

# Regulation of 11 $\beta$ -hydroxysteroid dehydrogenase isoforms and glucocorticoid receptor gene expression in the rat uterus

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## Abstract

Glucocorticoids are known to have diverse effects on the uterus, generally believed to be mediated by the glucocorticoid receptor (GR). To date, two isoforms of the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) have been identified, namely 11 $\beta$ HSD1 and 11 $\beta$ HSD2, which interconvert active and inactive glucocorticoids and regulate local levels of hormones available to the GR in target tissues. The aim of the present study was to examine the uterine expression of 11 $\beta$ HSD and GR mRNA. The interplay of these parameters is probably an important factor in determining actions of glucocorticoids on the uterus.

Using Northern analysis we investigated the uterine expression of 11 $\beta$ HSD1, 11 $\beta$ HSD2 and GR mRNA in relation to serum levels of sex steroid hormones and uterine progesterone receptor mRNA expression in an animal model. Immature female rats were treated with 10 IU pregnant mare serum gonadotrophin (PMSG) fol-

lowed by 10 IU human chorionic gonadotrophin (hCG) 48 h afterwards, and then killed at 0, 3, 6, 9, 12 and 24 h and 5 days after the hCG injection.

Expression of both 11 $\beta$ HSD1 and 11 $\beta$ HSD2 mRNA in total uterine RNA was found to be up-regulated by more than 50% at 48 h after PMSG injection when oestradiol levels were also high. Following hCG treatment the expression of 11 $\beta$ HSD1 and 11 $\beta$ HSD2 further increased to reach maximal levels at 24 and 12 h respectively. GR mRNA expression was down-regulated by more than 50% by PMSG but gradually recovered after hCG injection. The results show that mRNA expression of 11 $\beta$ HSD1, 11 $\beta$ HSD2 and GR in the uterus is developmentally regulated, suggesting that these key determinants of glucocorticoid action may play an important role in uterine function.

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## Introduction

Glucocorticoids have a wide range of effects on many organs and tissues. Pharmacological doses of glucocorticoids were shown to suppress the hypothalamic–pituitary–gonadal axis both in humans (Quigley & Yen 1980) and in the rat (Rivier & Vale 1984). They are also known to alter uterine responses to oestrogens. Glucocorticoids have been reported to suppress oestrogen-stimulated uterine blood flow, weight gain, synthesis of DNA and proliferation in the uterus (Campbell 1978, Monheit & Resnik 1981). However, Gunin (1998) recently demonstrated that triamcinolone treatment caused an increase in oestradiol-induced proliferation of stromal cells in the endometrium. The concentration of uterine oestrogen receptors, on the other hand, can be down-regulated by the administration of glucocorticoids (Atkinson & Adams 1988, Rabin *et al.* 1990).

Uterine events such as menstruation, implantation, cervical softening and parturition have a lot of similarities with non-reproductive inflammatory situations (Kelly

1996). At high concentrations, glucocorticoids inhibit most immunological responses and are well-known anti-inflammatory agents. It is thus likely that they participate in each of the above uterine events. For instance, synthesis of prostaglandins, known participants in inflammatory situations, was shown to be modulated by glucocorticoids in the uterus (Pakrasi *et al.* 1983).

Regulation of the actions of glucocorticoids is achieved by a number of mechanisms. Pituitary adrenocorticotrophic hormone maintains blood levels of glucocorticoids in balance. In plasma, cortisol and corticosterone bind to corticosteroid-binding globulin (CBG) and albumin. Plasma levels of CBG thus regulate the bioavailability of free glucocorticoids. At the cellular level, hormone availability to the glucocorticoid receptor (GR) is regulated by the expression of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD). Two isoforms of this enzyme have been identified (Monder & Shackleton 1984, Brown *et al.* 1993), namely type 1 (11 $\beta$ HSD1) and type 2 (11 $\beta$ HSD2). 11 $\beta$ HSD1 is widely distributed (Whorwood *et al.* 1992). Though the precise physiological role of 11 $\beta$ HSD1 is

