Prenatal glucocorticoids and long-term programming

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Abstract

Epidemiological evidence suggests that low birth weight is associated with an increased risk of cardiovascular, metabolic and neuroendocrine disorders in adult life. Glucocorticoid administration during pregnancy reduces offspring birth weight and alters the maturation of the lung and other organs. We hypothesised that prenatal exposure to excess glucocorticoids or stress might represent a mechanism linking foetal growth with adult pathophysiology. In rats, birth weight is reduced following prenatal exposure to the synthetic steroid dexamethasone, which readily crosses the placenta, or to carbenoxolone, which inhibits 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), the physiological fetoplacental 'barrier' to maternal glucocorticoids. As adults, the offspring exhibit permanent hypertension, hyperglycaemic, increased hypothalamic-pituitary-adrenal (HPA) axis activity and behaviour reminiscent of anxiety. Physiological variations in placental 11β-HSD2 activity correlate directly with foetal weight. In humans, 11β-HSD2 gene mutations cause low birth weight. Moreover, lowbirth-weight babies have higher plasma cortisol levels throughout adult life, indicating HPA axis programming. The molecular mechanisms may reflect permanent changes in the expression of specific transcription factors, key among which is the glucocorticoid receptor (GR) itself. The differential programming of the GR in different tissues reflects effects upon one or more of the multiple tissue-specific alternate first exons/promoters of the GR gene. Overall, the data suggest that both pharmacological and physiological exposure prenatally to excess glucocorticoids programmes cardiovascular, metabolic and neuroendocrine disorders in adult life.

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Introduction

It is now axiomatic that early-life environmental factors influence prenatal development and may cause structural and functional changes which persist for the lifespan. This organisational phenomenon is termed 'early-life programming'. Programming factors include nutrients and hormones. Sex steroid hormones, which are lipophilic and readily cross biological barriers, are powerful mediators of early-life organisational effects. We therefore suggested that similar programming effects might also follow prenatal exposure to other steroid hormones, notably glucocorticoids. Here the evidence for such actions is briefly reviewed.

Programming

The concept of early-life physiological 'programming' or 'imprinting' has been advanced to explain the associations between prenatal environmental events, altered foetal growth and development, and later pathophysiology (1-4). Programming reflects the action of a factor during a sensitive developmental period or 'window' to affect the development and organisation of specific tissues that are concurrently vulnerable, producing effects that persist throughout life. Of course, different cells and tissues are sensitive at different times, so the effects of environmental challenges will have distinct effects, depending not only the challenge involved but also upon its timing.

Programming has been examined in several settings. For hormones, a long and detailed literature has examined the 'pharmacology' of such systems (1). Such studies have employed exposure of pregnant dams or newborns to exogenous agents, including toxins, drugs and hormones, and have then examined the short- and long-term consequences.

One area that has made the transition to physiology has been the phenomenon of perinatal programming by sex steroids. In many vertebrate species, males show a short burst of androgen secretion around the time of birth. This permanently programmes steroid metabolising enzyme expression in the liver, the size, connection and neurochemistry of specific hypothalamic nuclei, and some sexual behaviours (5, 6). Oestrogens also exert organisational effects on the developing central nervous system (CNS) (7). Critically, these effects can be exerted only during specific perinatal U50 J R Seckl



periods, but they then persist throughout life, largely irrespective of any subsequent sex steroid manipulations. The mechanisms reflect sex steroid actions on the growth, maturation and remodelling of organs during critical perinatal periods. For instance in the rat, the sexually dimorphic nucleus of the preoptic hypothalamic area is larger in males. Testosterone inhibits apoptosis specifically between postnatal days 6 and 10 and selectively in this locus, thus producing the male adult phenotype (8). So, might glucocorticoids, used in several antenatal therapeutic settings, also have long-term effects on offspring physiology?

Glucocorticoid programming

Glucocorticoids and birth weight

Glucocorticoid treatment during pregnancy reduces birth weight in animal models, including non-human primates (9-13) and humans (12, 14). Birth weight



reduction is most notable when glucocorticoids are administered in the latter stages of pregnancy (10), presumably reflecting the catabolic actions of these steroids, actions most likely to become manifest as reduced birth weight during the period of maximum foetal somatic growth.

