Glucocorticoid metabolism and reproduction: a tale of two enzymes

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Within potential target cells, the actions of physiological glucocorticoids (cortisol and corticosterone) are modulated by isoforms of the enzyme 11 β -hydroxysteroid dehydrogenase (11 β HSD). To date, two isoforms of 11 β HSD have been cloned: 11 β HSD1 acts predominantly as an NADP(H)-dependent reductase to generate active cortisol or corticosterone, and 11 β HSD2 is a high affinity NAD⁺-dependent enzyme that catalyses the enzymatic inactivation of glucocorticoids. Whereas the regeneration of active glucocorticoids by 11 β HSD1 has been implicated in the cellular mechanisms of pituitary function, ovulation and parturition, the enzymatic inactivation of cortisol and corticosterone by 11 β HSD enzymes appears to be central to the protection of gonadal steroidogenesis, prevention of intra-uterine growth retardation, and lactation. Recent evidence indicates that follicular fluid contains endogenous modulators of cortisol metabolism by 11 β HSD1, the concentrations of which are associated with the clinical outcome of assisted conception cycles and are altered in cystic ovarian disease. In conclusion, the two cloned isoforms of 11 β HSD fulfil diverse roles in a wide range of reproductive processes from conception to lactation.

The definitive role of glucocorticoids, synthesized in the zona fasciculata of the adrenal cortex in response to adrenocorticotrophic hormone (ACTH), is to increase plasma glucose concentrations. However, these steroids exert diverse actions throughout the body, many of which have important implications for fertility. Before excretion in the urine or faeces, glucocorticoids must be rendered water soluble by the sequential actions of hepatic 5 α - or 5 β -reductase and 3 α - or 3 β -hydroxysteroid dehydrogenase (3 α HSD or 3 β HSD) which reduce C=C double bonds and ketones, respectively, to generate hydrophilic dihydro- and tetrahydro-steroid metabolites. It was recognized in the late 1950s that within potential target cells, the actions of glucocorticoids are modulated by 11 β -hydroxysteroid dehydrogenases (11 β HSD) (EC 1.1.1.146) which catalyse the reversible inactivation of cortisol and corticosterone to their inert 11-ketosteroid metabolites, cortisone and 11-dehydrocorticosterone, respectively (Bush *et al.*, 1968) (Fig. 1). Although the biochemistry of 11 β HSD is well established, it is only over the past decade that studies have started to

define the physiological significance of glucocorticoid metabolism by these enzymes. This review focuses on the emerging importance of 11 β HSD isoenzymes in the germ cells, testis, ovary, reproductive tracts, placenta and mammary glands. As this field of reproductive biology is comparatively new, this review summarizes the current understanding of the physiological roles played by 11 β HSD in diverse processes including reproductive suppression, ovulation, luteinization, cystic ovarian disease, the developmental potential of oocytes, intra-uterine growth retardation (IUGR), parturition and lactation. Where sufficient robust data have been published, this review summarizes the defined roles for the 11 β HSD enzymes. However, this review also includes working hypotheses which fit all of the data currently available, but which will be subject to change as further experimental evidence is reported.

Physiology and molecular biology of 11 β HSD

Before discussing specific roles for 11 β HSD in reproductive tissues, it is necessary to outline major milestones in the history of this enzyme. To date, two biochemically distinct isoforms of 11 β HSD, designated type 1 11 β HSD

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Table 1. Biochemical properties of cloned isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD)

	11βHSD1	11βHSD2
Gene	<i>HSD11B1</i>	<i>HSD11B2</i>
Enzyme cofactor	NADPH > NADP ⁺ (>>> NADH > NAD ⁺)	NAD ⁺ only
K _m – cortisol	17–27 μmol l ⁻¹	14–60 nmol l ⁻¹
(K _m – corticosterone)	(2 μmol l ⁻¹)	(1–10 nmol l ⁻¹)
K _m – cortisone	300 nmol l ⁻¹	∞
Direction of reaction	Bi-directional: 11-ketosteroid reductase > 11β- dehydrogenase	Uni-directional: 11β-dehydrogenase only

Fig. 1. Interconversion of cortisol and cortisone by enzymatic oxidation or reduction at carbon position 11 (C11), catalysed by the two cloned isoforms of 11β-hydroxysteroid dehydrogenase (11βHSD). By convention, bonds below the plane of the molecule (α bonds) are represented by dotted lines; bonds which have no definitive orientation are represented by solid lines; and bonds above the plane of the molecule (β bonds) are represented by solid triangles. Corticosterone and 11-dehydrocorticosterone have similar structures to cortisol and cortisone, respectively, but lack the α-hydroxyl groups at position C17.

(11βHSD1) and type 2 11βHSD (11βHSD2), have been cloned. (By convention, terms in standard upper case text refer to the enzyme proteins, 11βHSD1 and 11βHSD2, whereas the corresponding genes are referred to in italicized text, *HSD11B1* and *HSD11B2*, respectively.)

Both cloned isoforms of 11βHSD are members of the short-chain alcohol dehydrogenase superfamily of enzymes, which includes not only other mammalian and bacterial HSD enzymes, but also 15-hydroxy-prostaglandin dehydrogenase (PGDH), retinol dehydrogenase, and the hepatic alcohol dehydrogenase (Penning, 1997). Whereas the 11βHSD enzymes are closely related to other hydroxysteroid dehydrogenases, such as the 3βHSD, 17βHSD and 20αHSD enzymes, 11βHSD1 and 11βHSD2 are distinct gene products (encoded by the *HSD11B1* and *HSD11B2* genes, respectively) which share only 14% homology in their primary sequence (Albiston *et al.*, 1994). Although both enzymes have similar active sites, with a conserved catalytic triad comprising tyrosine, serine and lysine (Penning, 1997), 11βHSD1 acts as a dimer or tetramer whereas 11βHSD2 appears only to be active in its monomeric form. The biochemical properties of the two cloned 11βHSD enzymes are summarized (Table 1). 11βHSD2 has an absolute requirement for the oxidized form of nicotinamide adenine dinucleotide (NAD⁺) as an enzyme cofactor, whereas 11βHSD1 preferentially utilizes nicotinamide adenine dinucleotide phosphate (NADP⁺/NADPH).

11βHSD1 was originally isolated from liver (Lakshmi and Monder, 1988), where it acts as an NADPH-dependent reductase to generate active glucocorticoids from inert 11-ketosteroids (Fig. 1). After cloning of 11βHSD1 (Agarwal *et al.*, 1989; Tannin *et al.*, 1991), this reductive enzyme isoform was shown to be widely expressed, co-localizing with glucocorticoid receptors (Whorwood *et al.*, 1992; Seckl and Walker, 2001). On the basis of this pattern of expression, it is generally accepted that the principal role of 11βHSD1 is to regenerate cortisol or corticosterone to maximize activation of the glucocorticoid receptors (Seckl and Walker, 2001). However, whereas 11βHSD1 acts predominantly as a ketosteroid reductase in intact cells, this enzyme is inherently bi-directional; in cell homogenates provided with NADP⁺, 11βHSD1 can inactivate glucocorticoids, albeit with a low affinity (K_m for cortisol = 27 μmol l⁻¹) (Lakshmi and Monder, 1988; Agarwal *et al.*, 1989) (Table 1). Hence, the predominant direction of reaction catalysed by 11βHSD1 depends on the redox state of

NADP(H) in a particular cell. In liver cells, with an abundant supply of glucose (glycogen) and an active pentose phosphate pathway, most NADP will be in the reduced form, favouring the NADPH-dependent ketosteroid reductase activity of 11 β HSD1. However, in the steroidogenic cells of the testis, ovary and placenta, the activity of NADPH-dependent cytochrome P450 enzymes, required for the biosynthesis of steroids, favours the oxidation of NADPH to NADP⁺, thus promoting the oxidative activity of 11 β HSD1.