currently unclear, it has been suggested to play a role in maintaining expression of glucocorticoid-regulated genes (Jamieson *et al.* 1999). It is a bi-directional, NADP-dependent enzyme with predominant 11-ketoreductase activity and a low binding affinity for the active glucocorticoids cortisol and corticosterone (Lakshmi & Monder 1988, Monder & Lakshmi 1990, Stewart & Mason 1995). 11 $\beta$ HSD2, in contrast, is NAD-dependent and has strong 11 $\beta$ -dehydrogenase activity and high binding affinity for cortisol and corticosterone. It inactivates cortisol to cortisone in the human and corticosterone to 11-dehydrocorticosterone in the rat (Albiston *et al.* 1994, Stewart *et al.* 1994, Brown *et al.* 1996).

Glucocorticoid action on its target tissues depends not only on levels of active steroid that can gain access to the tissues, but also on GR levels and the local glucocorticoid metabolism profile. GR is a member of the steroid-thyroid hormone receptor superfamily (Evans 1988, Funder 1993). Classically, actions of glucocorticoids are mediated via GR, which interacts with specific hormone response elements (HREs) or other transcription factors to regulate gene transcription (Bamberger *et al.* 1996). To date, little is known about how the interaction of the above parameters is related to uterine function. In this study, we investigated the regulation of the uterine 11 $\beta$ HSD system and GR in relation to plasma levels of ovarian steroid hormones in a rat model.

## Materials and Methods

### *Animals and treatment*

Immature female rats of the Wistar strain between 21 and 25 days of age were housed in a temperature-controlled room illuminated for 12 h/day. The animals had free access to rat chow and water.

Rats were injected s.c. with 10 IU of pregnant mare serum gonadotrophin (PMSG; Sigma Chemical Co., Poole, Dorset, UK) followed by s.c. injection of 10 IU human chorionic gonadotrophin (hCG; Sigma) 48 h after PMSG treatment. These animals were then killed at 0, 3, 6, 9, 12 and 24 h and 5 days after the hCG injection. Control animals were killed when hCG was injected to other animals which had received PMSG. With this treatment, ovulation was noted to take place around 12 h after hCG injection. At 5 days, numerous corpora lutea were present in the ovaries.

Collected uteri were kept on ice in PBS. They were cleaned under a dissection microscope and then deep frozen in liquid nitrogen. All animal handling and treatment complied with guidance issued by the UK Home Office. Uteri from groups of 4–13 animals were pooled together to give sufficient material for Northern blot analysis. Serum samples were obtained from randomly selected animals to determine oestradiol and progesterone levels.

### *Hormone assays*

Oestradiol assays were performed as described previously (Hillier & De Zwart 1981). The intra-assay coefficient of variation was less than 10%. Plasma progesterone levels were determined with a solid-phase RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

### *RNA preparation*

Frozen uterine samples were homogenized in ice-cold 4 M guanidinium thiocyanate solution containing 25 mM sodium citrate, 0.5% (w/v) sarcosyl and 0.1 M  $\beta$ -mercaptoethanol (all from Sigma). Total RNA was extracted with phenol–chloroform as previously described (Chomczynski & Sacchi 1987), dissolved in formamide and kept at  $-70^{\circ}\text{C}$  until Northern blot analysis.

