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Changes in the Levels of 11β-Hydroxysteroid Dehydrogenase mRNA over the Oestrous Cycle in the Rat

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The NADP dependent enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) metabolizes glucocorticoids to their inactive 11-keto-metabolites in a wide range of tissues. To date very little is known about the regulation of this enzyme at the level of gene transcription. In this study we show significant changes in the uterine, renal, ovarian and hepatic levels of 11βHSD1 mRNA over the oestrous cycle. Uterine and renal message levels followed the same pattern, with the highest levels observed at dioestrus and the lowest levels at oestrus, a pattern that correlates with plasma oestrogen levels during the cycle. In both the uterus and kidney 11βHSD1 message levels more than halved from dioestrus to oestrus, while renal levels then doubled at metoestrus. In contrast, hepatic 11βHSD1 message levels at prooestrus were twice those observed at metoestrus. Ovarian levels remain constant until metoestrus when a marked decrease in message levels was seen. 11βHSD1 mRNA levels are thus differentially regulated in a tissue specific manner throughout the oestrous cycle.

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INTRODUCTION

11β-hydroxysteroid dehydrogenase (11βHSD) catalyses the conversion of glucocorticoids to their inactive 11-keto metabolites. Two distinct forms of 11βHSD enzymes have been identified to date based on differential co-factor dependency. The more recently identified enzyme, 11βHSD2, is NAD(H) dependent and has been demonstrated in the placenta and mineralocorticoid target tissues such as the kidney [1–3]. Currently, it is thought that the high affinity of 11βHSD2 for glucocorticoids enables aldosterone to occupy the non-selective mineralocorticoid receptor [4]. In contrast 11βHSD1 is NADP(H) dependent, has been identified in most tissues and has a K_m approx. 100-fold lower for corticosterone than 11βHSD2 [1]. Recent studies [5] suggest that a mutation in the 11βHSD1 gene may not be involved in the etiology of the syndrome of apparent mineralocorticoid excess, which is associated with a high urinary cortisol to cortisone ratio [6, 7]. The role of 11βHSD1 is, therefore, likely to be the modulation of glucocorticoid hormone action at the cellular level by regulating access of glucocorticoids to their own receptor.

The regulation of 11βHSD activity by sex steroids is well known. Early studies by Lax *et al.* [8] demonstrated that hepatic 11βHSD levels are almost completely suppressed by oestradiol in both male and female rats, while testosterone enhanced enzyme activity. However, after hypophysectomy activity levels are no longer under sex steroid modulation. More recent studies have shown no effect of sex hormones on 11βHSD activity in the kidney [9]. During pregnancy placental 11βHSD activity in the baboon has been observed to change in both level and direction, and these changes correlate with the rising levels of oestrogen throughout pregnancy [10]. Furthermore, both oestradiol and testosterone have been shown to modulate 11βHSD1 gene expression in the kidney and liver but not hippocampus [11]. To further investigate the hormonal regulation of the 11βHSD1 gene 11βHSD1 mRNA levels were determined during the oestrous cycle in the kidney, liver, ovary and uterus.

MATERIALS AND METHODS

Sprague–Dawley rats were maintained on a 12 h dark–light cycle and provided with water and rat chow *ad libitum*. Daily vaginal smears were used to determine

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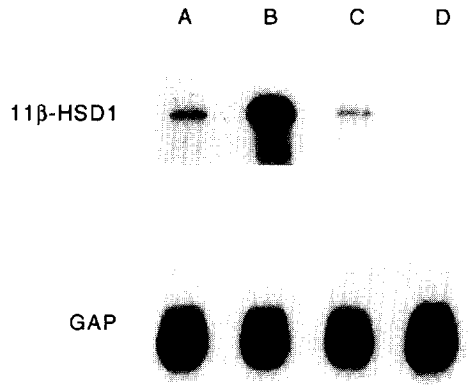

Fig. 1. 11βHSD1 mRNA and GAP mRNA in the liver (A), kidney (B), ovary (C) and uterus (D). Total RNA was analysed by the RNA protection assay and run on a 6% polyacrylamide sequencing gel and exposed for 18 h at −70°C with an intensifying screen.

the stage of the oestrous cycle in 11-week female rats. Rats were killed and tissues collected only after individual rats had been through three recognized cycles ($n = 4$ for each group). Total RNA was extracted from tissues according to the method of Chirgwin [12].