In human pregnancy, glucocorticoids are now used mainly in the management of women at risk of preterm delivery and, much more rarely, in the antenatal treatment of foetuses at risk of congenital adrenal hyperplasia (CAH). In some studies, antenatal glucocorticoids are associated with a reduction in birth weight (12, 14), although normal birth weight has been reported in infants at risk of CAH whose mothers received relatively low-dose dexamethasone *in utero* from the first trimester (15, 16). A recent study of pregnant women with asthma did not find changes in birth weight with use of inhaled and/or episodic oral glucocorticoids. Indeed, lack of glucocorticoid therapy is associated with a reduction in offspring birth weight (17). However, the



steroid dehydrogenase (11β-HSD) activity correlates with birth weight in rodents and, less certainly, in humans. This suggests that relative deficiency of this barrier to maternal glucocorticoids, but allowing active forms to cross to the foetus, correlates with foetal growth restraint. Centre panel: inhibition of 11β-HSD by maternal treatment with carbenoxolone (CBX; filled bar/solid line) reduces birth weight compared with control (open bar/broken line). Right panels: this produces higher blood pressure and plasma glucose levels across an oral glucose tolerance test (fasting and post-prandial) in the adult, 6-month old offspring.

Figure 2 Left panel: placental 11B-hydroxy-



Figure 3 Key targets of glucocorticoid programming include metabolic tissues, such as liver, visceral adipose tissue, skeletal muscle and pancreas, and regions of the brain important in cognition, mood and neuroendocrine control.

effects on placental function of inflammatory mediators in poorly controlled asthma, the predominant topical route of steroid administration and the use of prednisolone, which is rapidly inactivated by placental 11 β -hydroxysteroid dehydrogenase type 2 and poorly accesses the foetal compartment (see below), might underpin these apparently discordant results.

For endogenous glucocorticoids, human foetal blood cortisol levels are increased in intrauterine growth retardation and also in pre-eclampsia, implicating endogenous cortisol in retarded foetal growth (18, 19). Cortisol also affects placental size in experimental animals, the precise effect depending on the dose used and its timing during pregnancy (20).

Glucocorticoids and tissue maturation

Glucocorticoids have potent effects upon tissue development. Indeed, it is the accelerated maturation of organs, notably the lung (21), which underpins their widespread use in obstetric and neonatal practice in threatened or actual preterm delivery.

Underpinning such actions, glucocorticoid receptors (GR), which are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, are expressed in most foetal tissues from early embryonal stages (22, 23). Expression of the closely related, higher affinity mineralocorticoid receptor (MR) has a more limited tissue distribution in development and is present only at later gestational stages, at least in rodents (24). Additionally, GR are highly

expressed in the placenta (25), where they are thought to mediate metabolic and anti-inflammatory effects. Clearly, systems to transduce glucocorticoid effects upon the genome exist from early developmental stages, with complex cell-specific patterns of expression, and presumably sensitivity, to the steroid ligands (23).

Birth weight and foetal programming

Numerous studies, initially in the UK and then worldwide, have revealed an association between lower birth weight and the subsequent development of the common cardiovascular and metabolic disorders of adult life, notably hypertension, insulin resistance, type 2 diabetes and cardiovascular disease deaths (2, 26-34). These early-life events altering birth weight are important predictors of adult morbidity (28, 29, 35). In a study of 22000 American men, those born lighter than 2.2 kg had relative risks of adult hypertension (1.26) and type 2 diabetes (1.75)compared with average birth-weight adults (29). Moreover, the association between birth weight and later cardiometabolic disease appears largely independent of classical lifestyle risk factors (smoking, adult weight, social class, excess alcohol intake and sedentariness), which are additive to the effect of birth weight (2). The low birth weight-adult disease relationships are broadly continuous across birth weights within the normal range (2, 28, 29), although premature babies also have increased cardiovascular risk in adult life (36). Additionally, post-natal catchup growth also appears to be predictive of the risk of adult cardiovascular disease (31, 32, 37, 38), suggesting it is restriction of intrauterine growth which is important. While such effects might reflect classical genetic actions, some work has suggested that the smaller of twins at birth has higher blood pressure in later life (37), although this has not been consistently reported (39). Whatever the limitations of human twin observations, the occurrence of associations between early-life environmental manipulations and later physiology and disease risk in isogenic rodent models strongly implicates environmental factors, at least in part, in aetiology. It is intriguing that as blunt a measure of a disadvantageous intrauterine environment as birth weight has proved to show a relatively robust relationship with later pathophysiology. Nonetheless, it is generally accepted that birth weight and other anthropometric indices are just crude markers; presumably, many insults that may affect offspring biology do not alter gross birth weight. Inevitably, the epidemiological data have spawned a host of mechanistic studies in animal models. Two major environmental hypotheses have been proposed: foetal undernutrition and overexposure of the foetus to glucocorticoids (2-4).