### *cDNA templates and $^{32}\text{P}$ -labelled riboprobes*

Reverse transcription PCR (RT-PCR) generated the required DNA templates. Oligonucleotide primer pairs were obtained from Cruachem (Glasgow, UK). Single-strand cDNA of 11 $\beta$ HSD1 was reverse transcribed from total RNA obtained from immature rat kidney, whereas 11 $\beta$ HSD2 and GR cDNAs were from immature rat liver using Moloney murine leukemia virus reverse transcriptase (Stratagene Cloning Systems, La Jolla, CA, USA) at  $37^{\circ}\text{C}$  for 60 min. The resultant templates underwent PCR amplification (30 cycles) with Pfu-DNA polymerase (Stratagene). Each PCR cycle consisted of denaturing for 45 s at  $94^{\circ}\text{C}$ , annealing for 45 s at  $60^{\circ}\text{C}$  and 2 min extension at  $72^{\circ}\text{C}$  with the final extension for 10 min. The resultant PCR products were then cloned using a pCR-Script Amp SK(+) Cloning Kit (Stratagene) and sequenced to verify the authenticity of the products. The sizes of the templates were: 11 $\beta$ HSD1, 620 bp (nucleotides (nt) 109–728; Agarwal *et al.* 1989, GenBank accession no. J05107); 11 $\beta$ HSD2, 412 bp (nt 534–945; Zhou *et al.* 1995, GenBank accession no. U22424); and GR 772 bp (nt 1383–2154; Miesfeld *et al.* 1986, GenBank accession no. M14053). 18S rRNA was synthesized from cDNA containing the 80 bp fragment of a highly conserved region of human 18S rRNA (pT7 RNA 18S; Ambion, Austin, TX, USA). All RT-PCR-generated cDNAs were linearized with either EcoRI or NotI restriction enzyme (Promega, Madison, WI, USA).

The cDNA template of rat progesterone receptor (PR1) was generously provided by Dr Ok-Kyong Park-Sarge, University of Kentucky. This cDNA (approximately 550 bp) encodes the hormone-binding domain of the rat PR (Park & Mayo 1991).

All RNA probes were labelled with UTP (3000 Ci/mmol for 11 $\beta$ HSD1, 11 $\beta$ HSD2, GR and PR; 800 Ci/mmol for 18S; Amersham International,

Aylesbury, Bucks, UK) using either T3 or T7 RNA polymerase and reagents supplied by Promega.

#### Northern blot analysis

Total RNA (20  $\mu$ g per lane) was size fractionated by electrophoresis in 1% (w/v) agarose gel containing 2.2 M formaldehyde at 75 V for 3.5 h. Following electrophoresis, RNA was transferred onto a nylon membrane (Boehringer Mannheim, Mannheim, Germany) overnight in 20  $\times$  standard saline citrate (SSC; single-strength SSC = 0.15 M NaCl and 0.015 M sodium citrate) and then covalently cross-linked to the membrane by ultraviolet irradiation. Ethidium bromide staining confirmed the presence of undegraded 18S and 28S rRNA. RNA from three different sets of animals was run on gels to give nylon membranes in triplicate.

The nylon membranes were prehybridized and hybridized as described by Church & Gilbert (1984) with minor modifications. The prehybridization/hybridization solution consisted of 0.2 M sodium phosphate (pH 7.2), 1 mM EDTA, 1% (w/v) BSA, 7% (w/v) SDS and 45% (v/v) deionized formamide. Prehybridization was carried out for 2 h at 60 or 65  $^{\circ}$ C and hybridization for 18–24 h at the same temperature. Radiolabelled RNA probes were then added to the hybridization solution at a concentration of  $1 \times 10^6$  c.p.m./ml ( $0.5 \times 10^6$  c.p.m./ml for the 18S probe). Membranes were washed in stringency wash solution containing 40 mM sodium phosphate, 1 mM EDTA and 1% (w/v) SDS, twice at room temperature (5 min), followed by two 30 min washes at 60 or 65  $^{\circ}$ C. After autoradiography, the membranes were stripped by washing twice in boiling 0.1% SDS solution for 30 min at room temperature and rinsed briefly in 2  $\times$  SSC. They were then re-probed with another  $^{32}$ P-labelled riboprobe.