Failure of 11 β HSD1 to act as an 11-ketosteroid reductase in the liver manifests as 'apparent cortisone reductase deficiency' (ACRD) (Phillipou and Higgins, 1985; Jamieson *et al.*, 1999). As ACRD has been reported to date in only five women, each diagnosed with anovulatory infertility, hyperandrogenism and polycystic ovaries, the molecular basis and reproductive consequences of this condition will be discussed later. A second clinical syndrome of defective glucocorticoid metabolism is that of 'apparent mineralocorticoid excess' (AME) (Ulick *et al.*, 1979; Stewart *et al.*, 1988). In this syndrome, glucocorticoids are not inactivated, due to a defect in the oxidative activity of 11 β HSD, and so gain access to mineralocorticoid receptors which display little inherent specificity for their normal ligand, aldosterone (Krozowski and Funder, 1983; Arriza *et al.*, 1987). As glucocorticoids circulate at concentrations 1000-fold higher than aldosterone, the mineralocorticoid receptors are hyperstimulated, resulting in excessive sodium transport and significant changes in sodium-dependent fluid fluxes (Funder *et al.*, 1988). Increased resorption of water in the distal nephron and colon increases blood volume and pressure, suppressing plasma renin activity and the concentration of aldosterone (Ulick *et al.*, 1979; Stewart *et al.*, 1988).

After the cloning of the human *HSD11B1* gene, patients with the inherited form of AME were screened for mutations. The lack of any mutations in these patients (Nikkila *et al.*, 1993), and the low affinity of 11 β HSD1, implicated the existence of a second isoform of 11 β HSD to protect the non-specific mineralocorticoid receptors. In 1994, 11 β HSD2 was cloned (Agarwal *et al.*, 1994; Albiston *et al.*, 1994) and identified as the enzymatic 'gatekeeper' that excludes glucocorticoids from mineralocorticoid receptors (Fig. 2). Unlike 11 β HSD1, 11 β HSD2 has been localized to mineralocorticoid target cells in the kidney, colon and parotid glands, as well as to the pancreas and placenta (Mercer and Krozowski, 1992; Brown *et al.*, 1993; Albiston *et al.*, 1994). With physiological substrates, 11 β HSD2 acts exclusively as an NAD⁺-dependent, high affinity 11 β -dehydrogenase (K_m for cortisol = 60 nmol l⁻¹) (Mercer and Krozowski, 1992; Brown *et al.*, 1993; Albiston *et al.*, 1994) (Table 1). Although this enzyme can catalyse limited reduction of the synthetic steroid, 11-dehydrodexamethasone, it has no reductive activity against cortisone or 11-dehydrocorticosterone, even in the presence of excess NADH.

Fig. 2. Modulation of glucocorticoid access to the 'mineralocorticoid receptor' by 11 β -hydroxysteroid dehydrogenase 2 (11 β HSD2), a molecular 'gatekeeper'. Deficiency of 11 β HSD2 activity allows cortisol to gain access to the promiscuous mineralocorticoid receptor.

Although patients with the inherited form of AME have been shown to inherit recessive mutations in the *HSD11B2* gene that encodes 11 β HSD2 (Mune *et al.*, 1995; Ferrari *et al.*, 1996), such congenital defects are rare. By comparison, failure of 11 β HSD2 due to enzyme inhibition is relatively common. Important inhibitors of 11 β HSD2 include glycyrrhizic and glycyrrhetic acids (metabolites of liquorice) (Stewart *et al.*, 1987; Monder *et al.*, 1989), and, particularly relevant for the current review, progesterone (Lopez-Bernal *et al.*, 1980; Sun *et al.*, 1998; Burton and Waddell, 2002).

11 β HSD in the hypothalamus and anterior pituitary gland

In reproductive biology, any review should logically begin at the level of the hypothalamo-pituitary complex, where both cloned isoforms of 11 β HSD are known to be expressed and functional. In 11 β HSD1 knockout mice, which are unable to reduce 11-ketosteroids to active glucocorticoids, there is increased hypothalamic secretion of corticotrophin-releasing hormone (CRH) and increased ACTH drive to the adrenal gland, indicating that 11 β HSD1 is required in the hypothalamus or anterior pituitary corticotrophs for glucocorticoids to exert full negative feedback in the hypothalamo-pituitary-adrenal (HPA) axis (Harris *et al.*, 2001). A recent study confirmed that regeneration of glucocorticoids by 11 β HSD1 limits the sensitivity of corticotrophs to CRH by decreasing the expression of CRH receptors (Hanafusa *et al.*, 2002). Moreover, pilot studies in the present authors' laboratory

have found that one or more of the 11 β HSD enzymes is active in α T3-1 and L β T2 gonadotroph cells, and that 11 β HSD activities respond acutely to GnRH in these pituitary gonadotroph cell lines (Thurston *et al.*, 2003a).

Testicular 11 β HSD: fertility and reproductive suppression

Over the past three decades, it has emerged that glucocorticoids exert a number of deleterious effects on the interstitial Leydig cells of the testis, including direct inhibition of testosterone biosynthesis, suppression of LH receptor expression, and induction of Leydig cell apoptosis (Bambino and Hsueh, 1981; Monder *et al.*, 1994; Gao *et al.*, 2002). Shortly after the cloning of rat 11 β HSD1, Phillips *et al.* (1989) confirmed expression of this enzyme in rat Leydig cells. In subsequent studies, 11 β HSD1 was shown to alleviate inhibition of testosterone biosynthesis by corticosterone (Monder *et al.*, 1994). This protective role presumes that in rat Leydig cells, 11 β HSD1 acts predominantly as an 11 β -dehydrogenase. However, this assumption was challenged by Leckie *et al.* (1998) who found that 11 β HSD1 acts as an 11-ketosteroid reductase in rat Leydig cells maintained in primary culture. Detailed *in vitro* studies have since revealed that the predominant direction of testicular 11 β HSD1 activity depends on the age of the rat from which Leydig cells have been harvested, the duration of incubation with enzyme substrates, and the precise composition of the cell culture medium in which enzyme activities are assessed. Expression of 11 β HSD1 is highest, and the reductase activity of this enzyme predominates, in immature Leydig cells (Ge *et al.*, 1997a), on prolonged incubation with steroid substrates (Ge and Hardy, 2000), and in culture medium containing high concentrations of D-glucose or pyruvate (Ferguson *et al.*, 1999; Ge and Hardy, 2000). However, in adult Leydig cells, on acute incubation with corticosterone (< 1 h) or in Dulbecco's modified Eagle's medium without added glucose or pyruvate, expression of 11 β HSD1 mRNA is decreased and the oxidative activity of 11 β HSD predominates (Ge *et al.*, 1997a; Ferguson *et al.*, 1999; Ge and Hardy, 2000).