In evidence for the latter possibility, the major systems affected in the 'low-birth-weight baby syndrome' are glucocorticoid-sensitive targets. Notably, the syndrome

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is broadly familiar to endocrinologists since it resembles both the rare Cushing's syndrome of glucocorticoid excess and the common metabolic syndrome continuum of interassociated cardiovascular risk factors (type 2 diabetes/insulin resistance, dyslipidaemia and hypertension). These disorders may be linked by tissue glucocorticoid excess (40). Even the less recognised components of the small baby syndrome, such as osteoporosis (41), are also key features of Cushing's syndrome. Moreover, at least a proportion of these physiological systems are also glucocorticoid sensitive in early life, since cortisol also elevates foetal blood pressure when infused directly *in utero* in sheep (42) and at birth in sheep (43) and humans (44).

Physiology: placental 11β -hydroxysteroid dehydrogenase type 2

All the points above relate to pharmacological glucocorticoid exposures. So, is glucocorticoid overexposure in utero of any possible physiological relevance? While lipophilic steroids easily cross the placenta, foetal glucocorticoid levels are much lower than maternal levels (45, 46). This is thought to be due to 11β -HSD-2, which is highly expressed in the placenta. 11B-HSD-2 is an NAD-dependent 11B-dehvdrogenase which catalyses the rapid conversion of active physiological glucocorticoids (cortisol and corticosterone) to inert 11-keto forms (cortisone and 11-dehydrocorticosterone) (47). In the placenta, 11β -HSD-2 forms a potent (48, 49) barrier to maternal glucocorticoids (Fig. 1), although the barrier is apparently incomplete, as a proportion of maternal glucocorticoid crosses intact to the foetus (50). This 10-20% passage of active maternal glucocorticoid to the foetus perhaps reflects anatomical bypass of the enzyme, which is located in the syncytiocytotrophoblast in human placenta (51) and the labyrinthine zone in rodent placenta (52, 53). Indeed, in rodents the peak of the circadian rhythm of plasma corticosterone penetrates the 11 β -HSD-2 barrier to an appreciable extent (54), presumably adding to the provision of glucocorticoids to the foetus for normal key developmental processes such as maturation of the lung. Dexamethasone is a poor substrate for 11B-HSD-2 and therefore readily passes the placenta (51). Betamethasone is similarly a poor substrate. In contrast, 11β-HSD-2 rapidly inactivates prednisolone to inert prednisone, so this widely used steroid is unlikely to have full impact upon the foetus in vivo.

Placental 11β-HSD-2 and birth weight

Observational studies have related placental 11β -HSD-2 to birth weight. The activity of placental 11β -HSD-2 near term shows considerable interindividual variation in humans and rats (55, 56) (Fig. 2). A relative deficiency of 11β -HSD-2, with consequent reduced placental

inactivation of maternal steroids, has been hypothesised to lead to overexposure of the foetus to glucocorticoids, retard foetal growth and programme responses leading to later disease (3). In support of this idea, lower placental 11 β -HSD-2 activity in rats is associated with the smallest foetuses (55). Similar associations have been reported in humans (17, 56–58), although not all studies have concurred (59, 66). Additionally, markers of foetal exposure to glucocorticoids, such as cord-blood levels of osteocalcin (a glucocorticoid-sensitive osteoblast product that does not cross the placenta), also correlate with placental 11 β -HSD-2 activity (60).