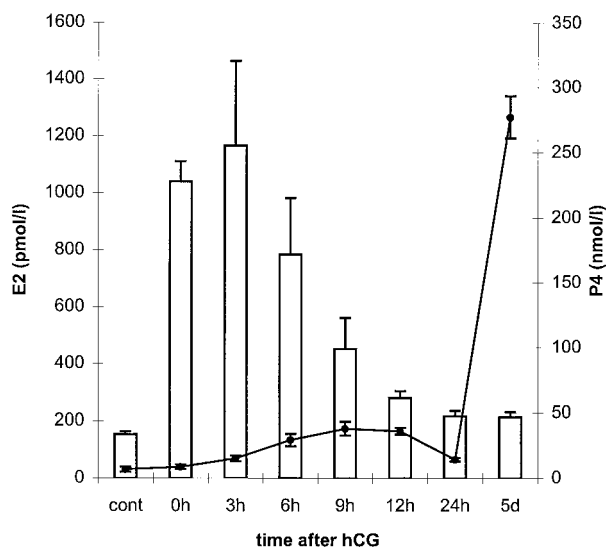
#### Data analysis

Quantification of radioactive signals of RNA by electronic autoradiography (Instant Imager, Packard, Downers Grove, IL, USA) was followed by exposure of the membranes to Kodak XAR-5 film (Eastman Kodak, Rochester, NY, USA) with intensifying screens for 16–48 h at  $-70^{\circ}$ C. The abundance of mRNA was normalized to the 18S rRNA signal. Results were finally expressed as percentages of the control values and analysed using one-way ANOVA with a paired Student's *t*-test.

## Results

#### Steroid hormone levels

To establish the relationship between ovarian steroid hormones and glucocorticoid response parameters, levels of serum oestradiol and progesterone were measured at each



**Figure 1** Serum oestradiol (E2) (bars) and progesterone (P4) (line) levels in the experimental animals. Immature female Wistar rats (21–25 days old) were treated with 10 IU PMSG followed by 10 IU hCG 48 h afterwards. Animals were killed at 0, 3, 6, 9, 12 and 24 h and 5 days after the hCG injection. Control animals (cont) were killed at the time of hCG injection to other experimental animals. Mean  $\pm$  S.E.M. ( $n=4$ ).

point of uterine collection. As shown in Fig. 1, the serum steroid hormone levels mimicked the changes that occur in the rat oestrous cycle, with an initial increase in oestradiol, followed by a secondary rise in progesterone around the time of ovulation at 12 h after hCG treatment.

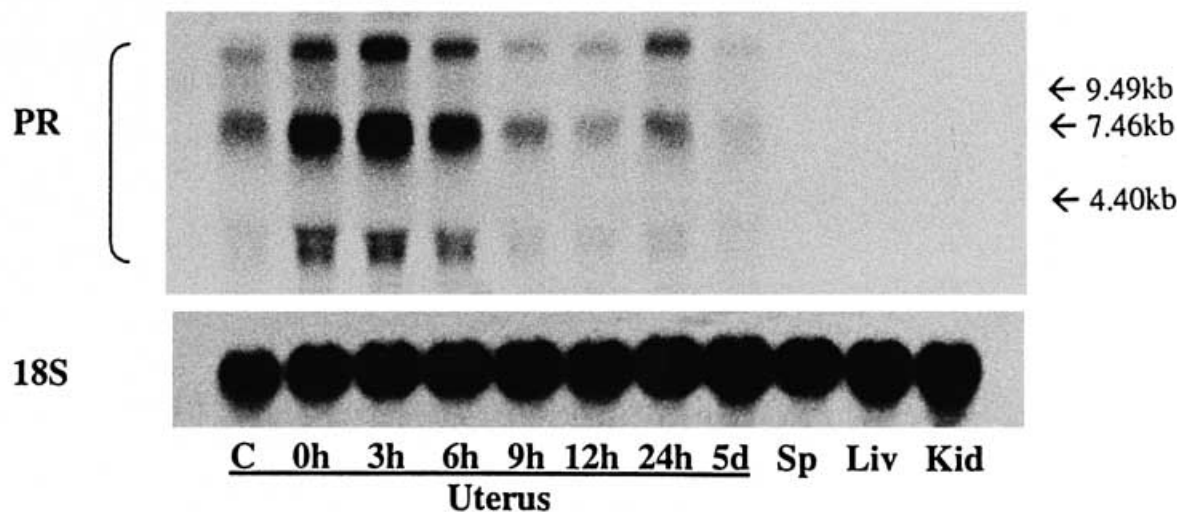
#### Expression of PR mRNA in total uterine RNA