In attempting to resolve disputes over the preferred direction of action of 11 β HSD1 in Leydig cells in rats, evidence has accumulated to indicate the existence of an enzyme activity that is biochemically distinct from either of the cloned isoforms of 11 β HSD. In addition to the low affinity, NADPH-dependent activity characteristic of 11 β HSD1, Leydig cells also exhibit an NADP⁺-dependent enzyme activity which acts preferentially as a high affinity dehydrogenase (K_m for corticosterone = 42 nmol l⁻¹) (Ge *et al.*, 1997b). Available evidence indicates that this represents a novel isoform of 11 β HSD, rather than a post-translationally modified form of 11 β HSD1.

Investigation of the hormonal regulation of 11 β HSD1 in testis Leydig cells (Gao *et al.*, 1997) established that

over 3 days in primary culture, both LH and epidermal growth factor (EGF) upregulated expression of 11 β HSD1 mRNA. In the case of EGF, this increase in 11 β HSD1 expression was accompanied by a significant increase in 11-ketosteroid reductase activity, whereas both LH and EGF decreased the net 11 β -dehydrogenase activity in cultured Leydig cells (Gao *et al.*, 1997). Recent studies have revealed that increase of intracellular calcium favours testicular 11-ketosteroid reductase activity, whereas activation of protein kinase C (PKC) inhibits the net reductase activity and instead stimulates the net oxidation of glucocorticoids in rat Leydig cells (Ge and Hardy, 2002). Analysis of the primary amino acid sequences of the 11 β HSD1 and 11 β HSD2 proteins reveals multiple phosphorylation sequences for calmodulin kinase, casein kinase II and various tyrosine kinases. However, whereas the catalytic domain of 11 β HSD1 contains a consensus phosphorylation site for PKC, there is no such sequence in 11 β HSD2, and neither 11 β HSD1 nor 11 β HSD2 contains a consensus phosphorylation sequence for protein kinase A (PKA). Given that the enzymes contain binding sites for both calcium and for adenosine triphosphate (ATP), it seems probable that in Leydig cells direct binding of calcium to 11 β HSD1 stimulates the reductase activity of this enzyme, whereas phosphorylation of the active site via PKC favours the oxidative activity of this enzyme.

With respect to the physiological relevance of 11 β HSD in Leydig cells, the fact that 11 β HSD1 protein cannot be detected before day 31 after birth (Schafers *et al.*, 2001), and the developmental switch from predominant reductase activity in mesenchymal-like Leydig cell progenitors to an oxidase in adult Leydig cells (Ge *et al.*, 1997a), indicates a role for testicular 11 β HSD enzymes in increased testosterone synthesis at puberty. Moreover, latest reports indicate that failure of testicular 11 β HSD to protect Leydig cells from the adverse effects of corticosterone contributes to decreased testosterone output in socially subordinate male rats whereas increased testicular 11 β HSD activities are observed in dominant males (Hardy *et al.*, 2002). As these trends mirror changes in serum LH concentrations within the first week of establishing the social hierarchy (Hardy *et al.*, 2002), the authors propose that LH upregulates the protective 11 β HSD system in sexually active dominant male rats (allowing their Leydig cells to maintain testosterone production in the face of increased plasma corticosterone), whereas suppression of LH in social subordinates contributes to the decrease in testicular 11 β HSD activities.

11 β HSD in spermatozoa and the male reproductive tract

Within testis Leydig cells, expression of 11 β HSD1 protein coincides with the maturation of elongate spermatids in neighbouring seminiferous tubules (Neumann *et al.*,

1993). 11 β HSD activity has also been reported in spermatozoa and other cellular components of human semen (Nacharaju *et al.*, 1997). Moreover, the net oxidative activities of 11 β HSD were 10-fold higher in semen from men with low sperm counts or a high percentage of morphologically abnormal spermatozoa compared with ejaculates with high sperm counts or good sperm morphology (Nacharaju *et al.*, 1997). Although the physiological role for 11 β HSD in spermatozoa has yet to be defined, it is relevant to note that human seminal plasma contains relatively high concentrations of cortisol (Brotherton, 1990) and that in men with increased serum cortisol concentrations (due to Cushing's disease), pathological changes occur in the seminiferous tubules (McKenna *et al.*, 1979).

Enzyme activity was localized histochemically to the entire length of the mouse epididymis in initial studies of testicular 11 β HSD (Rastogi *et al.*, 1976). In the epithelium of the vas deferens in pigs, glucocorticoids have been associated with increased sodium absorption (Phillips and Schultz, 2002), which would be expected to reduce seminal fluid volume, leading to a high viscosity luminal environment for the mature spermatozoa. In addition, corticosteroid-dependent sodium absorption will limit the activity of sodium-proton co-transporters in the epithelia of the male reproductive tract which participate in the control of seminal pH (Pushkin *et al.*, 2000). Hence, alterations in 11 β HSD-mediated glucocorticoid metabolism within the male reproductive tract modify the luminal environment for maturing spermatozoa.

11 β HSD in the oocyte

In a wide range of teleost species, cortisol, 11-deoxycortisol and related hydroxylated metabolites of progesterone stimulate oocyte maturation and developmental potential (for example see Kime *et al.*, 1992; Petrino *et al.*, 1993; Pinter and Thomas, 1999). To date, investigations of the direct effects of glucocorticoids on mammalian oocytes, confined to studies of pig oocytes, have found that glucocorticoids inhibit meiotic development (Yang *et al.*, 1999). In contrast, in follicular aspirates of women undergoing assisted conception, follicular glucocorticoid concentrations, which increase following the preovulatory LH surge (Andersen and Hornnes, 1994; Harlow *et al.*, 1997), have been positively correlated with oocyte maturity (Fateh *et al.*, 1989; Jimena *et al.*, 1992).

Metabolism of glucocorticoids by 11 β HSD in mammalian oocytes further implicates these steroids in mammalian oogenesis. Although 11 β HSD expression has been reported in human oocytes (Ricketts *et al.*, 1998; Smith *et al.*, 2000), the most convincing data have been obtained in the ovary in rats, wherein expression of 11 β HSD1 mRNA and protein were both very high in the oocyte (Benediktsson *et al.*, 1992). In interpreting this observation, it is important to note that in oocytes,

transcription of new mRNA is blocked between the preovulatory resumption of meiosis and the second mitotic division of the two-cell embryo. Hence, high expression of 11 β HSD1 in the preovulatory oocyte could reflect roles for this reductive isoform of 11 β HSD in the ovulatory oocyte, zygote or early embryo.

11 β HSD across the ovarian cycle: roles in ovulation and luteinization

As in Leydig cells of the testis, glucocorticoids act directly on ovarian cells to inhibit both gonadotrophin action and steroid biosynthesis (Hsueh and Erickson, 1978; Michael *et al.*, 1993). Moreover, before the cloning of 11 β HSD isoforms, it was established that in granulosa lutein cells in humans, 11 β HSD inactivates glucocorticoids, so increasing the concentration of cortisol required to inhibit LH-stimulated steroidogenesis by an order of magnitude (Michael *et al.*, 1993).

Within ovarian follicles in humans, 11 β HSD2 protein has been localized to theca cells with low 11 β HSD2 activity measured in theca cells isolated from immature follicles (Ricketts *et al.*, 1998; Yong *et al.*, 2000). As regards enzyme expression in granulosa cells, this is highly dependent on the functional phenotype and differentiation of the cells. In parallel studies of ovaries in rats and humans, Tetsuka *et al.* (1997, 1999a) demonstrated that granulosa cells that have not experienced an LH surge co-express mRNA encoding 11 β HSD2 and mineralocorticoid receptors. Thurston *et al.* (2003b) observed expression of 11 β HSD2 protein in bovine follicles at all stages of development. Given the pivotal role for 11 β HSD2 in regulating stimulation of sodium-dependent fluid fluxes via mineralocorticoid receptors, this implicates corticosteroids in the control of follicular fluid accumulation during the expansion of the antral follicle.

