·8 × 10⁵ c.p.m./µg for 18S rRNA.

Ribonuclease protection assay
Total RNA (3–5 µg) was hybridised with approximately 2 × 10⁴ c.p.m. each of 11βHSD1 and 18S rRNA probes for 16 h at 42 °C in 20 µl hybridisation buffer containing 80% deionised formamide, 40 mM PIPES (pH 6·7), 0·4 M NaCl and 1 mM EDTA (Tetsuka & Hillier 1996). The resultant protected RNA fragments were processed as described previously and size-fractionated by electrophoresis on 5% acrylamide gels containing 8 M urea. Total RNA from liver and rRNA were used as positive and negative controls, respectively.

Data analysis
The radioactive signals given by protected RNAs were quantified by electronic autoradiography (Instant Imager, Packard, Downers Grove, IL, USA). The background value (result for rRNA) was subtracted and mRNA abundance was normalised to the 18S rRNA value. Results were expressed as percentages of the control value. Quantitative experiments were carried out at least three times. Data from replicate experiments were pooled for analysis of variance with Student’s t-test for group comparisons. P values <0·05 were considered statistically significant.

Results

LH-responsive 11βHSD1 mRNA expression
Pretreatment of immature granulosa cells with rhFSH and testosterone to induce preovulatory maturation increased the expression of 11βHSD1 mRNA approximately two-fold (data not shown). To examine subsequent responsiveness to rhLH, mature granulosa cells were cultured for a further 12 h with increasing concentrations (0–100 ng/ml) of rhLH. In three experiments, there was a dose-related response (mean ± s.e.m.) to rhLH, producing 170·8 ± 15·8% (non-significant) stimulation by 1 ng/ml rhLH, increasing to 214·0 ± 14·7% (P<0·05) at 3 ng/ml. Higher doses of rhLH (10–100 ng/ml) showed no further significant effect (Fig. 1). Time-course studies confirmed the responses to rhLH 3 and 10 ng/ml to be maximal at 12 h (data not shown).

Post-receptor signalling
To determine which post-receptor pathway(s) mediate upregulation of 11βHSD1 mRNA, granulosa cells previously incubated with rhFSH and testosterone were incubated for a further 12 h in the presence of a stimulatory dose of rhLH (3 ng/ml), a ‘high’ dose of rhFSH (100 ng/ml) or a pharmacological activator of PKA or PKC signalling: forskolin (10 µM), db-cAMP (2 mM), LHRH (1 µM) or PMA (200 nM) (Fig. 2). Treatment with 3 ng/ml rhLH significantly increased the expression of 11βHSD1 mRNA (P<0·01). rhFSH and forskolin caused the greatest, but most variable, levels of stimulation (P<0·05). All other pharmacological activators of PKA and PKC signalling also tested positively (P<0·01).
To determine if IL-1α affects the expression of 11βHSD1 mRNA, rhFSH-pretreated granulosa cells were treated for a further 12 h with increasing concentrations (0·05–50 ng/ml) of the inflammatory mediator. IL-1α in concentrations ≥5 ng/ml significantly increased the expression of 11βHSD1 mRNA both in the absence and in the presence of 3 ng/ml rhLH (Fig. 3). Analysis of the interaction between IL-1α (5 ng/ml) and rhLH (3 ng/ml) in five separate experiments revealed that the combined response was additive (Fig. 4).

To assess the involvement of IL-1 receptors in mediating the action of IL-1α, rhFSH-pretreated granulosa cells were treated for 12 h with IL-1ra (5 ng/ml) in the presence and absence of IL-1ra (250 ng/ml). IL-1ra completely blocked the stimulatory effect of IL-1α in all three of three experiments (Fig. 5).

Discussion

These findings pinpoint a cellular level at which gonadotrophins and cytokines act to increase follicular expression...
of 11βHSD1 expression. Previously, we reported that, in human beings (Tetsuka et al. 1997) and rats (Tetsuka et al. 1999), administration of hCG (surrogate LH) to induce ovulation induces upregulation of 11βHSD1 mRNA expression in granulosa cells of ovulatory follicles. Other studies confirmed the presence of a high level of 11βHSD1 mRNA in human granulosa-lutein cells (Michael et al. 1997, Smith et al. 1997). However, it had not been determined if the action of hCG was direct on granulosa cells or mediated by paracrine interactions with thecal cells. Here, we have proved that LH is able to stimulate granulosa cell 11βHSD1 mRNA expression directly in the absence of any other LH-responsive cell type. This leaves open the possibility of other cell types contributing to the overall response to LH/hCG in vivo.