Uterine PR mRNA levels closely followed the changes in serum oestradiol levels, with peak PR mRNA expression at 0 and 3 h coinciding with peak serum concentrations of oestradiol, whereas high serum levels of progesterone at 9 and 12 h and 5 days after hCG were associated with relatively low expression of PR mRNA (Figs 2 and 3).

#### Expression of 11 $\beta$ HSD1, 11 $\beta$ HSD2 and GR mRNA in total uterine RNA

Expression of both 11 $\beta$ HSD1 and 11 $\beta$ HSD2 mRNA (Figs 4 and 5A) increased by more than 50% at 48 h after PMSG treatment when oestradiol levels were also high. Thereafter, 11 $\beta$ HSD1 mRNA levels rose gradually to approximately three times the control values at 24 h after hCG injection.

Changes of 11 $\beta$ HSD2 mRNA levels followed a pattern slightly dissimilar to that of 11 $\beta$ HSD1 mRNA levels. Up-regulation of 11 $\beta$ HSD2 mRNA expression to more than 250% of the control values at 12 h after hCG, i.e. 9 h



**Figure 2** Expression of PR mRNA in total uterine RNA: autoradiogram of Northern blot analysis. Duration of film exposure: PR, 16 h; 18S, 17 h. Similar loading of total RNA was confirmed by similar intensity of the 18S rRNA band in each lane. PR mRNA expression in spleen (Sp), liver (Liv) and kidney (Kid) was insignificant. C=control.

after peak serum oestradiol levels had been reached, was followed by a sharp decrease at 24 h.

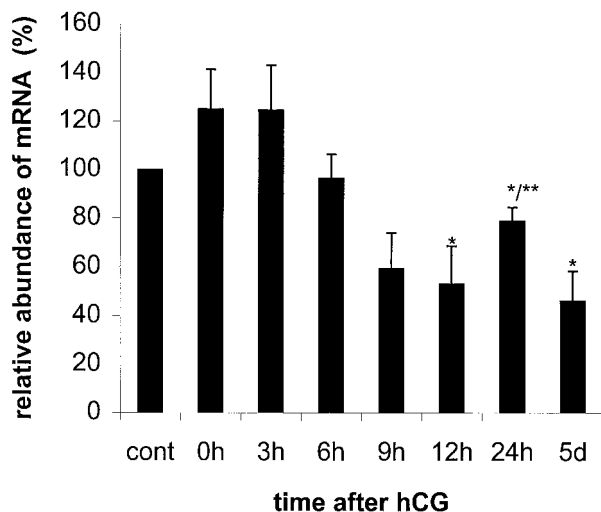
The pattern of GR mRNA expression (Fig. 5B) bore no direct relationship with the patterns of 11 $\beta$ HSD1 and 11 $\beta$ HSD2 mRNA levels but appeared to be inversely related to circulating levels of oestradiol. GR mRNA expression decreased by more than 50% after PMSG

treatment and gradually increased thereafter to approximately 75% of the control values at 5 days after hCG.

