RAPID COMMUNICATION

Chronic maternal stress inhibits the capacity to up-regulate placental 11β-hydroxysteroid dehydrogenase type 2 activity

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Abstract

This study investigated the effects of acute and chronic restraint stress during the third week of pregnancy on placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity in rats. Acute exposure to stress on gestational day 20 immediately up-regulated placental 11β-HSD2 activity by 160%, while chronic stress from day 14 to day 19 of pregnancy did not significantly alter basal 11β-HSD2 activity. However, the latter reduced the capacity to up-regulate placental 11β-HSD2 activity in the face of an acute stressor by 90%. Thus, immediate up-regulation of 11β-HSD2, the feto-placental barrier to maternal corticosteroids, may protect the fetus against stress-induced high levels of maternal corticosteroids, but exposure to chronic stress greatly diminishes this protection.

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Introduction

Animal studies of prenatal stress, environmental enrichment and maternal separation have shown that events early in life can alter the set points of the hypothalamic–pituitary–adrenal (HPA) and corticotropin-releasing factor (CRF) systems with permanent behavioural and endocrine consequences (Ladd *et al.* 2000, Welberg & Seckl 2001). The mechanisms underlying these programming effects are still unknown, but exposure to elevated levels of glucocorticoids during a time of rapid brain development may be a major factor (Welberg & Seckl 2001). Indeed, exposing pregnant rats to the synthetic glucocorticoid dexamethasone results in offspring with a hyperactive HPA axis and elevated CRF expression in the amygdala (Welberg *et al.* 2001). In addition, stress-induced elevations in maternal glucocorticoid levels have been shown to underlie at least some of the effects of prenatal stress (Barbazanges *et al.* 1996). However, access of maternal corticosterone to the fetus is regulated, in part, by the high-affinity, high-efficiency type 2 isoform of the placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2), that rapidly converts corticosterone (in rats) and cortisol (in humans) into their inactive metabolites (11-dehydrocorticosterone and cortisone respectively) (Seckl & Meaney 2004). Animal studies have shown that pharmacological inhibition of placental 11β-HSD2 during pregnancy results in offspring with HPA hyperactivity and an anxious phenotype with elevated CRF expression in the amygdala (Welberg *et al.* 2000). Placental 11β-HSD2 activity varies significantly between individuals, in both rats (Benediktsson *et al.* 1993) and humans (Stewart *et al.* 1995). Since pregnant dams produce far more corticosterone than fetuses, moderate decreases in 11β-HSD2 activity would result in relatively large increases in corticosterone reaching the fetal blood stream. Thus, in order to understand the programming effects of endogenous maternal corticosteroids it is crucial to understand the regulation of their access to the fetus.

Few studies have addressed the *in vivo* regulation of placental 11β-HSD2, but data from recent *in vitro* studies suggest that factors associated with stress may play a role: Catecholamines reduced 11β-HSD2 gene transcription via activation of alpha-adrenergic receptors (Sarkar *et al.* 2001), while the synthetic glucocorticoid dexamethasone increased 11β-HSD2 activity and gene transcription (van Beek *et al.* 2004) in cultured human trophoblasts. The present study aimed to investigate the effects of acute and chronic stress during the third week of pregnancy on placental 11β-HSD2 activity in rats.

Materials and Methods

Animals

Experiments were performed in accordance with NIH Guidelines for the care and use of laboratory animals and

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all protocols were approved by the Emory University Institutional Animal Care and Use Committee. Timed-pregnant Long–Evans rats (Charles River Laboratories, Inc, Wilmington, MA, USA) arrived in our facilities on the morning of day 13 of pregnancy (day 1 being the day after mating occurred). All animals used in Experiment 1 (see below) arrived in the same transport, as did all animals used in Experiment 2. Rats were housed in standard (W × L × H: 20 × 32 × 15 cm) cages with corn-cob bedding. Food and water were available *ad libitum*, and animals were maintained on a 12:12 light:dark cycle (lights on 0700 h).