In human and rat granulosa cells which have luteinized following exposure to an ovulatory dose of LH *in vivo*, there is no detectable expression of 11 β HSD2 mRNA or protein (Michael *et al.*, 1997; Tetsuka *et al.*, 1997, 1999a; Thurston *et al.*, 2003c). Instead, the predominant enzyme isoform in such cells is 11 β HSD1 (Michael *et al.*, 1997; Tetsuka *et al.*, 1997, 1999a), expression of which increases progressively as the cells undergo functional luteinization (Thurston *et al.*, 2003c). Moreover, the only cloned isoform of 11 β HSD expressed in the corpus luteum of pregnant rats (until such time as the gland undergoes functional regression) is 11 β HSD1 (Waddell *et al.*, 1996). This transition from expression of 11 β HSD2 in follicular granulosa cells to 11 β HSD1 in luteinized granulosa cells is accompanied by a switch in expression from mineralocorticoid receptors in the follicle to glucocorticoid receptors in luteinized cells (Tetsuka *et al.*, 1997, 1999a) (Fig. 3).

Hillier and colleagues have determined the molecular basis for the transition from 11 β HSD2 to 11 β HSD1

Fig. 3. Temporal pattern of expression of 11 β -hydroxysteroid dehydrogenase (11 β HSD) isoforms and corticosteroid receptors across ovarian cycles in rats and humans. The upper panel depicts changes in oestradiol (purple line) and progesterone (brown line) to illustrate the phase of the ovarian cycle. (The broken purple line indicates that oestradiol is not secreted in the luteal phase for non-primate species). The horizontal coloured bars indicate the stages of the ovarian cycles in rats and humans at which follicular granulosa cells or luteinized granulosa or luteal cells express a particular isoform of 11 β HSD or a particular corticosteroid receptor. Note the transient expression of 11 β HSD2 in luteal cells coincident with functional regression of the corpus luteum.

during luteinization, concluding that 11 β HSD1 upregulation at ovulation is induced by gonadotrophins. As ovulation is an inflammatory event characterized by increased synthesis of interleukins and prostaglandins (Espey, 1980; Terranova and Rice, 1997; Ando *et al.*, 1998), increased generation of anti-inflammatory glucocorticoids by the reductase activity of 11 β HSD1 at ovulation may present a physiological mechanism to limit the ovarian inflammatory process (Hillier and Tetsuka, 1998; Andersen, 2002). Certainly, glucocorticoids inhibit the synthesis of both prostaglandins and pro-inflammatory cytokines in the ovary (Goppelt-Strube, 1997; Telleria *et al.*, 1998). As expression of 11 β HSD1 is upregulated in granulosa cells by LH and by pro-inflammatory cytokines such as interleukin 1 β (IL-1 β) (Evagelatou *et al.*, 1997; Tetsuka *et al.*, 1999b), the synthesis of glucocorticoids via 11 β HSD1 may be increased by gonadotrophins or cytokines as an integral aspect of the inflammatory cascade of ovulation. Consistent with this hypothesis, the total and free concentrations of cortisol in human follicular fluid increase in response to the preovulatory LH surge (Fateh *et al.*, 1989; Andersen and Hornnes, 1994; Harlow *et al.*, 1997; Yong *et al.*, 2000). The increase in free cortisol also involves competitive displacement of cortisol from cortisol-binding globulin by the high concentrations of progesterone within the ovulatory follicle (Andersen and Hornnes, 1994; Harlow *et al.*, 1997; Andersen, 2002).

Whilst accepting the roles for LH and interleukins in the transition from expression of 11 β HSD2 to expression

of 11 β HSD1 at ovulation, this periovulatory switch in enzyme isoforms at luteinization could also involve steroidal regulation of enzyme expression. Specifically, 11 β HSD2 expression in follicular granulosa cells may be dependent on local synthesis of oestrogens, whereas progesterone may suppress expression of 11 β HSD2 or induce expression of 11 β HSD1 in luteinizing cells. Evidence to support a role for progesterone was obtained in a study of the pregnant rat corpus luteum. In this transient endocrine gland, expression of 11 β HSD1 is high until the last day of pregnancy when the corpus luteum undergoes functional regression. At this time, 11 β HSD1 expression decreases markedly with a resurgence in expression of 11 β HSD2 (Waddell *et al.*, 1996) (Fig. 3). The simplest interpretation of this observation is that upregulation of 11 β HSD1 and suppression of 11 β HSD2 at luteinization is dependent on progesterone, such that the decline in progesterone output with luteal regression causes a parallel loss of 11 β HSD1 expression accompanied by an increase in 11 β HSD2 expression. In a recent study of human granulosa lutein cells undergoing functional luteinization *in vitro*, this possibility has been examined. Whereas suppression of progesterone synthesis increased both the oxidative and reductive activities of 11 β HSD (reflecting decreased competitive inhibition by progesterone), expression of 11 β HSD1 protein was unaffected by progesterone suppression and increased progressively as cells luteinized (Thurston *et al.*, 2003c). Hence, upregulation of 11 β HSD1 expression in luteinizing cells does not rely on local synthesis of progesterone.

Tetsuka *et al.* (2003) reported data for the bovine ovary which differ fundamentally from the observations of ovarian cells in humans and rats. Bovine granulosa cells in large antral follicles expressed only mRNA encoding 11 β HSD1 whereas 11 β HSD2 mRNA was not detected by RT-PCR. In the corpus luteum in cows, both 11 β HSD1 and 11 β HSD2 were co-expressed, with 11 β HSD1 mRNA predominating in the active corpus luteum and 11 β HSD2 predominating in the regressing corpus albicans (Tetsuka *et al.*, 2003). Hence, although enzyme expression does change with the functional activity of follicles and corpus luteum, the cow does not show the absolute switch from expression of 11 β HSD2 to 11 β HSD1 at ovulation. The species specificity of this phenomenon further proves that the transition between 11 β HSD isoforms as granulosa cells in rats and humans luteinize cannot be ascribed solely to changes in follicular and luteal steroid production. In large antral follicles in cows, the synthesis of oestradiol is not accompanied by expression of 11 β HSD2, and in the corpus luteum in cows, 11 β HSD2 is expressed in the absence of oestradiol and in the presence of progesterone.

With respect to luteinizing granulosa cells in humans, the issue of whether 11 β HSD1 acts in these cells as either a reductase or an oxidase has been disputed by

independent studies (Michael *et al.*, 1997; Thomas *et al.*, 1998). It seems likely that in granulosa lutein cells, as in rat Leydig cells, the predominant direction of action is influenced by subtle factors such as cell differentiation or age and differences between culture media. In studying the detailed kinetics of 11 β HSD in granulosa lutein cell homogenates in humans, an NADP⁺-dependent enzyme activity that appeared to act predominantly as an 11 β -dehydrogenase with a high affinity for cortisol (K_m = 400 nmol l⁻¹) was identified (Michael *et al.*, 1997). The biochemical properties of this ovarian enzyme activity are very similar to the high affinity 11 β HSD activity described in Leydig cells in rats (Ge *et al.*, 1997b).

