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REVIEW

Karen M. Moritz · Wee Ming Boon · E. Marelyn Wintour

Glucocorticoid programming of adult disease

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Abstract Fetal exposure to elevated levels of glucocorticoids can occur naturally when maternal glucocorticoids are elevated in times of stress or when exogenous glucocorticoids are administered. Epidemiological studies and animal models have shown that, whereas short-term benefits may be associated with fetal glucocorticoid exposure, long-term deleterious effects may arise. This review compares the effects of exposure to natural versus synthetic glucocorticoids and considers the ways in which the timing of the exposure and the sex of the fetus may influence outcomes. Some of the long-term effects of glucocorticoid exposure may be explained by epigenetic mechanisms.

Keywords Glucocorticoids (synthetic/natural) · Fetal exposure · Fetal sex · Short-term benefits · Long-term problems · Epigenetic mechanisms · Disease

Introduction

Maternal exposure to high circulating concentrations of glucocorticoids has been shown in numerous epidemiological studies and animal models to alter normal development in the fetus and to result in altered function or disease in the offspring. Glucocorticoid administration is probably the second most widely used model of in utero programming of adult disease after protein/calorie restriction. Evidence supports elevated levels of maternal (and thus fetal) glucocorticoids being the mechanism through which other models, such as maternal calorie/protein restriction, mediate their effects, at least in some species. Investigators have used natural or synthetic glucocorticoids for discrete periods during development to mimic effects of maternal stress. In addition, research has also focused on the effects of glucocorticoid administration to women threatening preterm delivery. This practice has been in clinical use for over 30 years and has been one of the major interventions leading to improved outcome for premature babies. However, until recently, possible deleterious effects have not been examined.

Many recent reviews have been concerned with the effects of perinatal steroid treatment on immediate and long-term consequences for the fetus (Andrews et al. 2004; Fowden and Forhead 2004; Jobe and Soll 2004; Seckl 1997; Weaver et al. 2004a). In this review, we will focus on a few areas that have not been extensively covered previously. These include (1) the diversity of responses observed between exposures to natural versus synthetic glucocorticoids and some possible explanations, (2) the importance of the timing of the glucocorticoid exposure on long-term outcome, particularly with reference to the state of maturation of various organ/systems, such as the kidney, (3) potential mechanisms whereby the effects may differ depending on the sex of the fetus and (4) epigenetic mechanisms that may explain long-term effects of short-term exposure to glucocorticioids in a "sensitive" period. This may give valuable insights into programming outcomes for offspring born to mothers who were "stressed" and thus exposed to naturally occurring glucocorticoids during particular stages of pregnancy compared to those exposed to synthetic glucocorticoids for medicinal purposes.

Possible means of fetal exposure to excess glucocorticoid in utero during long gestation in mammals

Fetuses may be exposed to excess glucocorticoid when the mother is stressed (Andrews et al. 2004; Barbazanges et al. 1996; Kanitz et al. 2003; Laplante et al. 2004; Lesage et al.

K. M. Moritz (✉)
Department of Anatomy and Cell Biology,
Monash University,
3800 Clayton, Australia
e-mail: karen.moritz@med.monash.edu.au
Tel.: +61-3-99052761
Fax: +61-3-99052766