11βHSD1 mRNA levels were determined in the liver, kidney, ovary and uterus by RNA protection assays as previously described [13]. All results were standardized against glyceraldehyde-3-phosphate dehydrogenase (GAP) mRNA levels as an internal control. Levels of mRNA were determined for individual rat tissues except for the ovaries which were pooled into two groups of two. Results are expressed as mean ± SEM. Statistical analysis was by one-way analysis of variance.

RESULTS

Figure 1 shows the levels of 11βHSD1 mRNA detected in the liver, kidney, ovary and uterus by the RNA protection assay. All four tissues analysed expressed 11βHSD1; the most abundant mRNA levels were in kidney, and markedly lower levels were seen in the liver, uterus and ovary. All four tissues showed changes in 11βHSD1 message levels over the oestrous cycle (Fig. 2). In the kidney message levels halved from dioestrus to oestrus, followed by a return to dioestrus levels at metoestrus [Fig. 2(A)]. The level of a truncated 11βHSD1 message previously described in the kidney [13] changed in parallel with the full length mRNA (not shown). The hepatic message levels of 11βHSD1 were more than twice as high at prooestrus than at metoestrus [Fig. 2(B)]. In contrast, ovarian 11βHSD1 mRNA levels remained constant until oestrus followed by an apparent decrease at metoestrus [Fig. 2(C)], but statistical analysis was not performed as it was necessary to pool samples before RNA extraction. In the uterus message levels fell markedly between dioestrus and oestrus [Fig. 2(D)].

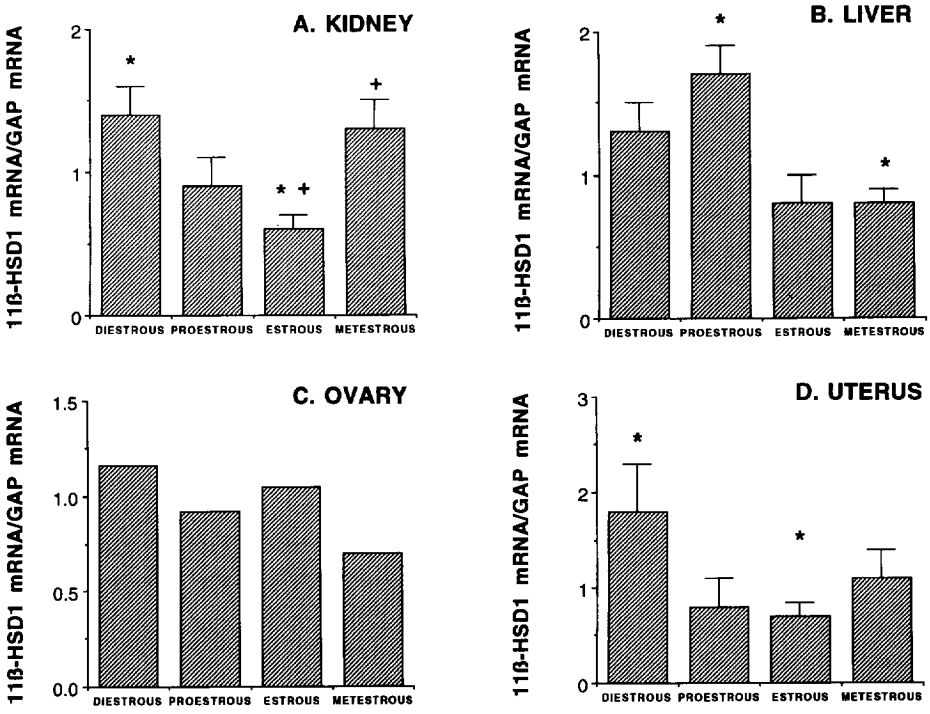

Fig. 2. Levels of 11βHSD1 mRNA throughout the oestrous cycle. Autoradiograms of varying exposure times (6–24 h at −70°C with an intensifying screen) were analysed by densitometry. All results have been normalized to GAP mRNA levels and are presented as mean ± SEM. Except for the ovary, RNA levels were determined for individual rats, with $n = 4$ for each stage of the oestrous cycle. ($^{*+}P < 0.05$).

DISCUSSION

This study clearly indicates that the levels of 11βHSD1 mRNA change in the kidney, liver and uterus during the oestrous cycle. The changes observed for the kidney and the uterus can be correlated in part with the plasma oestrogen levels during the oestrous cycle [14]. At prooestrus there is a significant increase in circulating levels of oestrogen and at this stage of the cycle both uterine and renal 11βHSD1 mRNA levels begin to fall and do not rise again until metoestrus when oestrogen levels are significantly lower.