Experimental protocol

Experiment 1 was performed to establish whether acute or chronic stress affected placental 11β-HSD2 activity. Pregnant rats were randomly assigned to one of three treatment groups: (1) rats in the chronic-stress group (CS, $n=7$) were restrained in flat-bottom rodent restrainers (approximately 8 × 25 cm) for 45 min on day 14 of pregnancy, and then twice daily for 30 or 45 min, once in the morning and once in the afternoon, until day 19 of pregnancy. On day 20 of pregnancy CS rats were deeply anesthetized with isoflurane, immediately after which caesarean section was performed; (2) rats in the acute-stress group (AS, $n=6$) were left undisturbed until day 20 of pregnancy. On that day, AS rats were restrained for 45 min and immediately afterwards anesthetized with isoflurane and subjected to caesarean surgery; (3) a control group of unstressed rats (NS, $n=7$) was left undisturbed until the morning of day 20 of pregnancy, when NS rats were also anesthetized and subjected to caesarean section.

Experiment 2, carried out separately from Experiment 1, was performed to establish whether chronic stress exposure altered placental 11β-HSD2 activity in response to an acute stressor. Here, pregnant rats were weighed and then assigned to one of two groups: (1) rats in the chronic + acute stress group (CAS, $n=6$) were restrained from day 14 of pregnancy onwards as the CS rats in Experiment 1 described above, but underwent a final, acute, 45 min restraint session on day 20 of pregnancy immediately prior to anaesthesia, weighing and caesarean section; (2) a control group of unstressed rats (NS, $n=6$) was left undisturbed until the morning of day 20 of pregnancy when they also underwent anaesthesia, weighing and caesarean section.

Sample collection In both experiments, dams were sacrificed on day 20 of pregnancy between 0900 and 1100 h. Once rats were anesthetized, the abdominal cavity was opened, the uterus exposed and two (Experiment 1) or four (Experiment 2) feto-placental units were quickly dissected. Each feto-placental unit was weighed (Experiment 2 only), after which fetus and placenta were separated. The placenta was then rapidly frozen on powdered dry ice and stored at −70 °C until further processing.

Tissue Processing To determine the effect of maternal stress on placental 11β-HSD2 activity, in both Experiment 1 and 2 two placentas per pregnancy were homogenized together, after which enzyme activity was measured as described below, yielding one value per pregnancy. To determine the correlation between 11β-HSD2 activity and feto-placental weight, the additional two placentas per pregnancy obtained in Experiment 2 were weighed and then processed individually for enzyme activity measurements. Placentas were homogenized using a Powergen Model 125 homogenizer (Fisher Scientific, Atlanta, GA, USA) in 1 ml buffer (1 × PBS containing 0·25 M sucrose) per placenta. Protein concentrations of the samples were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

11β-HSD2 assay Enzyme activity was estimated using a radiometric conversion assay according to protocols described previously (Benediktsson *et al.* 1993), with small variations. Briefly, 1 mg/ml protein of placental homogenate was incubated with 500 nM NAD and 12 nM of [1,2,6,7] ^{3}H-corticosterone (specific activity, 75·6 Ci/mmol) in a final volume of 500 μl 1X PBS containing 0·25 M sucrose at 37 ° C for 30 min. The reaction was terminated by the addition of 2 ml ethyl acetate (Fisher Scientific). Steroids were extracted using ethyl acetate and separated by means of thin-layer chromatography using chloroform-ethanol (92:8) as solvent (Ethanol: Fisher Scientific). Spots corresponding to corticosterone and 11-dehydrocorticosterone were visualized under ultra violet light, cut out, transferred to vials containing liquid scintillant, and their radioactivity was measured in a beta-counter (1209 Rackbeta; LKB/Wallac/Perkin Elmer, Boston, MA, USA). All samples were assayed in duplicate. Blank controls were included in all assays, as well as samples assayed as described above but with addition of 40 μM carbenoxolone, an inhibitor of 11β-HSD. Activity of 11β-HSD2 in each sample was estimated by calculating the fractions of ^{3}H-dehydrocorticosterone and ^{3}H-corticosterone (NEN/Perkin Elmer). Unless otherwise specified, all reagents obtained from Sigma.

Data analysis Conversion levels are expressed as a percentage of (unstressed) control values. Data from Experiment 1 were analyzed using one-way ANOVA. When the overall response was significant, *post-hoc* comparisons were performed using the Tukey HSD test. Data from Experiment 2 were analyzed using unpaired *t*-tests. Significance was set at $P<0·05$.