W. M. Boon · E. M. Wintour
Department of Physiology,
Monash University,
3800 Clayton, Australia

2004; Weinstock 1997), when placental concentrations of the protective enzyme, 11βhydroxysteroid dehydrogenase 2 (11βHSD2), are reduced (Seckl 1997) or when exogenous steroids are administered to the mother, as in asthma (Clifton and Murphy 2004). In the rat, substantial evidence has been obtained showing that protein undernutrition of the mother increases maternal adrenal glucocorticoid secretion (Lesage et al. 2002) and that the "programming" effects of low protein are attributable to fetal exposure to such steroids (Langley-Evans 1997; McMullen and Langley-Evans 2005). Such increases in maternal plasma glucocorticoids do not appear to occur in undernourished sheep (Bispham et al. 2003), although perinatal undernutrition can cause premature maturation of the fetal hypothalamic-pituitary-adrenal (HPA) axis (Bloomfield et al. 2004) and be associated with premature parturition (Bloomfield et al. 2003). Placental 11βHSD2 concentrations are decreased in a variety of circumstances, some of which are associated with low birth weight in the human (Kajantie et al. 2003; Murphy et al. 2002). Pre-eclampsia and hypoxia are also associated with decreased levels of this enzyme in the placenta (Alfaidy et al. 2002). The placental production of 11βHSD2 is also known to be decreased by angiotensin II (Lanz et al. 2003) and alcohol (Wilcoxon et al. 2003). Rats made diabetic by streptozotocin treatment and then allowed to become pregnant produce low-birth-weight offspring. The placental and fetal kidney levels of 11βHSD2 mRNA are significantly decreased (Fujisawa et al. 2004). As shown in Table 1, the effects can differ depending on the sex of the fetus, reflecting the fact that placental gene expression, of glucocorticoid receptor (GR) and 11βHSD2, varies depending on the sex of the fetus (Clifton and Murphy 2004).

Evidence that synthetic and natural glucocorticoids, when administered to the pregnant mother, can have differing effects on the fetus

A comparison of the effects of natural and synthetic glucocorticoids is summarized in Table 1. Natural and synthetic glucocorticoids, administered to the mother, do not necessarily have the same effect, either short- or long-term, on the offspring. Betamethasone, administered to the pregnant rat in the last week of pregnancy, does not result in hypertensive adult offspring (McDonald et al. 2003), whereas dexamethasone does (Levitt et al. 1996). Cortisol (not the natural glucocorticoid for the rat) given throughout pregnancy to the maternal rat does not cause hypertension in the adult offspring, whereas dexamethasone does (Celsi et al. 1998). Similarly, dexamethasone, but not cortisol acetate, causes an increase in 5α-reductase activity in the pre-optic area of the hypothalamus in 10-day-old male rat pups (Reznikov et al. 2004). Maternally administered betamethasone causes fetal lung maturation but decreases fetal body growth in lambs, whereas maternally administered cortisol has no effect on either parameter (Jobe et al. 2003). Treatment of pregnant sheep at mid-gestation with betamethasone is reported to produce hypertension in 6-month-old lambs (Figueroa et al. 2004), whereas treatment with dexamethasone does not (Dodic et al. 1998).

The long-term effects of early prenatal cortisol/dexamethasone treatment in sheep also show such differences. Both produce hypertension in the adult sheep offspring (Dodic et al. 1998, 2002b) and both produce a decrease in nephron number after nephrogenesis is completed (Wintour et al. 2003a,b; Moritz et al. 2004). However, as shown in

Table 1 Comparison of the effects of maternal glucocorticoid (*GC*) exposure on the blood pressure (*BP*) of offspring in various species (*PP* postpartum, *MAP* mean arterial pressure, *NC* no change)

Species (reference)	Treatment	Time of treatment	Time of analysis	Blood pressure of offspring
Rat (McMullen and Langley-Evans 2005)	Protein restriction ± metyrapone	1–14 days pregnancy (for drug) 1–22 for diet	4 weeks PP	Increase in systolic BP male, female; dependent on GC-male only
Rat (Langley-Evans 1997)	Protein restriction ± metyrapone	Low protein (1–22) ± metyrapone (1–14)	7 weeks PP	Increase in systolic BP both sexes GC-dependent in both sexes
Rat (Ortiz et al. 2003)	Dexamethasone	Days 15,16	3 weeks PP	Increase in systolic BP female, not male
	Dexamethasone	Days 13–14, 15–16, 17–18	6 months	Increase in systolic BP male, not female
Rat (Woods et al. 2004)	Modest protein restriction	All pregnancy	22 weeks PP	Hypertension male only
	Severe protein restriction	All pregnancy		Hypertension both sexes
Sheep (Dodic et al. 2002a)	Dexamethasone	26–28 days	16–24 months	Hypertension in both male and female
Sheep (Dodic et al. 2002b)	Cortisol	26–28 days	18 months	Hypertension in both male and female
Guinea pig (Liu et al. 2001)	Dexamethasone	40–41, 50–51, 60–61 days	9–10 weeks	No change in MAP in either sex; reduced basal and activated HPA axis in males only
Guinea pig (Banjanin et al. 2004)	Dexamethasone	40–41, 50–51, 60–61 days	21–22 weeks	Increase in MAP in males only; NC in basal HPA axis in males