11 β HSD in the ovarian surface epithelium

Historically, studies of ovarian function have overlooked the surface epithelium in favour of more dynamic ovarian events and structures. However, emerging evidence indicates that this single-layered epithelium participates in repair of the ovarian surface after the 'wounding' of ovulation. Recent studies have shown that human ovarian surface epithelial (OSE) cells express 11 β HSD1, which is upregulated by pro-inflammatory cytokines, such as IL-1 α (Yong *et al.*, 2002). Regeneration of cortisol by the reductive activity of 11 β HSD1 may create an anti-inflammatory environment as part of a co-ordinated response to the preovulatory LH surge (Yong *et al.*, 2002). In addition, ovulation also involves collagenolysis and tissue remodelling at the ovarian surface with apoptosis of OSE cells from the follicular apex (Murdoch *et al.*, 1999). Therefore, the remaining 'bystander' OSE cells surrounding the ovulatory follicle and, after ovulation, the ovulatory rupture site, may play functional roles both in the tissue breakdown that precedes ovulation and in repair of the damaged ovarian surface. These 'bystander' OSE cells are spared from apoptotic damage (Murdoch *et al.*, 1999), indicating that a highly localized inflammatory environment is present at ovulation. In the OSE cells, 11 β HSD1 will participate in the local generation of cortisol at and around the site of ovulation to facilitate increased anti-inflammatory and surface repair mechanisms (Yong *et al.*, 2002). In human OSE cells *in vitro*, cortisol inhibits the induction of metalloproteinase activity by pro-inflammatory cytokines (Rae *et al.*, 2003).

Ovarian 11 β HSD and assisted conception

Michael *et al.* (1995) reported an inverse correlation between levels of oxidation of cortisol by 11 β HSD in granulosa lutein cells in humans and the clinical outcome of gonadotrophin-stimulated assisted conception cycles. Whereas none of the *in vitro* fertilization-embryo transfer (IVF-ET) cycles characterized by detectable net conversion of cortisol to cortisone resulted in conception,

the probability of establishing a clinical pregnancy in IVF-ET cycles characterized by undetectably low ovarian 11 β HSD activities was 64% (Michael *et al.*, 1995). As ovarian 11 β HSD activities were only weakly associated with the probability of oocyte fertilization *in vitro* (Michael *et al.*, 1995), it was concluded that ovarian 11 β HSD activities in the granulosa lutein cells reflected the development potential of ova after fertilization. In patients studied over consecutive treatment cycles, ovarian 11 β HSD activities appeared to be a feature of the ovarian response to treatment in a particular IVF-ET cycle for a given patient (Michael *et al.*, 1995).

In an independent study, Thomas *et al.* (1998) found no significant difference between mean ovarian 11 β HSD activities in conception versus non-conception IVF cycles, although the probability of conception was threefold higher in patients with low ovarian 11 β HSD activities than in patients whose granulosa lutein cells exhibited high rates of cortisol-cortisone interconversion. Subsequently, the ratio of cortisol:cortisone in follicular fluid was assessed as a reflection of 11 β HSD activities within follicles *in vivo*. In gonadotrophin-stimulated IVF-ET cycles, high follicular cortisol:cortisone ratios have been found to be associated with a greater than 50% probability of conception, whereas low follicular cortisol:cortisone ratios are typically associated with a probability of establishing a pregnancy through IVF-ET of less than 10% (Michael *et al.*, 1999; Thurston *et al.*, 2003d). Although a study by Andersen *et al.* (1999) found no significant correlation between IVF outcome and follicular cortisol:cortisone ratios, Keay *et al.* (2002) reported a significant association between increased follicular cortisol:cortisone ratios and the probability of conception in women undergoing natural cycle IVF. High cortisol:cortisone ratios in follicular fluid aspirated from women who conceive by IVF-ET would reflect either a low net oxidation of cortisol to cortisone in the mature follicle (in agreement with the present authors' initial observations) or an increase in the opposing reduction of cortisone to cortisol.

Ovarian modulators of 11 β HSD: IVF outcome, ovarian cysts and apparent cortisone reductase deficiency

In initial studies, granulosa lutein cells were stored for up to 3 days in follicular fluid before being isolated and cultured. Thurston *et al.* (2002, 2003e) have showed that follicular fluid from women, cows and pigs contains at least two distinct classes of compound capable of selectively modulating the NADP(H)-dependent activities of the 11 β HSD1 isoform. Specifically, follicular fluid contains a hydrophilic compound(s) that can stimulate 11 β HSD1 activity by up to threefold within 1 h, and a hydrophobic component(s) that acutely inhibits 11 β HSD1 activity by up to 84%.

Fig. 4. Independent effects of ovarian modulators of 11 β -hydroxysteroid dehydrogenase (11 β HSD) on 11 β HSD1 activity and the developmental potential of the oocyte. Green lines with a positive symbol indicate a stimulatory effect on enzyme activity or on the developmental potential of the oocyte or embryo. Red lines with a negative symbol indicate an inhibitory effect on enzyme activity or on the developmental potential of the oocyte or embryo.

Within human follicular fluid, high activity of the hydrophilic stimulator(s) of 11 β HSD1 correlates with a low intrafollicular cortisol:cortisone ratio and predicts a low probability of conception, whereas high activity of the hydrophobic inhibitor(s) of 11 β HSD1 are associated with a high cortisol:cortisone ratio and an increased likelihood of establishing a clinical pregnancy (Thurston *et al.*, 2003d). Hence, in the present authors' initial studies, low 11 β HSD activities may have reflected the activity of enzyme modulators present in the follicular fluid samples in which cells were stored before culture. The working model is that the association between the follicular content of enzyme stimulator(s), enzyme inhibitor(s) and IVF outcome reflects independent effects of these as yet unidentified compounds on ovarian glucocorticoid metabolism and the developmental competence of oocytes in maturing follicles (Fig. 4).

In ovaries in cows and pigs, aspirates from spontaneous ovarian cysts contain much lower contents of the hydrophilic stimulator(s) of NADP(H)-dependent glucocorticoid metabolism and higher contents of the hydrophobic inhibitor(s) of 11 β HSD1 than does follicular fluid aspirated from large antral follicles (Thurston *et al.*, 2003e). If this picture within follicular and ovarian cyst fluid is reflective of circulating modulators of 11 β HSD1 activities, this could explain, at least in part, the association between polycystic ovaries and ACRD in

Fig. 5. Contrasting paracrine and endocrine actions of hydrophobic ovarian inhibitors of 11 β -hydroxysteroid dehydrogenase 1 (11 β HSD1) produced by a maturing ovarian follicle versus an ovarian cyst. Red lines indicate a paracrine or endocrine inhibition of cortisol-cortisone inter-conversion by 11 β HSD1 in the ovary, liver or pituitary gland. Broken lines indicate relatively weak inhibition; solid lines indicate relatively strong inhibition.

hyperandrogenic, anovulatory women (Fig. 5). Certainly, in patients with ACRD, no mutations have yet been identified in the *HSD11B1* gene (Nikkila *et al.*, 1993; Jamieson *et al.*, 1999), indicating a post-transcriptional defect in the reductase activity of hepatic 11 β HSD1. The present authors, based on latest findings, would attribute ACRD to a post-translational inhibition of hepatic 11 β HSD1 activity in cystic ovarian disease, possibly due to increased secretion of a hydrophobic inhibitor of 11 β HSD1 from the ovarian cysts. Given that 11 β HSD1 is required in the pituitary gland for glucocorticoids to suppress ACTH synthesis and secretion (Harris *et al.*, 2001), the present authors propose that in cystic ovarian disease, increased circulating concentrations of the hydrophobic inhibitor(s) of 11 β HSD1 not only alters hepatic glucocorticoid metabolism, but also impedes negative feedback within the HPA axis. The consequent increase in pituitary ACTH secretion would then, in turn, contribute to the stimulation of adrenal hyperandrogenism typical of cystic ovarian disease (Fig. 5). Although it is yet to be proved definitively that the

inhibitor(s) of 11 β HSD1 present in follicular or cyst fluid arise locally within the ovaries, the fact that first degree male relatives of women with ACRD show normal hepatic cortisone reductase activity (Jamieson *et al.*, 1999) supports the view that the major inhibitor of 11 β HSD1 is produced in a tissue or gland found only in females.