Results

Experiment 1

Analysis of variance on conversion levels revealed a significant effect of maternal stress on placental 11β-HSD2

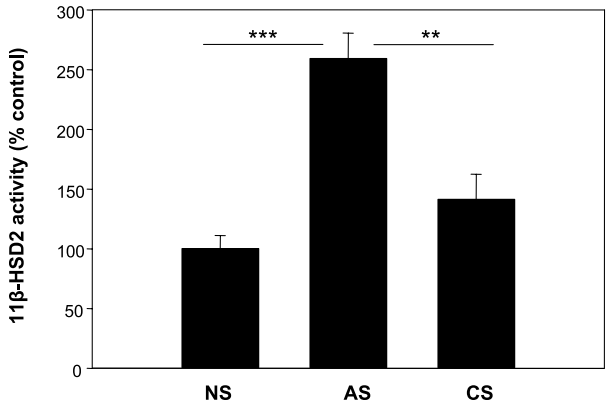

Figure 1 Effect of acute or chronic exposure to stress during pregnancy on placental 11β-HSD2 activity. Stress took place between days 14 and 20 of pregnancy; feto-placental units were harvested on day 20 of gestation. NS=no stress (n=7); AS=acute stress (n=6); CS=chronic stress (n=7); **P<0·001; ***P<0·0005

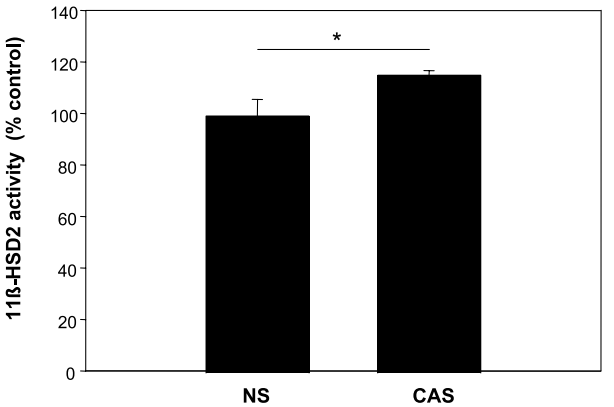

Figure 2 Effect of exposure to combined chronic and acute exposure to stress during pregnancy on placental 11β-HSD2 activity. Stress took place between days 14 and 20 of pregnancy; feto-placental units were harvested on day 20 of gestation. NS=no stress (n=6); CAS=chronic+acute stress (n=6); *P<0·05

activity (F=19·57, P<0·0001). *Post-hoc* analysis showed that acute stress increased 11β-HSD2 activity by 160% compared with activity in unstressed pregnant rats (P<0·0005), and by 84% compared with activity in chronically-stressed pregnant rats (P<0·001). Chronic stress did not significantly affect placental 11β-HSD2 activity (P=0·26) 16 h after the last exposure to stress (Figure 1).

Experiment 2

As shown in Table 1, NS and CAS dams had similar body weights on day 13 and day 20 of pregnancy, and their percentage weight gain was not significantly different. CAS reduced weights of whole feto-placental units on gestational day 20, but not placental weights (Table 1).

A t-test showed that combined chronic and acute stress increased placental 11β-HSD2 activity by 16% compared with activity in placentas from unstressed pregnancies (t=2·33, P<0·05) (Figure 2).

Correlations between placental 11β-HSD2 activity and weights of the feto-placental units as measured on gestational day 20 are shown in Table 2. Enzyme activity correlated negatively with feto-placental weight (Figure 3) and with frozen placental weight, although only the

Table 1 Body weight gain and feto-placental weights in unstressed dams (NS, n=6) and in dams exposed to chronic + acute stress (CAS, n=6). Stress took place between days 14 and 20 of pregnancy, feto-placental units of NS (n=23) and CAS (n=24) pregnancies were harvested on day 20 of pregnancy. Frozen placental weights were also measured in NS (n=12) and CAS (n=12) pregnancies

	NS	CAS	*P*-value
Body weight GD13 (g)	260±8	275±7	0·17
Body weight GD20 (g)	298±10	319±8	0·16
Weight gain (% start weight)	17·3±0·9	16·0±1·4	0·45
Feto-placental weight (g)	2·73±0·04	2·62±0·03	0·03*
Placental weight (mg)	428±16	431±19	0·90

NB: One feto-placental unit in the NS group was not weighted. *denotes a significant difference. GD; Gestational day.