Programming of the kidney by glucocorticoids

Gestation	Dex	Cortisol
End	↓Nephrons	↓Nephrons
Mid	↓ AT1	↓↓↓ AT1,AT2,
Late	↑↑AT1, AT2, A'ogen	↑↑AT1,AT2, ↑↑↑A'ogen
Adults	Hypertension due to ↑CO	Hypertension due to ↑PR

Fig. 1 Summary of changes in the fetal kidney at various ages after early prenatal exposure to glucocorticoids (*Dex* dexamethasone, *Cortisol* cortisol) between days 26–28 of gestation (*AT1* angiotensin type 1 receptor, *AT2* angiotensin type 2 receptor, *A'ogen* angiotensinogen, *CO* cardiac output, *PR* peripheral resistance) in the sheep (term = 150 days)

Fig. 1, the hypertension in the case of prenatal dexamethasone exposure is dependent on increased cardiac output (Dodic et al. 1999), whereas that attributable to cortisol is dependent upon increased peripheral resistance (McAlinden et al. 2004). In addition, the long-term changes in gene expression in various sections of the brain also differ (Fig. 2). In late gestation, 100 days after fetal exposure to maternally administered dexamamethasone, hypothalamic angiotensinogen gene expression rises significantly and angiotensin type 1 receptor (AT1) expression increases in the medulla oblongata (Dodic et al. 2002a). These changes have functional consequences in adult male sheep, which show increased pressure sensitivity to intracerebroventricular (ICV) infusion of low doses of angiotensin II (unpublished results). No changes have been observed in the expression of

Programming of the brain by glucocorticoids

	DEX	CORTISOL
Medulla Oblongata	AT1↑	AT1→
Hypothalamus	A'ogen ↑↑↑	A'ogen →
Hippocampus	AT1, A'ogen, GR, MR →	AT1,A'ogen,GR,MR ↑↑
Response to ICV ang II	Increased	Unchanged

Fig. 2 Summary of changes in the fetal brain at 130 days of gestation after early prenatal exposure to glucocorticoids (*DEX* dexamethasone, *CORTISOL* cortisol) between days 26–28 of gestation (*AT1* angiotensin type 1 receptor, *MR* mineralocorticoid receptor, *GR* glucocorticoid receptor, *A'ogen* angiotensinogen, *ICV ang II* intracerebroventricular angiotensin II infusion) in the sheep (term = 150 days)

angiotensinogen, ATI, mineralocorticoid receptor (MR) or GR in the hippocampus (Dodic et al. 2002a). In contrast, prenatal exposure to cortisol produces no change in the hypothalamic or medullary expression of these genes, with no increased sensitivity to ICV angiotensin II, but does upregulate all of the genes in the hippocampus of the late gestation fetus (Dodic et al. 2002b). One can only speculate as to whether this also causes altered growth/maturation/function in the adult hippocampus (Montaron et al. 2003). In guinea pigs treated late in gestation with dexamethasone, the (older) adult male offspring have an increased hippocampus to brain weight, increased MR in the dentate gyrus and increased blood pressure (Banjanin et al. 2004). Aldosterone ICV infusion causes hypertension in some rat models, but not in sheep (Gomez Sanchez 1991; Tresham et al. 1990). However, some rats are more susceptible than others to ICV aldosterone effects on blood pressure. In particular, chronic central infusion of aldosterone leads to sympathetic hyperactivity and to hypertension in Dahl salt-sensitive but not resistant rats (Huang et al. 2005). The hypertensive offspring of the ewes treated early with cortisol may show a similar response in increased blood pressure and sympathetic activity but this has not yet been tested. There are indications of some sympathetic overactivity in the adult male sheep (McAlinden et al. 2004).