Humans with 11β -HSD-2 deficiency are rarely reported. However, babies homozygous (or compound heterozygous) for deleterious mutations of the 11B-HSD-2 gene have very low birth weight (61), averaging 1.2 kg less than their heterozygote siblings. Although an initial report suggested that 11β-HSD-2-null mice have normal foetal weight in late gestation (62), this appears to have reflected the 'genetic noise' of the crossed $(129 \times MF1)$ strain background of the original 11B-HSD-2-null mouse. Indeed, preliminary data suggest that in congenic mice on the C57Bl/6 strain background 11β-HSD-2 nullizygosity lowers birth weight (63). Additionally, there may also be species differences. Thus, the mouse shows dramatic lategestational loss of placental 11β-HSD-2 gene expression (24), whereas this occurs later in rat gestation (53), and in humans, placental 11β -HSD-2 activity increases throughout gestation (56). Because maternal glucocorticoid levels are much higher than those of the foetus, subtle changes in placental 11β-HSD-2 activity may have profound effects on foetal glucocorticoid exposure (48, 49).

A common mechanism may underlie foetal programming through maternal undernutrition and glucocorticoid exposure. Dietary protein restriction during rat pregnancy selectively attenuates 11 β -HSD-2, but, apparently, not other placental enzymes (64–66). Indeed, in the maternal protein restriction model, offspring hypertension can be prevented by treating the pregnant dam with glucocorticoid synthesis inhibitors, and can be recreated by concurrent administration of corticosterone, at least in female offspring (67).

Glucocorticoid programming effects and mechanisms (Fig. 3)

Glucocorticoid programming of the brain

Maternal and/or foetal stressors alter developmental trajectories of specific brain structures with persistent effects (for reviews, see (68, 69). Glucocorticoids are important for normal maturation in most regions of the developing CNS (70), initiating terminal maturation, and remodelling axons and dendrites, and for cell survival (71). Prenatal glucocorticoid administration retards brain weight at birth in sheep (72),

delaying maturation of neurons, myelination, glia and vasculature (73, 74). Exposure to glucocorticoids *in utero* has widespread acute effects upon neuronal structure and synapse formation (75), and may permanently alter brain structure (76). In rhesus monkeys, treatment with antenatal dexamethasone caused dose-dependent neuronal degeneration of hippocampal neurons and reduced hippocampal volume, effects which persisted at 20 months of age (77). Foetuses receiving multiple lower-dose injections showed more severe damage than those receiving a single large injection. Human and animal studies have demonstrated that altered hippocampal structure may be associated with a number of consequences for memory and behaviour (78-80).

Given such widespread effects of glucocorticoids, it is unsurprising that GR and MR are highly expressed in the developing brain with complex ontogenies to allow selectivity of effects (81, 82). Whether the receptors are occupied by endogenous glucocorticoids until late gestation is less certain, as there is also plentiful 11β-HSD-2 in the CNS at midgestation (24, 83), which presumably 'protects' vulnerable developing cells from premature glucocorticoid action. 11B-HSD-2 expression is dramatically switched off at the end of midgestation in the rat and mouse brain, coinciding with the terminal stage of neurogenesis (24, 84). Similarly, in human foetal brain, 11β -HSD-2 appears to be silenced between gestational weeks 19 and 26 (51, 85). Thus, there appears to be an exquisitely timed system of protection and then exposure of developing brain regions to circulating glucocorticoids.

The hypothalamic-pituitary-adrenal (HPA) axis

The hypothalamic-pituitary-adrenal (HPA) axis, and its key limbic regulator the hippocampus (86), are particularly sensitive to glucocorticoids and their perinatal programming actions (68, 87-89). Prenatal glucocorticoid exposure permanently increases basal plasma corticosterone levels in adult rats (90, 91). This is apparently because the density of both types of corticosteroid receptor, GR and MR, are permanently reduced in the hippocampus, changes anticipated to attenuate HPA axis feedback sensitivity. Maternal undernutrition in rats (92) and sheep (93) also affects adult HPA axis function, suggesting that HPA programming may be a common outcome of prenatal environmental challenge, perhaps acting in part via alterations in placental 11β -HSD-2 activity, which is selectively downregulated by maternal dietary constraint (64, 65). Consequent plasma glucocorticoid excess exacerbates hypertension and hyperglycaemia in such prenaenvironmental programming models tal (67).Moreover, tissue glucocorticoid action is further increased by the documented elevations in hepatic

and visceral adipose tissue glucocorticoid sensitivity (10, 94).