Table 2 Correlations (R-values) between placental 11β-HSD2 activity and weights of feto-placental units. Stress took place between days 14 and 20 of pregnancy; feto-placental units were harvested on day 20 of gestation

	Feto-placental weight	Placental weight
All units (n=24)	−0·47*	−0·31
Unstressed (NS, n=12)	−0·31	−0·31
Stressed (CAS, n=12)	−0·61*	−0·57

*correlation significant at P<0·05.

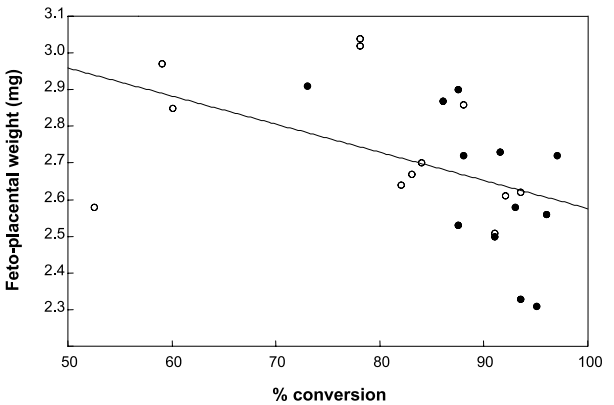

Figure 3 Correlation between feto-placental weight and placental 11β-HSD2 activity. Feto-placental units were harvested on gestational day 20 from unstressed (white circles, n=12) and CAS pregnancies (black circles, n=12). R=-0·46, P=0·02.

former reached statistical significance. These correlations disappeared when only values from unstressed pregnancies were taken into account, but became stronger when considering only values from stressed pregnancies (Table 2).

Discussion

This is, to our knowledge, the first study that assessed placental 11β-HSD2 activity in response to maternal stress. Placental 11β-HSD2 is thought to act as a barrier against maternal glucocorticoids and thus to protect the fetus from potential harmful effects that the elevated levels of these steroids can exert during development (Seckl & Meaney 2004). The finding that placental 11β-HSD2 activity increased in response to an acute stressor is important since it shows that plasma corticosteroids produced by the dam in response to a single stressor are not necessarily harmful to the fetus, provided that enzyme activity is high enough to 'inactivate' them. Importantly, however, the present study also showed that the capacity to adapt placental 11β-HSD2 activity in response to an acute stressor was greatly reduced by previous exposure to chronic stress.

In the present study CAS reduced feto-placental weight on day 20 of gestation. Intrauterine growth retardation is a common finding in prenatal stress studies (Patin *et al.* 2002, Lesage *et al.* 2004), as well as after administration of synthetic glucocorticoids during pregnancy (Welberg *et al.* 2001). This indicates that stress-induced glucocorticoids indeed reached the fetus in this study. Moreover, feto-placental weight correlated negatively with 11β-HSD2 activity, especially in the CAS group, suggesting that the units with the largest growth retardation were exposed to the highest levels of maternal corticosterone and up-regulated their placental 11β-HSD2 activity in an attempt to prevent more corticosterone entering the fetal blood.

CAS-induced reduction in feto-placental weight (around 100 mg) cannot be accounted for by the parallel reduction in placental weight, as NS and CAS placentas differed only by about 3 mg. Thus, although fetal weights were not measured, it is likely that CAS reduced both placental and fetal weights while slightly increasing placental 11β-HSD2 activity.

The combination of increased placental 11β-HSD2 activity and decreased *in utero* growth appears to partly contradict an earlier report in which placental 11β-HSD2 activity correlated positively with birth weight and negatively with placental weight (Benediktsson *et al.* 1993), but several explanations are possible. In the present study, the correlation between feto-placental weight and enzyme activity is not necessarily a causal one, as the increased placental 11β-HSD2 activity in CAS pregnancies probably resulted from both the acute stress exposure immediately before harvesting and previous exposures, while the placental (and likely, fetal) growth retardation in CAS was due to the chronicity of the stressor. In contrast, in Benediktsson *et al.* (1993), fetuses and placentas were taken from unstressed pregnancies, thus linking 'basal' placental 11β-HSD2 activity with birth weight. This correlation probably does describe a causal relationship, as artificially inhibiting placental 11β-HSD2 activity without stressing the pregnant dams also reduced intrauterine growth (Welberg *et al.* 2000), as did chronic exposure during pregnancy to synthetic glucocorticoids, which are not metabolized by 11β-HSD2 (Welberg *et al.* 2001).