Why are the programming effects of early treatment with dexamethasone and cortisol different with respect to the cardiovascular system and the brain?

1) This may be an effect of differing biological potencies on the GR. Cortisol is inactivated to the 11-keto-compound, cortisone, by the enzyme 11βHSD2. Dexamethasone is a substrate for this enzyme but 11-keto-dexamethasone binds to, and transactivates GR, as efficiently as does dexamethasone (Rebuffat et al. 2004).
2) Dexamethasone may exert effects on the brain and peripheral circulation on a different receptor, e.g. the pregnane-X-receptor (PXR, particularly the isoform PXR2), which does not recognize cortisol to any appreciable extent (Kliewer et al. 2002). PXR shows striking differences in activation profiles across species; the sheep receptor has not been studied yet. Whether PXR is expressed at the time at which the steroid treatment is given (26–28 days out of total pregnancy, 145–150 days) remains unknown. However, P-glycoproteins mdr1a/1b, which are particularly abundant in rat brain microvessels, are known to be activated by PXR liganded with dexamethasone (Bauer et al. 2004; Mei et al. 2004). These proteins are ATP-driven drug export pumps and help form part of the blood-brain barrier to many chemicals/drugs, including dexamethasone. Therefore, the hippocampus, which is behind the blood-brain barrier, might not "see" dexamethasone but would "see" cortisol. Whether a blood-brain barrier exists before the first month of ovine fetal development is debatable and no organized hippocampus exists at that time (Dodic et al.

2002b). However, this may help to explain the differing effects of synthetic and natural glucocorticoids when they are given later in gestation in other studies.

3) Another receptor in the brain that might be activated by cortisol is MR; this receptor can bind dexamethasone but is not transactivated by the synthetic steroid (Rogerson et al. 2003). The hippocampus expresses MR in the sheep (Dodic et al. 2002a); the activation of hippocampal MR may occur with natural glucocorticoids (cortisol in the sheep), because there is no hippocampal expression of the inactivating enzyme, 11βHSD2. Indeed, in the adult sheep and rat, MR is thought to be responsible for mediation of the effects of glucocorticoids on neurogenesis (Montaron et al. 2003; Richards et al. 2003).
4) In the case of the human, at least, there are multiple isoforms of the GR that are thought to account for the differing tissue specificities to different glucocorticoid ligands (Lu and Cidlowski 2004). The existence of multiple isoforms in the sheep remains to be explored.

"Early" versus "late" glucocorticoid exposure in long-gestation mammals

In long-gestation mammals, such as human and sheep, the effect of "stress" or excess glucocorticoid exposure can be shown to depend on the timing of the insult, more than on the type of glucocorticoid (natural or synthetic). Late-gestation (during the last third of pregnancy) exposure to betamethasone, via maternal injection, causes an increase in insulin resistance in the offspring at 3, 6 and 12 months but no hypertension (Moss et al. 2001). Indeed, the blood pressure is lower than normal at 3 months, increasing to normal by 6 months. When dexamethasone has been used as the maternal glucocorticoid treatment, again no change has been seen in mean arterial pressure at 5 months of age (Molnar et al. 2003). However, when small vessels from a skeletal muscle have been examined in vitro, evidence of endothelial dysfunction and vasodilatory compensation has been obtained. Late gestation famine exposure in the "Dutch Hunger Winter" has also been associated with an increased risk of glucose intolerance, insulin resistance and diabetes type 2 (Ravelli et al. 1998). When dexamethasone or cortisol is infused early into the ewe (from 26–28 days of gestation; term is 145–150 days), all offspring are hypertensive from 3–5 months (Dodic et al. 1998, 2002a,b; Roghair et al. 2005). In the case of dexamethasone treatment, the hypertension is associated with an increased cardiac output and no change in peripheral sensitivity to infused angiotensin II (Dodic et al. 1998, 1999; Roghair et al. 2005). The in vitro responsiveness of mesenteric arteries of hypertensive offspring is actually less responsive to angiotensin II, whereas the coronary arteries show increased vasoconstriction to ang II and other second-messenger-dependent vasoconstrictors (Dodic et al. 1998; Roghair et al. 2005). As discussed elsewhere, the "sensitive period" for cardiovascular programming seems to be related to the timing of metanephric kidney development rather than to the stage of pregnancy. Glucocorticoids given to the guinea pig in late gestation are also associated with changes in the regulation of the HPA axis, particularly in young adult males, with hypertension developing only in older adult males (Banjanin et al. 2004; Liu et al. 2001).