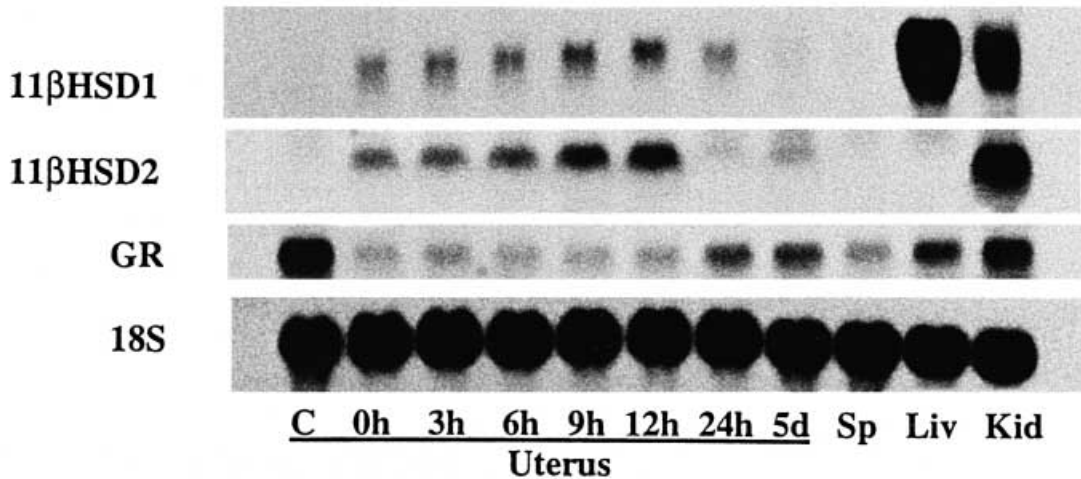
## Discussion

The present study demonstrates that PR, 11 $\beta$ HSD1, 11 $\beta$ HSD2 and GR mRNA expression is developmentally regulated in the rat uterus. These results show a hitherto unknown relationship between parameters of uterine glucocorticoid responsiveness and uterine functional status, as detected by circulating ovarian steroid hormone levels. Changes in serum concentrations of oestradiol and progesterone in the current animal model mimicked those of the rat oestrous cycle. Peak oestradiol levels at 3 h after hCG were followed 6 h afterwards by peak progesterone levels. This sequence can be likened to the pro-oestrus midday oestradiol surge followed by a surge of progesterone in the late afternoon (Butcher *et al.* 1974, Smith *et al.* 1975). The high serum concentrations of progesterone at 5 days after hCG, presumably secreted from the numerous corpora lutea noticed at that time, can be likened to those found during pseudopregnancy.

To ascertain if uterine tissues of the animals responded to various steroid hormone levels as predicted from reported studies (Kraus & Katzenellenbogen 1993, Graham & Clarke 1997), we investigated uterine PR mRNA expression. It is known that uterine PR expression can be increased by oestrogen administration, whereas progesterone down-regulates PR expression in the cycling rat. In our animal model, the pattern of PR mRNA levels



**Figure 3** Quantitative analysis of PR mRNA expression. Mean  $\pm$  S.E.M. ( $n=3$ ). Values significantly different from the controls are marked \* ( $P<0.05$ ). The value at 24 h is significantly different from that at 5 days (\*\*,  $P<0.05$ ). cont=control.



**Figure 4** Expression of 11 $\beta$ HSD1, 11 $\beta$ HSD2 and GR mRNA in total uterine RNA: autoradiogram of Northern blot analysis. Duration of film exposure: 11 $\beta$ HSD1, 48 h; 11 $\beta$ HSD2, 19 h; GR, 21 h; 18S, 17 h. Similar loading of total RNA was confirmed by similar intensity of the 18S rRNA band in each lane. Sp, spleen (negative control); Liv, liver (positive control for 11 $\beta$ HSD1); Kid, kidney (positive control for 11 $\beta$ HSD2). C=control.