11 β HSD in the uterus

In the uterus, both cloned isoforms of 11 β HSD co-operate to control access of glucocorticoids to the glucocorticoid receptors (rather than the mineralocorticoid receptors). Initial studies of rat uteri found that 11 β HSD1 mRNA expression varied across the oestrous cycle from a peak at dioestrus to a nadir at oestrus, consistent with suppression of 11 β HSD1 by oestrogens (Albiston *et al.*, 1995). Subsequent studies confirmed that uterine expression of both 11 β HSD1 and 11 β HSD2 is indeed oestrogen sensitive (Burton *et al.*, 1998). However, studies of 11 β HSD1 expression in liver and hippocampus established that in these tissues, suppression of 11 β HSD1 mRNA by oestradiol is mediated indirectly via increased plasma growth hormone (Low *et al.*, 1994). The suppression of 11 β HSD1 expression by growth hormone is in turn mediated by insulin-like growth factor 1 (IGF-I) (Moore *et al.*, 1999). As the IGF system is active in the uterus, expression of 11 β HSD1 here is likely to be controlled by both direct effects of oestradiol and indirect effects mediated via IGF-I. As in the ovary, expression of 11 β HSD1 mRNA in the uterus in rats can also be increased by gonadotrophins (Ho *et al.*, 1999), although indirect effects secondary to stimulation of ovarian steroidogenesis cannot be excluded.

Within the myometrium, glucocorticoids exert important effects on such parameters as myometrial contractility (Challis *et al.*, 2000) and expression of oestrogen receptors (Wu *et al.*, 1996). In the uteri in rats, 11 β HSD1 and 11 β HSD2 have both been co-localized with glucocorticoid receptors in the myometrial smooth muscle cells (Burton *et al.*, 1996a; Waddell and Burton, 2000; Burton and Waddell, 2002). Myometrial expression of 11 β HSD2 mRNA and protein both increase from day 10 of pregnancy, peak at day 16 of gestation, and are significantly suppressed within 24 h after birth (Burton and Waddell, 2002). However, in pregnancy, NAD⁺-dependent oxidation of corticosterone is suppressed (rather than increased) relative to the non-pregnant rat myometrium, and remains constantly low throughout pregnancy, returning to pre-conception values within 24 h of parturition. This apparent paradox reflects competitive inhibition of myometrial 11 β HSD2 by progesterone throughout pregnancy (Burton and Waddell, 2002). During pregnancy, the expression of 11 β HSD1 mRNA and protein in the rat myometrium also increases markedly

to a maximum on day 22 of gestation (that is 1 day before birth), decreasing to non-pregnant values by day 1 after birth (Burton *et al.*, 1996a; Waddell and Burton, 2000; Burton and Waddell, 2002). Unlike 11 β HSD2, the 30-fold increase in expression of 11 β HSD1 at term is accompanied by a 10-fold increase in the 11-ketosteroid reductase activity which peaks on the day before parturition (Burton *et al.*, 1996a; Waddell and Burton, 2000). As the oxidative activity of myometrial 11 β HSD2 remains constant throughout pregnancy, the pre-partum induction of 11 β HSD1 favours net regeneration of active glucocorticoids within the myometrium (Burton and Waddell, 2002). As discussed below, this change from inactivation to regeneration of glucocorticoids within the myometrium assists in parturition. However, this role for myometrial 11 β HSD is species specific as there is little detectable expression of 11 β HSD1 mRNA in the myometrium in sheep from day 60 of gestation to 2 days before term (Yang *et al.*, 1996).

Although the data reviewed above indicate up-regulation of myometrial 11 β HSD1 by progesterone as pregnancy progresses, Waddell and Burton (2000) observed that in unilaterally pregnant rats there was only limited endocrine upregulation of 11 β HSD1 mRNA in the contralateral, non-gravid uterine horn, concluding that full induction of myometrial 11 β HSD1 expression in the gravid horn was reliant on paracrine factors. This comprehensive study established that paracrine upregulation of 11 β HSD1 expression and activity in the pregnant uterine horn was attributable to the placenta rather than to uterine distension or to the presence of a fetus. Waddell and Burton (2000) concluded that placental hormones (for example placental cytokines) are required for local upregulation of myometrial 11 β HSD1 in pregnancy.

In the uterine endometrium, in myomorph rodents and sheep, 11 β HSD1 is absent from the endometrial stroma and glandular epithelium, but has been localized to the luminal epithelium where it is co-expressed with glucocorticoid receptors (Yang *et al.*, 1996; Burton *et al.*, 1998; Thompson *et al.*, 2002). As in the myometrium, endometrial expression of 11 β HSD1 mRNA is increased in the luteal phase of the oestrous cycle and in pregnancy, reflecting upregulation by progesterone (Yang *et al.*, 1996). In cultured human endometrial stroma cells, the expression and activity of 11 β HSD1 was increased by treatment of cells with progesterone for 3 days. Although treatment with oestradiol alone had no effect, co-treatment of stromal cells with oestradiol potentiated the induction of 11 β HSD1 by progesterone. This observation led Arcuri *et al.* (1996) to conclude that upregulation of 11 β HSD1 is a feature of endometrial decidualization.

In the endometrium of pregnant rats and mice, 11 β HSD2 is expressed only in the sub-epithelial stroma, and there is no detectable expression in the uterine epithelia (Burton and Waddell, 2002; Thompson *et al.*,

2002). In contrast, in the endometrium of non-pregnant women, there is limited expression of 11 β HSD2 in the stroma, but this oxidative enzyme isoform is highly expressed in both the glandular and luminal epithelia (Smith *et al.*, 1997). Although endometrial 11 β HSD2 activity increases in the secretory phase of the menstrual cycle, this almost certainly reflects expansion of the glandular epithelium under the action of progesterone (Smith *et al.*, 1997). Although roles for endometrial 11 β HSD have been proposed in pregnancy (particularly for the decidua), the physiological roles for glucocorticoid metabolism in the non-pregnant endometrium have yet to be defined.

11 β HSD in pregnancy

Early studies of glucocorticoid metabolism focused on human placenta as a source of enzyme activity (Osinski, 1960; Murphy *et al.*, 1974). As the complex role for 11 β HSD in this organ has been discussed in several excellent reviews (for example Yang, 1997; Burton and Waddell, 1999; Bertram and Hanson, 2002), coverage in the current review will focus on major points of note and recent developments.