Effects of glucocorticoids on the developing kidney

The developing metanephric kidney is particularly susceptible to the effects of elevated glucocorticoids (Moritz et al. 2002). To date, most evidence suggests that exposure of the developing fetus to elevated glucocorticoids causes a reduction in nephron endowment if the exposure occurs early in kidney development. In the rat, maternal injections of dexamethasone on embryonic days 15 and 16 (E15,16) or E17,18 causes a 20%–30% reduction in nephron number when examined in offspring at 2 or 6 months of age (Ortiz et al. 2001, 2003). No effect is observed if the treatment is given earlier (E11,12 or E13,14) or later (E19,20 or E20,21). Extremely high doses of dexamethasone (100 mg/kg) given throughout pregnancy in the rat cause a 40% decrease in nephron number (Celsi et al. 1998), although, in the same study, cortisol (not the natural glucocorticoid in the rat) exposure is reported as having no effect. Nevertheless, none of these studies in the rat has used the optimal methodology for determining glomerular (and thus nephron) number.

In the sheep, maternal dexamethasone treatment at 26–28 days causes a reduction in nephron number of approximately 40% in offspring at 7 years of age (Wintour et al. 2003b). Surprisingly, in these animals, no significant glomerulosclerosis occurs, although evidence of interstitial fibrosis has been found in some animals (Wintour et al. 2003b). More recently, we have shown that both dexamethasone and cortisol treatment (between 26 and 28 days) causes a reduction in nephron endowment at 140 days of gestation (Moritz et al. 2004), a time at which nephrogenesis is complete but prior to the development of hypertension in this model (Moritz et al. 2002). Studies in the sheep have been performed by using unbiased stereology; the results suggest that nephron deficit may be a major contributor to the development of altered cardiovascular function in the adult sheep following early prenatal exposure to glucocorticoids.

In order to understand the way in which the kidney may have been affected by glucocorticoids, we have started to examine gene expression levels of factors important in normal renal development and those that mediate renal function. An intact renin–angiotensin system (RAS) is critical for normal kidney growth and a local RAS is present within the developing ovine kidney from as early as 40 days of gestation (Wintour et al. 1996). Ovine fetuses exposed to glucocortoicoids early in pregnancy have increased expression levels of both AT1 and AT2 at 130 days of gestation, the time of completion of nephrogenesis (Moritz et al. 2002); at 140 days, the angiotensinogen gene is also up-regulated (Wintour et al. 2004). Subsequent in vivo studies have revealed that fetuses exposed to dexamethasone have normal basal renal function late in gestation but,

in response to an infusion of angiotensin II, do not increase glomerular filtration rate or urine flow rate to the same degree as fetuses exposed to saline (Moritz et al. 2002). More recently, we have shown that a decrease in renal expression of AT1 occurs at mid-gestation (75 days) in fetuses exposed to either dexmethasone or cortisol early in pregnancy (Wintour et al. 2004). This finding suggests that the RAS has been inhibited intra-renally during development and that this has contributed to the reduction in nephron endowment. The increase in AT1 mRNA expression seen at 130 days is likely to be a compensatory increase at the completion of nephrogenesis and may well influence future renal function.