Another important difference between the present study and that by Benediktsson *et al.* (1993) is that the latter measured body weight at term, whereas here, feto-placental weights were recorded on day 20 of gestation, three days before expected delivery. Crucially, both expression and activity of placental 11β-HSD2 drop dramatically between gestational days 20 and 22 (Burton *et al.* 1996, Waddell *et al.* 1998) in the labyrinth zone of the placenta, the site of maternal–fetal transfer, and this will likely change existing correlations between weight and enzyme activity on those days. Taken together, it is likely that in the present study CAS-induced maternal corticosterone reached the fetal blood stream *in spite of* increased enzyme activity.

An alternative explanation for our finding that chronic stress reduced the capacity to respond the acute stress with an up-regulation of placental 11β-HSD2 activity may be that repetition of the restraint procedure caused it no longer to be perceived as stressful. This interpretation would be supported by the fact that CAS dams did not show reduced weight gain during pregnancy, in contrast to previous findings (Darnaudery *et al.* 2004). Since maternal plasma corticosterone was not measured, it is impossible to verify the stressfulness of repeated exposure to restraint in this study. However, other studies have shown that repeated restraint during pregnancy reliably elevated maternal plasma corticosterone levels (Ward & Weisz 1984, Barbazanges *et al.* 1996, Weinstock 2005) and repeated restraint has been used many times as a prenatal stressor with long-term effects on the offspring (Barbazanges *et al.* 1996, Lesage *et al.* 2004). In addition, as mentioned before, feto-placental weights from CAS pregnancies were smaller than those from unstressed pregnancies, confirming the common finding of fetal growth retardation in prenatal stress paradigms (Patin *et al.* 2002, Lesage *et al.* 2004). It is important to note that dams used in the present study arrived in our facilities on day 12 of pregnancy, and an effect of the stress of the transportation on basal placental 11β-HSD2 activity or its response to chronic or acute stress cannot be ruled out.

Although no other studies have investigated regulation of placental 11β-HSD2 by stress *per se*, it has been shown that its gene expression in human trophoblast cells is rapidly inhibited by catecholamines via activation of alpha-adrenergic receptors (Sarkar *et al.* 2001). Moreover, glucocorticoids can both up- and downregulate placental

11β-HSD2 mRNA expression, depending on species, timing and mode of administration (Kerzner *et al.* 2002, Ma *et al.* 2003, van Beek *et al.* 2004). Another study reported a reduction in ovine placental 11β-HSD2 activity in response to chronically elevated glucocorticoid levels (Clarke *et al.* 2002). Importantly, none of the above studies investigated *immediate* regulation of placental 11β-HSD2 *activity* in response to glucocorticoids, although this presumably would be the most efficient way to inactivate stress-induced corticosteroids from maternal blood.

A recent investigation showed that activity of the renal 11β-HSD2 enzyme, which is identical to that in the placenta, is up-regulated within at least two hours (the earliest time point studied) by corticosterone injections, but also by stress in adrenalectomized rats (Zallocchi *et al.* 2004). This indicates that 11β-HSD2 activity can be regulated by adrenal steroids as well as by extra-adrenal factors, although the exact mechanism remains to be determined. Thus, the capacity of placental 11β-HSD2 activity to rapidly increase in response to stress in combination with an attenuated maternal HPA reactivity during pregnancy (Neumann *et al.* 1998) may function to control access of corticosteroids to the fetal blood stream, ensuring the proper level of glucocorticoids necessary for normal growth and maturation.

In conclusion, this study showed that immediate up-regulation of 11β-HSD2, the feto-placental barrier to maternal corticosteroids, may protect the fetus against stress-induced elevations of maternal corticosteroids, but exposure to chronic stress greatly diminishes this protection.