HPA axis programming also illustrates an important variable: it often differs between male and female offspring of the same litter. Sex-specific programming of the HPA axis has been reported for prenatal stress in rats (95, 96). In male guinea pigs, short-term prenatal exposure to dexame has one significantly elevates subsequent basal plasma cortisol levels, whereas similarly exposed females have reduced HPA responses to stress. In contrast, males exposed to longer courses of prenatal glucocorticoids exhibit reduce plasma cortisol levels in adulthood, while females similarly exposed have higher plasma cortisol levels as adults in the follicular and early luteal phases of their oestrus cycles. In primates, offspring of mothers treated with dexamethasone during late pregnancy have elevated basal and stress-stimulated cortisol levels (97).

Programming behaviour

Overexposure to glucocorticoids *in utero* leads to alterations in adult behaviour. Late gestational dexamethasone in rats apparently impairs coping in adverse situations later in life (91). Prenatal glucocorticoid exposure also affects the developing dopaminergic system (98, 99), with implications for understanding the developmental contributions to schizoaffective, attention-deficit hyperactivity and extrapyramidal disorders. Stressful events in the second trimester of human pregnancy are associated with increased incidence of offspring schizophrenia (100). Prenatal exposure to dexamethasone may exert more widespread effects, since it also increases the susceptibility of the cochlea to acoustic noise trauma in adulthood (101).

Behavioural changes in adults exposed prenatally to glucocorticoids may be associated with altered functioning of the amygdala, a structure key to the expression of fear and anxiety. Intra-amygdala administration of corticotrophin-releasing hormone (CRH) is anxiogenic (102). Prenatal glucocorticoid exposure increases adult CRH levels specifically in the central nucleus of the amygdala, a key locus for its effects on fear and anxiety (91, 103). Prenatal stress similarly programmes increased anxiety-related behaviours with elevated CRH in the amygdala (104). Moreover, corticosteroids facilitate CRH mRNA expression in this nucleus (105) and increase GR and/or MR in the amygdala (91, 103). The amygdala stimulates the HPA axis via a CRH signal (106). Therefore, an elevated corticosteroid signal in the amygdala, due to hypercorticosteronaemia in the adult offspring of dexamethasone-treated dams, may produce the increased CRH levels in adulthood. A direct relationship between brain corticosteroid receptor levels and anxiety-like behaviour is supported by the phenotype of transgenic

mice with selective loss of GR gene expression in the brain, which show markedly reduced anxiety (107).

CNS programming mechanisms

In the 'neonatal handling' paradigm (70, 108–109), short (15 min daily) handling of rat pups during the first 2 weeks of life (109) permanently increases hippocampal GR levels. This potentiates the HPA axis sensitivity to glucocorticoid negative feedback and lowers plasma glucocorticoid levels throughout life, a state compatible with good adjustment to environmental stress (110, 111). The model is of physiological relevance, since handling enhances maternal care-related behaviours. Natural variation in such maternal behaviour correlates similarly with the offspring HPA physiology and hippocampal GR expression (112). Handling acts via ascending serotonergic (5HT) pathways from the midbrain raphe nuclei to the hippocampus (113). 5HT induces GR gene expression in foetal hippocampal neurons in vitro (114) and in neonatal (115) and adult hippocampal neurons in vivo (116). The 'handling' induction of 5HT requires thyroid hormones that are elevated by the stimulus in rats and guinea pigs (117). At the hippocampal neuronal membrane, recent findings implicate the ketanserin-sensitive 5HT₇ receptor subtype, which is regulated by glucocorticoids (118) and positively coupled to cAMP generation, in the handling effects (119). In vitro, 5HT stimulation of GR expression in hippocampal neurons occurs via 5HT₇ receptors and is mimicked by cAMP analogues (114, 120, 121). In vivo, handling stimulates hippocampal cAMP generation (122), which induces expression of specific transcription factors, most notably NGFI-A and AP-2 (119). NGFI-A and AP-2 bind to the GR gene promoter (123). This pathway might also be involved in some prenatal programming paradigms affecting the HPA axis, since last-trimester dexamethasone exposure increases 5HT transporter expression in the rat brain (124, 125), an effect predicted to reduce 5HT availability in the hippocampus and elsewhere. Crucial recent data show that NGFI-A binds to the GR promoter, inducing a specific GR transcript (126) (see below).