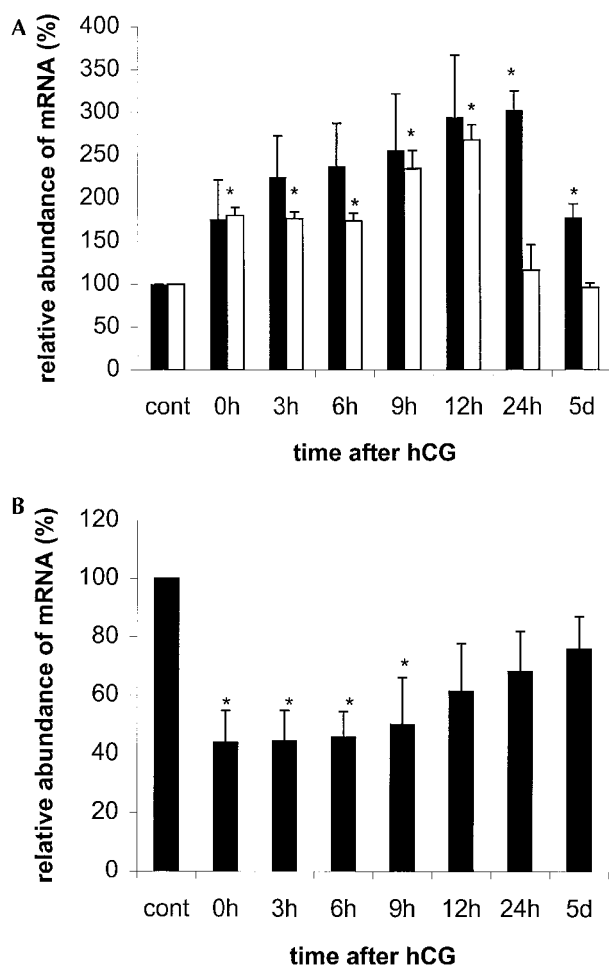
confirmed that the changing hormonal environment modulated PR mRNA expression in the experimental animals in a predicted manner. We detected at least three separate bands of PR mRNA in the uterus, the sizes of which correspond to those reported in the literature (Kraus & Katzenellenbogen 1993).

The effects of gonadotrophins on uterine function are mediated by ovarian steroid hormones but the influence of which on the uterine 11 $\beta$ HSD system remains unclear. Burton *et al.* (1998) showed that immunoreactivity for 11 $\beta$ HSD1 found in uterine homogenates from ovariectomized rats was increased by oestrogen replacement with or without progesterone. Progesterone replacement alone had no effect on the uterine 11 $\beta$ HSD1 signal. The same study reported that immunoreactivity for 11 $\beta$ HSD2 was increased by oestrogen replacement, whereas progesterone replacement alone had a slight stimulatory effect on 11 $\beta$ HSD2 immunostaining in stromal cells. Another study (Albiston *et al.* 1995), however, demonstrated in the cycling rat that at proestrus when circulating levels of oestrogen were high, 11 $\beta$ HSD1 mRNA expression began to fall and did not rise again until metoestrus when oestrogen levels were significantly lower. In our study, PMSG treatment up-regulated mRNA expression of both 11 $\beta$ HSD isoforms, most likely an indirect effect via rising oestradiol levels. Yet falling oestradiol levels between 3 and 12 h after hCG treatment were not associated with a corresponding decrease in either 11 $\beta$ HSD1 or 11 $\beta$ HSD2 mRNA expression. It is noteworthy that the increasing levels of 11 $\beta$ HSD1 and 11 $\beta$ HSD2 mRNA expression between 3 and 12 h after hCG coincided with the early rise of serum progesterone concentrations. However, high progesterone levels at 5 days were not associated with high

11 $\beta$ HSD1 or 11 $\beta$ HSD2 mRNA expression. Therefore a direct effect of progesterone on uterine 11 $\beta$ HSD mRNA expression remains to be established. The demonstration that expression of 11 $\beta$ HSD mRNA is related to ovarian hormone changes does not necessarily mean that enzyme protein or activity in the uterus follows a similar pattern. It should be borne in mind that the half-life of 11 $\beta$ HSD mRNA could affect tissue levels of mRNA and enzyme protein concentrations in the uterus. Translational and post-translational regulations are also potential factors in determining the local 11 $\beta$ HSD protein concentrations.