In the placenta of all species studied to date, there are two biochemically distinct 11 β HSD activities which have been attributed to the co-expression of both 11 β HSD1 and 11 β HSD2 (Murphy, 1981; Lakshmi *et al.*, 1993; Yang, 1995; Burton *et al.*, 1996b; Pepe *et al.*, 1996; Sun *et al.*, 1997a; Thompson *et al.*, 2002). 11 β HSD1 is predominantly expressed in the decidua, chorion, amnion and vascular endothelial cells, whereas 11 β HSD2 is expressed in both the decidua and in the placenta (Murphy, 1981; Sun *et al.*, 1997a; Burton and Waddell, 1999; Driver *et al.*, 2001; Thompson *et al.*, 2002). In the baboon placenta, 11 β HSD1 and 11 β HSD2 are co-expressed in the syncytiotrophoblast (Pepe *et al.*, 1996), and in the mouse placenta, both cloned isoforms of 11 β HSD are co-expressed with glucocorticoid receptors in the labyrinthine zone in the later stages of gestation (Thompson *et al.*, 2002). In all species, this localization of 11 β HSD2 at the materno-fetal interface reflects the role for 11 β HSD2 as a mechanism to limit transfer of glucocorticoids between the maternal and fetal circulations.

At different stages of gestation, the balance between the reductase activity of 11 β HSD1 and the oxidative activity of 11 β HSD2 in the placenta changes in a species-specific manner. In ewes and guinea-pigs, placental 11 β -dehydrogenase activity decreases as pregnancy progresses (Yang, 1997; Sampath-Kumar *et al.*, 1998), whereas in human, baboon, pig and rat placentas, 11 β -dehydrogenase activity increases in the later stages of gestation (Lopez-Bernal *et al.*, 1980; Pepe *et al.*, 1988; Klemcke and Christenson, 1996; Burton and Waddell, 1999). Although the net oxidative activity of 11 β HSD increases at term in the rat placenta, this reflects increa-

sing expression of 11 β HSD2 in the basal zone of the placenta (the major site of placental hormone synthesis), which masks a decrease in the expression and activity of 11 β HSD2 in the labyrinthine zone (the site of fetomaternal exchange) (Burton *et al.*, 1996b).

As in other reproductive tissues, the expression and activities of placental 11 β HSD isoforms is hormonally regulated. For example, in the placenta in sheep, suppression of 11 β HSD2 activity at term is mediated by cortisol (Clarke *et al.*, 2002). In placental and chorionic trophoblasts in humans, both progesterone and oestradiol selectively inhibit the oxidative activity of 11 β HSD2 without affecting 11 β HSD1 reductase activity (Sun *et al.*, 1998). In addition, progesterone represses the expression of 11 β HSD2 mRNA (Sun *et al.*, 1998). Expression or activity of this high affinity 11 β -dehydrogenase is also inhibited in human term placental trophoblasts by noradrenaline (Sarkar *et al.*, 2001), nitric oxide (mediated, at least in part, via cyclic GMP) (Sun *et al.*, 1997b), prostaglandins and leukotriene B₄ (which inhibit enzyme activity at the post-transcriptional level via increased intracellular calcium) (Hardy *et al.*, 1999, 2001). In contrast to these suppressive actions, placental 11 β HSD2 is selectively upregulated by cyclic AMP (Sun *et al.*, 1998). As 11 β HSD2 does not contain a consensus phosphorylation sequence for the cyclic AMP-dependent kinase, PKA, the selective upregulation of placental 11 β HSD2 must be attributable either to a direct effect of cyclic AMP on the enzyme (via interaction with the ATP-binding site) or to an effect on enzyme translation or stability. (The endocrine regulation of 11 β HSD1 expression and activity in the placenta is of primary importance at parturition, and so will be discussed below.)

Recent studies have focused on the regulation of placental 11 β HSD2 by hypoxia. Although two studies have shown that hypoxia represses expression of 11 β HSD2 (without affecting 11 β HSD1) in term human trophoblasts and villous explants (Alfaidy *et al.*, 2002; Hardy and Yang, 2002), Driver *et al.* (2002) found that hypoxia upregulates 11 β HSD2 and have identified a hypoxia-inducible factor 1 response element in the 11 β HSD2 promoter. Moreover, decreased oxygen tension indirectly influences the activities of placental 11 β HSD by altering the redox balances of pyridine nucleotide cofactors in favour of NADPH (so favouring the reductive activity of 11 β HSD1) and NADH (thereby limiting the NAD⁺-dependent oxidative activity of 11 β HSD2). In either event, the placental hypoxia associated with pre-eclampsia alters the trans-placental passage of glucocorticoids from mother to fetus and the local balance of glucocorticoid regeneration from 11-ketosteroids (Alfaidy *et al.*, 2002).

Endogenous inhibitors of glucocorticoid metabolism have been described in human urine (Morris *et al.*, 1992). Pending identification, these compounds were named 'glycyrrhetic acid-like factors' (GALFs), reflecting their ability to inhibit both 11 β HSD and 5 β -reductase:

characteristic actions of the liquorice derivative, glycyrrhetic acid. The urinary GALF content, which is higher in women than men, increases progressively during pregnancy (Morris *et al.*, 1992), consistent with production of GALF by the ovary or placenta. Although GALF and identified inhibitor(s) of 11 β HSD1 from follicular fluid share similar biophysical properties (Thurston *et al.*, 2002), it can only be speculated at present that the urinary GALFs and ovarian enzyme inhibitor(s) may be related (if not the same) compounds.

11 β HSD, fetal development and intra-uterine growth retardation

The widely accepted view is that in the placenta, 11 β HSD2 serves as a barrier to limit transfer of active glucocorticoids from mother to fetus. Hence, if placental 11 β HSD2 activity is compromised, increased transport of glucocorticoids (before activation of the fetal HPA axis) stimulates premature differentiation of fetal tissues, limiting subsequent tissue growth and culminating in growth retardation. This model was first advanced by Benediktsson *et al.* (1993) after observing a direct correlation between placental 11 β HSD activities and fetal weights in rats at term. That placental 11 β HSD activities are decreased in pregnancies complicated by IUGR was subsequently corroborated in studies of pregnancies in humans (Shams *et al.*, 1998; McTernan *et al.*, 2001). However, it is important to note that within the fetus, both cloned isoforms of 11 β HSD are widely expressed in a tissue-specific manner (Murphy, 1981; Stewart *et al.*, 1994). Hence, any decrease in placental inactivation of glucocorticoids may not necessarily influence the concentrations to which individual fetal tissues are exposed. It seems far more likely that the exposure of fetal tissues to glucocorticoids is determined at the intracrine level by the activities of 11 β HSD1 and 11 β HSD2, rather than at the relatively distant level of the placenta.

Recent studies have demonstrated that maternal nutrient restriction at defined stages of pregnancy results in both fetal growth retardation and in significant decreases in the expression and activity of placental 11 β HSD2 (Bertram *et al.*, 2001; Lesage *et al.*, 2001). Although this would permit increased passage of active glucocorticoid into the fetal circulation, it is not possible to comment on the physiological significance of the decrease in placental glucocorticoid metabolism versus changes in the expression or activities of 11 β HSD within affected fetuses.

Although the relative impact of placental versus fetal 11 β HSD in modulating fetal exposure to glucocorticoids remains contentious, 11 β HSD enzymes in tissues such as the decidua are able to influence fetal development *in utero*. Specifically, 11 β HSD1 acts in the decidua to increase the concentration of active glucocorticoid in amniotic fluid. As the amniotic fluid bathes the fetal

lungs, a high cortisol:cortisone (or corticosterone:11-dehydrocorticosterone) ratio in amniotic fluid promotes maturation of the fetal lungs. To guard against premature lung maturation, the fetal lung expresses the oxidative 11 β HSD2 enzyme throughout the first and second trimester (Murphy, 1981; Stewart *et al.*, 1994), but in late gestation, this oxidative enzyme is replaced by the reductive 11 β HSD1 enzyme, so allowing glucocorticoid derived from the amniotic fluid to stimulate synthesis of lung surfactant.