Cardiovascular and metabolic programming

Blood pressure

Of all the human data, the link between birth parameters and adult blood pressure is perhaps best documented and established. Cortisol infusion into the foetus *in utero* elevates blood pressure in sheep (42). Betamethasone given to pregnant baboons raises blood pressure in the foetus (127). Excess cortisol also directly elevates blood pressure at birth in humans (44) and sheep (43). For programming to occur, such effects need to persist.

Treatment of pregnant rats with dexamethasone reduces birth weight, a deficit reversed by weaning at 21 days of age. However, both male and female adult offspring of dexamethasone-treated pregnancies have elevated blood pressure (55). Similarly, adult hypertension is produced in sheep exposed to excess glucocorticoid in utero, either maternally administered dexamethasone or cortisol (128-132). The timing of glucocorticoid exposure appears to be important; exposure to glucocorticoids during the final week of pregnancy in the rat is sufficient to produce permanent adult hypertension (90, 133), whereas the sensitive window for such effects in sheep is earlier in gestation (134). Such differences may be primarily due to the complex species-specific patterns of expression of GR, MR and the isoenzymes of 11β-HSD (23, 24), which regulate maternal glucocorticoid transfer to the foetus and modulate glucocorticoid action in individual tissues.

Near identically, inhibition of 11β -HSD by treatment of pregnant rats with carbenoxolone causes reduced birth weight along with increased passage of maternal corticosterone to the foetal circulation (135, 136) (Fig. 2). As with dexamethasone, prenatal carbenoxolone-exposed rats develop adult hypertension (135). These effects of carbenoxolone are independent of changes in maternal blood pressure or electrolytes, but do require the presence of maternal glucocorticoids; the offspring of adrenalectomised pregnant rats are protected from carbenoxolone effects upon birth weight or adult physiology (135, 136). It must be noted that carbenoxolone is non-selective and inhibits both 11β-HSD isozymes and related dehydrogenases, and disrupts gap junctions at high concentrations (137). However, 11β -HSD-2 knockout mice also have low birth weight, and preliminary data suggest that null mice show several CNS aspects of the prenatal glucocorticoid 'programming' phenotype. Since the brain expresses little or no 11 β -HSD-2 in adult life (83, 138), the data imply a programming effect (139). Certainly, the developing CNS has high expression of 11β-HSD-2 during critical developmental windows (84).

The mechanisms of glucocorticoid-programmed adult hypertension probably involve a variety of processes. Prenatal glucocorticoid exposure leads to irreversible reductions in nephron number in rodents (140) and sheep (141). Antenatal glucocorticoid exposure also affects foetal and adult vascular responses to vasoconstrictors, enhancing endothelin-induced vasoconstriction and attenuating endothelium-dependent vasorelaxation in sheep (142, 143), indicating microvascular dysfunction. These effects appear to be vascular bed specific (144). Renin-angiotensin system receptor density and tissue synthesis are also affected by antenatal steroid exposure (145), notably in the foetal kidney (146), where angiotensinogen and the AT1 and AT2 receptors are increased after

dexamethasone, accompanied by a reduced glomerular filtration rate response to angiotensin II. Finally, key barocontrol centres in the brainstem are altered by prenatal glucocorticoid exposure (130). It is likely that a similar adult phenotype may be produced by distinct perinatal processes which differ with the timing of the exposure in a species and inevitably between species. It is presumably what is at a critical stage of development at the time of an environmental insult that governs the target affected.