Previous studies have indicated that oestrogen modulates 11βHSD1 activity levels in a number of tissues [8, 10, 11]. The present study suggests that regulation is at the transcriptional level and/or at the level of message stability. However, an oestrogen response element has not been identified in the upstream region of either the rat or human genes [15, 16]. Studies on the liver clearly show that the activity of 11βHSD1 is inhibited by oestrogen, although the effect is not a direct one as the pituitary clearly plays a role in mediating the actions of this hormone [8]. Sexual dimorphism in the secretory pattern of growth hormone is known to result in the differential induction of hepatic enzymes [17], but it is interesting to note that in the present study a decrease in hepatic 11βHSD mRNA levels was observed at oestrus during which there are two surges in plasma prolactin levels partly mediated by oestrogens [14]. Thus, the previously observed pituitary dependent oestrogen regulation of hepatic 11βHSD1 activity may be at least in part modulated by prolactin.

In a recent study oestrogens were shown to have opposite effects on total 11βHSD activity and 11βHSD1 message levels in the kidney of gonadectomized rats, underlining the confounding effect of multiple isoforms of the enzyme [11] and suggesting that earlier studies on 11βHSD1 activity may need to be re-evaluated. Studies by Baggia *et al.* [10] on the placental activity of 11βHSD1 in baboons have demonstrated that the level of activity increases and the direction of conversion changes from reductase to oxidase with increasing levels of oestrogen throughout pregnancy. However, the placenta is now known to contain both 11βHSD1 and 11βHSD2 activities [3, 18] and therefore the effect of oestrogen on 11βHSD1 activity may be masked. Similar observations can be made about the effect of sex steroids in renal tissue where no significant changes were observed [9].

There is mounting evidence to suggest that glucocorticoid action is modulated by physiological events associated with the oestrous cycle. Both the corticotropin-releasing factor (CRF) rhythms and plasma cortisol levels are dependent on stages of the oestrous cycle in the rat [19], and CRF secretion has been shown to be modulated by 11βHSD [20]. Hypothalamic CRF content is markedly higher during proestrus and oestrus in the morning than in the afternoon, but not significantly different during dioestrus. Plasma corticosterone levels follow a circadian rhythm, although during prooestrus and oestrus the concentrations of corticosterone are markedly elevated compared with the other stages of the cycle [19]. These observations are consistent with the present study where a significant decrease in message levels, and thus dehydrogenase activity, was observed in kidney and uterus between dioestrus and oestrus; such a decrease would tend to result in relatively higher local levels of active glucocorticoids in these tissues at oestrus. Furthermore, in the liver, where reductase activity predominates, 11βHSD1 mRNA levels are elevated during prooestrus. Tissue specific modulation of 11βHSD1 mRNA levels may thus be maintaining the elevated levels of corticosterone observed at this stage of the cycle.

There is considerable evidence for a role of glucocorticoids in the reproductive cycle. In rats glucocorticoids have been shown to inhibit the LH response to stimulation by gonadotrophin-releasing hormone [21], to compromise the positive feedback effects of oestrogen [22] and to modulate the ovulation rate [23, 24]. In humans cortisol has been shown to inhibit the steroidogenic effect of LH in the granulosa-lutein cells of some patients [25]. In the present study there was an apparent fall in ovarian 11βHSD1 mRNA levels at metoestrus, suggesting that variations in 11βHSD1 activity may play a regulatory role in ovarian steroidogenesis and ovulation. The well established association of adrenal hyperactivity with ovarian dysfunction indicates that glucocorticoids play an important role in ovulation. More recent studies have also provided evidence for a role for 11βHSD in ovulation. Immunohistochemical and *in situ* hybridization studies performed on the rat ovary have localized the 11βHSD1 enzyme to oocytes and luteal bodies [26], while 11βHSD activity has been shown to be a potential prognostic indicator of success in the *in vitro* fertilization embryo transfer technique [27].

In conclusion we have observed tissue-specific changes in the levels of 11βHSD1 mRNA over the oestrous cycle. The changes in the uterus and kidney correlate with plasma oestrogen levels. These results suggest that glucocorticoid action may be modulated during the oestrous cycle in a tissue-specific manner.

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