11 β HSD and parturition

Over the past 5 years, studies conducted primarily by Challis and colleagues have implicated the reductive activity of 11 β HSD1 in the mechanism of parturition (Fig. 6). Specifically, it has been proposed that glucocorticoids generated by 11 β HSD1 in the myometrium and decidua increase (rather than suppress) uterine concentrations of prostaglandins which stimulate contractions of the myometrium to expel the fetus (reviewed by Challis *et al.*, 2000). This unusual pro-inflammatory (rather than anti-inflammatory) action of glucocorticoids at term involves upregulation of prostaglandin H synthase 2 accompanied by suppression of PGDH in the chorion (Patel *et al.*, 1999; Whittle *et al.*, 2001) (Fig. 6). PGDH, which normally oxidizes prostaglandins to inactive metabolites, belongs to the same enzyme superfamily as the 11 β HSD enzymes, and shares over 90% primary sequence homology at the active site with 11 β HSD1 (Penning, 1997).

Within the placenta, prostaglandins increase both the expression and activity of 11 β HSD1 (Alfaidy *et al.*, 2001). The consequent increase in the concentration of active glucocorticoids is further enhanced by the abilities of prostaglandins E_2 and $F_{2\alpha}$ to inhibit the oxidative 11 β HSD2 isoform in the placenta (Hardy *et al.*, 1999). Recent studies have shown that glucocorticoids can also upregulate placental 11 β HSD1 expression (Sun *et al.*, 2002) and suppress the inactivation of glucocorticoids by placental 11 β HSD2 (Clarke *et al.*, 2002), thereby accelerating the paracrine glucocorticoid–prostaglandin spiral (Fig. 6).

At term, this positive feed-forward loop may be activated by glucocorticoids generated as the fetal HPA axis matures (Whittle *et al.*, 2001). However, this mechanism could also participate in the initiation of pre-term labour following an increase in either maternal or fetal glucocorticoids (and catecholamines) under conditions of maternal or fetal stress. In addition, this paracrine positive feed-forward loop provides a mechanism whereby increase of uterine prostaglandin concentrations (for example in response to an intra-uterine infection) would prematurely trigger parturition. As placental 11 β HSD2 appears to be sensitive to oxygen tension, changes in glucocorticoid metabolism have also been implicated in the mechanism of pre-term labour

Fig. 6. A potential glucocorticoid–prostaglandin paracrine feed-forward loop contributing to the mechanism of parturition. Black lines indicate an enzyme-catalysed reaction; solid black lines are used for major metabolic pathways; and broken lines indicate minor pathways. Solid green lines with a positive symbol indicate a stimulatory effect on the expression or activity of the appropriate enzyme, or on myometrial contraction. Broken red lines with a negative symbol indicate an inhibitory effect on the expression or activity of the appropriate enzyme. AA: arachidonic acid; 11βHSD1: 11β-hydroxysteroid dehydrogenase 1; PGDH: 15-hydroxyprostaglandin dehydrogenase; PGHS-2: prostaglandin H synthase 2.

in hypoxic states such as pre-eclampsia (Alfaidy *et al.*, 2002).

11βHSD and the mammary gland

Within the mammary gland, oxidative 11βHSD activity is 20-fold higher in adipose cells than in mammary epithelial cells (Quirk *et al.*, 1990). Moreover, whereas 11βHSD activities are comparably high in mammary cells from virgin and pregnant rats, the ability of 11βHSD to inactivate glucocorticoids decreases by over 75% during lactation (Quirk *et al.*, 1990). As glucocorticoids induce the expression of milk proteins such as casein and lactalbumin (Ono and Oka, 1980), the postpartum decrease in glucocorticoid oxidation is a prerequisite for the endocrine induction of lactogenesis (Quirk *et al.*, 1990), whereas inactivation of glucocorticoids within the mammary gland prevents futile antagonism between the positive effects of glucocorticoids and the suppressive actions of progesterone during pregnancy. As to the mechanism for the decrease in mammary 11βHSD activities during lactation, this is unlikely to reflect changes in gonadal or placental steroids as enzyme activities were the same in virgin versus pregnant rats. Instead,

the decrease in mammary 11βHSD activities probably reflects unique changes to the endocrine milieu of the mammary gland post partum (for example increased prolactin levels).

Hundertmark *et al.* (1997) reported inactivation of physiological and synthetic glucocorticoids by 11βHSD in the MCF-7 and ZR-75-1 human breast cancer cell lines. As the cells exhibited only limited 11-ketosteroid reductase activity, and because the oxidation of glucocorticoids could be inhibited by glycyrrhetic acid, these authors concluded that 11βHSD2 is the predominant enzyme isoform in the human breast. Moreover, inhibition of 11βHSD activity with glycyrrhetic acid potentiated the anti-proliferative action of prednisolone (Hundertmark *et al.*, 1997), confirming that oxidation modulates the actions of glucocorticoids in mammary cells.

Concluding remarks

The evidence outlined in this review indicates that the metabolism of glucocorticoids by isoforms of 11βHSD is important in a wide range of reproductive tissues and

Table 2. Overview of the confirmed and speculative physiological roles for 11 β -hydroxysteroid dehydrogenases (11 β HSD) in reproductive tissues

Tissue or organ	Cell	11 β HSD isoform	Proposed physiological role
Pituitary	Corticotrophs	1	Maximizes negative feedback on ACTH
	Gonadotrophs	1 + 2	Unknown
Testis	Leydig cells	1 (+?)	Protects testosterone biosynthesis from inhibition by glucocorticoids
	Spermatozoa	Unknown	Unknown
Epididymis/vas deferens	Not specified	1	Modulates control of sodium content/viscosity of seminal plasma
Ovary	Theca cells	2	Unknown
	Granulosa cells	2 (+?)	Protects oestradiol biosynthesis from inhibition by glucocorticoids
	Oocyte	1	Modulates effects of glucocorticoids on oocyte maturation
	Luteal cells	1 (+?)	Protects progesterone biosynthesis from inhibition by glucocorticoids
	Surface epithelial cells	1	Facilitates anti-inflammatory effects of glucocorticoids at ovulation
Myometrium	Smooth muscle cells	1 + 2	Modulates actions of glucocorticoids on contractility and oestrogen receptors
Endometrium	Epithelial cells + sub-epithelial stroma	1 + 2	Unknown
Placenta	Decidua + chorion + amnion + vascular endothelial cells	1	Facilitates effects of glucocorticoids on prostaglandin synthesis or metabolism to induce parturition (implicated in pre-term labour)
	Decidua + syncytiotrophoblast	2	Limits transfer of cortisol from the maternal to the fetal circulation (defective in IUGR)
Mammary gland	Adipose cells	2	Modulates actions of glucocorticoids on lactogenesis

1: 11 β HSD1; 2: 11 β HSD2; (+?): the possible existence of an as yet uncloned isoform of 11 β HSD; ACTH: adrenocorticotrophic hormone; IUGR: intra-uterine growth retardation.

a number of key reproductive processes (summarized in Table 2). The apparent significance of glucocorticoid inactivation or reactivation by 11 β HSD in reproductive suppression, ovulation, luteinization, cystic ovarian disease, the developmental potential of oocytes, IUGR, parturition and lactation indicates significant roles for glucocorticoids in each of these fundamental processes.

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