The heart

A core finding in low-birth-weight human populations is an increased risk of cardiovascular death in adults (33, 147). This may reflect the sum of increased cardiovascular risk factors, but primary cardiac programming might also contribute. Indeed, prenatal glucocorticoid exposure alters the development of cardiac noradrenergic and sympathetic processes (148), increases cardiac adenylate cyclase reactivity (149) and alters metabolic processes in the heart such as the glucose transporter 1. *akt*/protein kinase B. specific uncoupling proteins and PPARy, the nuclear receptor for thiazolidinediones and fatty acids (150, 151). Antenatal glucocorticoid exposure increases adult calreticulin in the heart (152); this is important since overexpression of cardiac calreticulin is associated with cardiac dysfunction and death. Thus, increased coronary heart disease deaths in low-birth-weight populations may reflect programmed primary cardiac dysfunction as well as the increased prevalence of cardiovascular risk factors.

Programming of glucose-insulin homeostasis and metabolism

Prenatal overexposure to exogenous or endogenous glucocorticoids 'programmes' permanent hyperglycaemia – particularly hyperinsulinaemia – in the adult offspring in the rat (10, 133, 136), effects confined to the last third of gestation. Prenatal stress has similar persisting effects (153). Gestational 11β -HSD inhibition has similar adult hyperglycaemic effects. Earlier dexamethasone exposure or post-partum treatments do not programme hypergylcaemia/hyperinsulinaemia in the rat; thus, there is a tight window for this effect (10, 154). Maternal glucocorticoid administration has an effect on cord glucose and insulin levels in the sheep foetus (155), and these effects persist into adulthood (131, 134). The 'window' of sensitivity is earlier in proportion to gestation than in the rat. Importantly, in the sheep, antenatal glucocorticoid exposure alters adult glucose metabolism whether or not there is prior foetal growth restriction (156). As expected, programming clearly relates to foetal exposure to excess glucocorticoids in utero, rather than any primary effect of intrauterine growth retardation per se.

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Glucocorticoids regulate expression of critical hepatic metabolic enzymes, notably phosphoenolpyruvate carboxykinase (PEPCK), which catalyses a rate-limiting step in gluconeogenesis. In rats, exposure to excess glucocorticoid in utero leads to offspring with permanent elevations in PEPCK mRNA and enzyme activity from a few days postnatally, selectively in the gluconeogenic periportal region of the hepatic acinus (10). Overexpression of PEPCK in hepatoma cells impairs insulin suppression of gluconeogenesis (157). Transgenic overexpression of PEPCK in the liver impairs glucose tolerance (158). The PEPCK gene is under complex transcriptional control (159). Intriguingly, increased expression of GR itself occurs in the liver of dexamethasone-programmed rats (10, 160). Moreover, rats exposed to dexamethasone in utero have greater plasma glucose responses to exogenous corticosterone, suggesting increased tissue sensitivity to glucocorticoids (10). Similar increases in hepatic GR are seen in the offspring of undernourished ewes (161), suggesting that the process is conserved.

Intriguingly, prenatal dexamethasone not only has effects in the immediate offspring as adults, but also elevates PEPCK and insulin levels in their own offspring (162). Such intergenerational effects are becoming more widely recognised (163). The mechanisms are uncertain, but appear to follow both male and female lines, suggesting epigenetic processes.

Pancreas

Prenatal undernutrition impairs pancreatic β -cell development (164, 165), reducing β -cell mass and causing glucose intolerance. Foetal pancreatic insulin content correlates inversely with foetal corticosterone levels (166). Maternal malnutrition elevates maternal and foetal corticosterone levels, and preventing the corticosterone increase in food-restricted dams restores β -cell mass. The mechanisms by which gluco-corticoids modulate pancreatic development are not clear, but dexamethasone downregulates β -cell Pdx-1 and induces C/EBP β , key factors in the induction and repression respectively of insulin expression (167).

Fat

Antenatal dexamethasone exposure in rats programmes fat metabolism (94), causing marked increase in GR expression selectively in visceral adipose tissue in adult rats (94) and sheep (161). Elevated GR expression in visceral adipose tissue may contribute to both adipose and hepatic insulin resistance. These changes in GR expression do not appear to be the result of metabolic derangement in the adult animal, and correction of the hypercorticosteronaemia and insulin sensitisation are not sufficient to normalise the programmed changes in GR (160). Leptin concentrations in human foetal cord blood correlate directly with body weight and adiposity at birth (168–172). Antenatal treatment with dexamethasone in pregnant rats reduces foetal plasma and placental leptin (133, 173), and placental expression of the Ob-Rb receptor which mediates leptin action (173). Intriguingly, concomitant treatment of malnourished pregnant and lactating rats with leptin appears to reverse, in part, the adult metabolic effects of antenatal challenge, at least for maternal malnutrition (174). In contrast, adiponectin (acrp30, adipoQ), an abundant adipokine that is associated negatively with fat mass (175) and positively with insulin sensitivity (176), apparently does not relate to birth weight (177).