In the same sheep model, glucocorticoid exposure has also been found to increase the mRNA expression levels of MRs and GRs at 130 days of gestation (Hantzis et al. 2002). In vivo studies have however revealed that this causes minimal impact on the normal response to infused aldosterone or cortisol (Moritz et al. 2005), thereby highlighting that not all "programming" changes influence function.

Linking the role of epigenetics in the programming effects of glucocorticoids

Some insights into the potential mechanisms by which programming phenomena might occur come from the field of epigentetics. Egger (2004) has defined epigenetics as "all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself". Epigenetics has been implicated in many facets of biology, the most notable being the pathology of diseases such as mental retardation in Rubinstein–Taybi, fragile-X, Prader–Willi and Angelman's syndromes (Ausio et al. 2003; Nicholls et al. 1998; Oostra and Willemsen 2002), various cancers including leukaemia, head and neck carcinomas, endometrial and colon cancers (Kane et al. 1997; Risinger et al. 2003; Rush et al. 2004; Tokumaru et al. 2004) and obesity in Pradi–Willi syndrome (Goldstone 2004). Epigenetic mechanisms also form the basis of some gene activation/repression activities contributing to developmental gene control, such as genetic imprinting (John and Surani 2000; Reik and Walter 1998), and to the silencing of retrotransposons and are involved in the maintenance of chromosome integrity (Bourc'his and Bestor 2004).

Although many epigenetic factors affect the biology of an organism, currently known epigenetic control mechanisms primarily include DNA methylation and histone modification pathways that are interlinked and that serve as landmarks of active and inactive chromatin. DNA methylation is mediated by two main gene sets. The first is DNA-cytosine methyltransferase 1 (Dnmt1), a maintenance gene that restores methylation at previously methylated CpG sites. The second set with known functions contains Dnmt3a and Dnmt3b, which are crucial to the de novo methylation of CpG sites and which are especially important after genome-wide postzygotic demethylation that serves to establish nuclear totipotency during development and the elimination of inherited epigenetic information (Reik et al. 2001). Methylated CpG sites are then open to binding by six different methyl-CpG-binding proteins that serve to recruit transcription factors and chromatin remodelling peptides to repress gene expression (Millar et al. 2002; Prokhortchouk et al. 2001; Wade 2001). Histone modifications form part of the gene repression chain of events. This is achieved by the the assembly of variant histones or post-translational modifications (e.g. methylation, acetylation) to lysine residues at the amino-tail of the histone core (Henikoff et al. 2004) by transcriptional coactivators (histone acetyltransferases) or transcriptional corepressors (histone deacetylases, HDACS; de Ruijter et al. 2003; Hassig and Schreiber 1997). For repression, the HDAC-complex of proteins are recruited to the methylated CpG islands via methyl-CpG binding proteins (de Ruijter et al. 2003; Hassig and Schreiber 1997).

Levels of methylation at the 5′ end of the GR gene promoter in the hippocampus have been shown to be inversely proportional to the extent to which rat pups receive pup licking and grooming and arched-back nursing (LG-ABN) by their mothers (Weaver et al. 2004b). The reduced level of methylation at the GR promoter is correlated with higher GR transcription. The glucocorticoid negative feedback system of the GR then inhibits corticotropin-releasing-factor synthesis, which in turn dampens HPA responses to stress. The offspring of high-LG-ABN mothers therefore show lower levels of HPA activity, which manifests as less fearful pups.