The implication of our results is that both activation of glucocorticoid through 11 $\beta$ HSD enzyme activity and levels of GR are likely to have roles in modulating glucocorticoid action in the uterus. The changes in GR mRNA expression in this study (Fig. 5B) followed a pattern different from those of 11 $\beta$ HSD1 and 11 $\beta$ HSD2. To our knowledge, this is the first time that PMSG treatment has been shown to down-regulate uterine GR mRNA expression in the immature rat. The down-regulation of GR mRNA after PMSG could be attributed to direct or indirect suppression of GR mRNA transcription by the rising oestradiol levels. An alternative explanation is a non-proportional increase in the number of cells with low expression of GR mRNA relative to the number of cells with high expression, leading to an apparent down-regulation of GR mRNA expression in total uterine RNA.

The dissimilar patterns of the above glucocorticoid response parameters suggest that it is the interaction of the local 11 $\beta$ HSD system and GR expression which determines uterine response to glucocorticoids. Uterine events such as menstruation, implantation, cervical softening and



**Figure 5** Quantitative analysis of expression of (A) 11 $\beta$ HSD1 (filled bars) and 11 $\beta$ HSD2 (open bars) mRNA and (B) GR mRNA. Mean  $\pm$  S.E.M. ( $n=3$ ). Values significantly different from the controls are marked \* ( $P<0.05$ ).

parturition have a lot of similarities with non-reproductive inflammatory processes (Kelly 1996). By virtue of their well-known anti-inflammatory actions, glucocorticoids are potentially important regulators of these uterine processes. At 24 h after hCG, when most of the animals had ovulated, the up-regulation of 11 $\beta$ HSD1 mRNA compared with the relatively low 11 $\beta$ HSD2 mRNA expression (Fig. 5A) could result in an accumulation of active glucocorticoids in the uterus. This is attributed to the fact that the major action of 11 $\beta$ HSD2 is inactivation of active glucocorticoids. GR mRNA expression was also relatively high at this time. Whether these active glucocorticoids prepare the uterus at this stage for implantation awaits further investigations.

Besides GR, PR is also implicated in the mediation of glucocorticoid anti-inflammatory effects. Steroid hormone receptors modulate transcription of target genes by

interacting with HREs on chromosomes. Studies have shown that both GR and PR can recognize and bind to the same HRE (Strähle *et al.* 1987, Tsai *et al.* 1988). Strähle *et al.* (1989) suggested that the lack of PR in the liver prevents progestins from activating glucocorticoid-responsive genes and that differential expression of hormone receptors is a mechanism by which steroid-specific gene activation is achieved. However, in organs co-expressing both GR and PR, e.g. the uterus, the mechanism by which hormone-specific transcription is achieved is still not fully understood, though mechanisms involving interaction of GR with other transcription factors in the absence of specific HRE binding have been described (Bamberger *et al.* 1996).

Our study confirms that the rat uterus expresses both GR and PR. Between 9 and 12 h after hCG, high serum progesterone concentrations coincided with peak 11 $\beta$ HSD2 mRNA expression. A speculative function of 11 $\beta$ HSD2 in the uterus during this period is the exclusion of active glucocorticoids from binding to GR. Progesterone-bound PR could therefore selectively modulate gene transcription via the non-selective HREs. Metabolism of active glucocorticoids by 11 $\beta$ HSD2 might provide a means of promoting progesterone specificity in tissues such as the uterus that co-express PR and GR.

In conclusion, we have shown that the rat uterus expresses key components of the glucocorticoid response system – 11 $\beta$ HSD types 1 and 2, and GR – all of which are developmentally regulated in response to gonadotrophin treatment. The patterns of response suggest that the ovarian steroids oestradiol and progesterone mediate some if not all of these changes. Further studies are required to substantiate the role of oestradiol and progesterone in the regulation of these parameters of glucocorticoid response and to delineate their physiological importance.

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