Upregulation of granulosa cell 11βHSD1 by LH/hCG is accompanied by downregulation of 11βHSD2 in vivo (Tetsuka et al. 1997, 1999). As, in contrast to 11βHSD1, 11βHSD2 is principally oxidative (Stewart & Mason 1995, Edwards et al. 1996, Seckl & Chapman 1997, White et al. 1997), its negative regulation would be expected to promote glucocorticoid activation, thereby supplementing the reductase function of 11βHSD1. During the present study, we found that the relatively high level of 11βHSD2 mRNA initially present in immature granulosa cells rapidly declined in vitro, regardless of hormone treatment (data not shown). This is in accord with previous evidence that the two 11βHSD isoforms are differentially regulated.
during follicular maturation in vivo. However, the regulatory mechanism giving rise to downregulation of 11βHSD2 in the face of upregulation of 11βHSD1 remains to be determined.

The ability of IL-1β to mimic LH-induced upregulation of 11βHSD1 mRNA expression by granulosa cells is consistent with other evidence for the involvement of proinflammatory factors in ovulation (Espey 1980, Terranova & Rice 1997, Adashi 1998). Work on the control of 11βHSD1 expression in glomerular mesangial cells has shown that exposure to proinflammatory cytokine increases the expression and reductase activity of 11βHSD1, serving to augment the anti-inflammatory effect of glucocorticoids (Escher et al. 1997). Increased production of tumour necrosis factor α and IL-1β (Terranova & Rice 1997, Adashi 1998) with upregulation of phospholipase A2 (Ben-Shlomo et al. 1997), cyclooxygenase 2 (Morris & Richards 1995, Hellberg et al. 1996, Ando et al. 1998) and 11βHSD1 (Tetsuka et al. 1997) are common features of ovulation. Glucocorticoid receptor mRNA expression in rat granulosa cells is not measurably influenced by LH/hCG treatment in vivo (Tetsuka et al. 1999). Thus local activation of glucocorticoids by 11βHSD could be a major determinant of glucocorticoid action in the ovulatory follicle.

The finding that IL-1β directly stimulates granulosa cell 11βHSD1 mRNA in vitro does not take account of any cell–cell interactions that might influence cytokine responsiveness in vivo. Others have shown that association with thecal-interstitial cells strongly influences IL-1β-responsive prostaglandin synthesis by granulosa cells in vitro (Kokia et al. 1992). It will be of interest to determine if a similar synergy occurs between these cell types with respect to IL-1β-responsive 11βHSD1 mRNA expression.

Whereas LH and IL-1β can each stimulate granulosa 11βHSD1 expression directly, the principal post-receptor signalling pathways involved are likely to differ. Actions of LH/hCG on granulosa cells classically involve PKA-mediated signalling leading to increased expression of cAMP-responsive genes (Morris & Richards 1995), including IL-1β (Adashi 1998). However, granulosa cell luteinisation also involves PKC- and tyrosine kinase(s)-mediated signalling pathways (Morris & Richards 1995), which are more likely to be activated by IL-1β. As shown here, pharmacological agonists of both the PKA (forskolin, db-cAMP) and PKC (PMA, LHRH) signalling pathways upregulate 11βHSD1 expression in vitro. These pharmacological data complement the finding that combined treatment with LH and IL-1β causes additive stimulation of 11βHSD1 mRNA expression in vivo (Figs 3 and 4), suggesting that more than one post-receptor signalling mechanism is likely to regulate granulosa cell 11βHSD1 expression in vivo.

Regardless of the signalling pathways involved, it is of interest that an LH-inducible proinflammatory component (IL-1β) in the periovulatory cascade has the capacity to set in train a means by which inflammation might be quelled — that is, through 11βHSD1-mediated formation of anti-inflammatory glucocorticoid. Further work is required to determine if the spatio-temporal expression of genes encoding inflammatory mediators in the wall of the periovulatory follicle is consistent with this postulated sequence of events in vivo.

In conclusion, our results suggest a mechanism by which the potentially deleterious inflammatory component of follicular rupture is ultimately self-limiting. LH can act directly to upregulate 11βHSD1 gene expression in granulosa cells in vitro and its action is mimicked by the proinflammatory mediator, IL-1β. This supports a working hypothesis that upregulation of 11βHSD1 expression during granulosa cell luteinisation (with reciprocal downregulation of 11βHSD2 gene expression) is a mechanism to augment local production of anti-inflammatory glucocorticoids, serving to minimise inflammatory tissue damage at the time of rupture and encourage rapid healing of the ovarian surface in anticipation of the next ovulation.

Acknowledgements

This study was supported by the Medical Research Council (Programme Grant 8929853).

References


Harlow CR, Jenkins JM & Winston RM 1997 Increased follicular fluid total and free cortisol levels during the luteinizing hormone surge. *Fertility and Sterility* 68 48–53.


Received 10 May 1999
Revised manuscript received 24 July 1999
Accepted 28 July 1999