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References

Barbazanges A, Piazza PV, Le Moal M & Maccari S 1996 Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *Journal of Neuroscience* **16** 3943–3949.

van Beek JP, Guan H, Julan L & Yang K 2004 Glucocorticoids stimulate the expression of 11 beta-hydroxysteroid dehydrogenase type 2 in cultured human placental trophoblast cells. *Journal of Clinical Endocrinology and Metabolism* **89** 5614–5621.

Benediktsson R, Lindsay RS, Noble J, Seckl JR & Edwards CR 1993 Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet* **341** 339–341.

Burton PJ, Smith RE, Krozowski ZS & Waddell BJ 1996 Zonal distribution of 11 beta-hydroxysteroid dehydrogenase types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy. *Biology of Reproduction* **55** 1023–1028.

Clarke KA, Ward JW, Forhead AJ, Giussani DA & Fowden AL 2002 Regulation of 11 beta-hydroxysteroid dehydrogenase type 2 activity in ovine placenta by fetal cortisol. *Journal of Endocrinology* **172** 527–534.

Darnaudery M, Dutriez I, Viltart O, Morley-Fletcher S & Maccari S 2004 Stress during gestation induces lasting effects on emotional reactivity of the dam rat. *Behavioural Brain Research* **153** 211–216.

Kerzner LS, Stonestreet BS, Wu KY, Sadowska G & Malee MP 2002 Antenatal dexamethasone: effect on ovine placental 11 beta-hydroxysteroid dehydrogenase type 2 expression and fetal growth. *Pediatric Research* **52** 706–712.

Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ & Plotsky PM 2000 Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Progress in Brain Research* **122** 81–103.

Lesage J, Del-Favero F, Leonhardt M, Louvart H, Maccari S, Vieau D & Darnaudery M 2004 Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. *Journal of Endocrinology* **181** 291–296.

Ma XH, Wu WX & Nathanielsz PW 2003 Gestation-related and betamethasone-induced changes in 11 beta-hydroxysteroid dehydrogenase types 1 and 2 in the baboon placenta. *American Journal of Obstetrics and Gynecology* **188** 13–21.

Neumann ID, Johnstone HA, Hatzinger M, Liebsch G, Shipston M, Russell JA, Landgraf R, Douglas AJ 1998 Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. *Journal of Physiology* **508** 289–300.

Patin V, Lordi B, Vincent A, Thoumas JL, Vaudry H & Caston J 2002 Effects of prenatal stress on maternal behavior in the rat. *Brain Research. Developmental Brain Research* **139** 1–8.

Sarkar S, Tsai SW, Nguyen TT, Plevyak M, Padbury JF & Rubin LP 2001 Inhibition of placental 11 beta-hydroxysteroid dehydrogenase type 2 by catecholamines via alpha-adrenergic signaling. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **281** R1966–R1974.

Seckl JR & Meaney MJ 2004 Glucocorticoid programming. *Annals of the New York Academy of Sciences* **1032** 63–84.

Stewart PM, Rogerson FM & Mason JI 1995 Type 2 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal adrenal steroidogenesis. *Journal of Clinical Endocrinology and Metabolism* **80** 885–890.

Waddell BJ, Benediktsson R, Brown RW & Seckl JR 1998 Tissue-specific messenger ribonucleic acid expression of 11 beta-hydroxysteroid dehydrogenase types 1 and 2 and the glucocorticoid receptor within rat placenta suggests exquisite local control of glucocorticoid action. *Endocrinology* **139** 1517–1523.

Ward IL & Weisz J 1984 Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. *Endocrinology* **114** 1635–1644.

Weinstock M 2005 The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity* **19** 296–308.

Welberg LAM & Seckl JR 2001 Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology* **13** 113–128.

Welberg LAM, Seckl JR & Holmes MC 2000 Inhibition of 11 beta-hydroxysteroid dehydrogenase, the foeto-placental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. *European Journal of Neuroscience* **12** 1047–1054.

Welberg LAM, Seckl JR & Holmes MC 2001 Prenatal glucocorticoid programming of brain corticosteroid receptors and

corticotrophin- releasing hormone: possible implications for behaviour. *Neuroscience* **104** 71–79.
Zallocchi ML, Matkovic L, Calvo JC & Damasco MC 2004 Adrenal gland involvement in the regulation of renal 11 beta-hydroxysteroid dehydrogenase 2. *Journal of Cellular Biochemistry* **92** 591–602.

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