Interestingly, the difference in methylation is not observed at E20 or postnatal day 1 (P1) but is significantly different at P6, P21 and P90, confirming that the nurturing behaviour of the mother at post-natal stages, and not factors during pregnancy, "programs" epigenetic changes in these pups. Cross-fostering of low-LG-ABN pups with high-LG-ABN mothers results in less fearful pups and lower levels of methylation at the GR promoter, again reinforcing the conclusions of Weaver et al. (2004b) that LG-ABN behaviour is the determinant of the epigenetic effects observed. As the effect of methylation is mediated by histone modifications (acetylation or deacetylation), infusion of the histone deacetylase inhibitor to low-LG-ABN pups maintains high levels of histone acetylation at the GR promoter and eliminates the HPA response and behavioural differences from pups with high-LG-ABN. Thus, behavioural programming alters the epigenetic state of a gene over the lifespan of the organism, is reversible via pharmacological means and, as Weaver et al. (2004a,b) have elegantly expressed, "the effects of chromatin structure serve as an intermediate process that imprints dynamic environmental experiences on the fixed genome, resulting in stable alterations in phenotype".

Other workers (Drake et al. 2004) have shown that fetal exposure to 100 μg/kg of the synthetic glucocorticoid, dexamethsone, during the last quarter of pregnancy in rats results in low birth weight, elevated activity of the key hepatic gluconeogenic enzyme phosphoenolpyruvate carboxylase (PEPCK) and subsequent hyperinsulinaemia and hyperglycaemia. The subsequent development of glucose dyshomeostasis is thought to be caused by the permanent

increase in PEPCK. Interestingly, these effects are not limited to the F1 generation of offspring but persisted into the F2 generation without further treatment of the F1 animals. F2 offspring resulting from F1 crosses (from either maternal, paternal or both dexamethasone-treated parents) have the same pathology, suggesting that both the maternal and paternal lines are capable of transmitting the epigenetic effects caused by dexamethasone treatment. The effects of fetal programming were however lost at the F3 generation. The observed intergenerational transmission of epigenetic modifications show that not all epigenetic information is erased during gametogenesis and embryogenesis. The complete erasure of epigenetic information may not necessarily be crucial to the development of totipotency in the gamete.

The epigenetic effects of uteroplacental insufficiency and intrauterine growth retardation (IUGR) have been investigated by performing bilateral uterine artery ligation of pregnant rats at day 19 of pregnancy (MacLennan et al. 2004). This treatment triggers a wide array of effects in the fetus, including genome-wide DNA hypomethylation in the liver, increased acetylation of histone H3, hypoglycaemia, acidosis, hypoinsulinaemia and hypoxia. The mechanistic observations associated with this model of IUGR include increased levels of *S*-adenosylhomocysteine (SAH), homocysteine and methionine and decreased mRNA levels of methionine adenosyltransferase and cystathionine-β-synthase. SAH is currently known to promote DNA hypomethylation and, thus, histone hyperacetylation. The large changes in levels of methylation and acetylation are important to the physiology of the programmed animals, as CpG-rich regions (in which methylation occurs) reside in 60% of gene promoters utilized by RNA polymerase II and the level of histone acetylation alters the positioning of histone–DNA contacts and the affinity with which they bind to DNA.

The other metabolites affected by this model of IUGR and having roles in DNA methylation include *S*-adenosylmethionine (SAM), methionine adenosyltransferase (reduced by hypoxia), cystathionine-β-synthase (CBS) and methylenetetrahydrofolate reductase (both affected by insulin). MacLennan et al. (2004) have speculated that their bilateral uterine artery ligation model is linked to the folate IUGR model, which shows increases in SAH and homocysteine levels and DNA hypomethylation. They propose that bilateral uterine artery ligation may have caused a decrease in folate leading to increased SAH, hypoglycaemia leading to decreased CBS and oxidative stress and hypoxia. As DNA hypomethylation and histone H3 hyperacetylation occur in their model of IUGR, these epigenetic mechanisms have been implicated in the physiological and metabolic changes observed.

Conclusions

Fetal exposure to glucocorticoids, either endogenous or exogenously administered, is likely to have long-term consequences for the health of an individual. The long-term effects will be dependent upon the stage of gestation at which glucocorticoid exposure occurs and the sex of the developing fetus. Whereas genetics play a role in the biology of an organism, the effects of "fetal programming" produced by glucocorticoids, perturbations to nutrient supply or maternal behaviour are clearly mediated by epigenetic mechanisms that are capable of programming the fetus for life.

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