The GR gene: a common programming target?

Transgenic mice with a reduction of 30-50% in tissue levels of GR have striking neuroendocrine, metabolic and immunological abnormalities (178). The level of expression of GR is thus critical for cell function. GR gene expression shows tissue-specific regulation. The GR promoter is complex, with multiple, tissue-specific, alternate, untranslated first exons in rats (179) and mice (180), most within a transcriptionally active 'CpG island'. All these mRNA species give rise to the same receptor protein, as only exons 2-9 encode the protein. The alternate untranslated first exons are spliced onto the common translated sequence beginning at exon 2. In the rat, two of the alternate exons are present in all tissues which have been studied; however, others are tissue-specific (179). This permits considerable complexity of tissue-specific variation in the control of GR expression without allowing any tissue to become GR depleted.

Neonatal handling permanently programmes increased expression of only one of the six alternate first exons (exon 1_7) utilised in the hippocampus (179). Similar effects are seen in the offspring of mothers which show particularly 'attentive' forms of maternal care (112). Exon 1_7 contains sites appropriate to bind the very third messenger/intermediate early gene transcription factors (AP-2, NGF1-A) induced by the neonatal manipulation (119).

The next key problem is to understand how discrete perinatal environmental events can permanently alter gene expression. Key recent evidence suggests selective methylation/demethylation of specific promoters of the GR gene. The putative NGFI-A site around exon 1_7 is subject to differential and permanent methylation/ demethylation in association with variations in maternal care (126). The changes in GR promoter DNA methylation pattern are associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter (126). Treatment of the adult offspring brain with a histone deacetylase inhibitor removes the epigenetic differences in histone acetylation and DNA methylation, and hence the NGFI-Abinding changes. This is associated with normalisation EUROPEAN JOURNAL OF ENDOCRINOLOGY (2004) 151

of hippocampal GR expression and HPA axis responses to stress. The findings suggest a causal relation between the epigenetic modifications induced by early-life events in the GR gene promoter and the permanent programming of GR expression in the adult hippocampus. This process may analogously produce tissue-specific effects in peripheral organs. Indeed, in liver-derived cells, GR may mediate differential demethylation of target gene promoters, effects which persist after steroid withdrawal (181). During development, such target promoter demethylation occurs before birth and may fine-tune the promoter to 'memorise' regulatory events occurring during development. This novel mechanism of gene control by early-life environmental events that then persist throughout the lifespan remains to be confirmed in other systems.

Human clinical observations

Glucocorticoids such as dexamethasone and betamethasone are commonly used to treat foetuses at risk of preterm delivery. Such synthetic glucocorticoids enhance lung maturation and reduce mortality in preterm infants; a single course of prenatal corticosteroid is associated with a significant reduction in the incidence of intraventricular haemorrhage and a trend toward less neurodevelopmental disability (182). However, a survey of British obstetric departments showed that 98% were prescribing repeated courses of antenatal glucocorticoids (183). There is little evidence of the safety and efficacy of such a regime (184). Recent overviews suggest that there is no evidence of additional benefit from repeated courses of glucocorticoid therapy in pregnancy (185, 186), but that clear conclusions are prevented by the lack of prospective, randomised, controlled trials and by variations in the protocols employed (type of glucocorticoid, route and timing of administration, and number of treatment courses). Antenatal glucocorticoid administration has also been linked with higher blood pressure in adolescence (187) and subtle effects on neurological function, including reduced visual closure and visual memory (188). Multiple doses of antenatal glucocorticoids given to women at risk of preterm delivery were associated with reduced head circumference (12) and an increased risk of externalising behaviour problems, distractibility and inattention (189).

In addition, women at risk of bearing foetuses at risk of CAH often receive low-dose dexamethasone from the first trimester to suppress foetal adrenal androgen overproduction. Birth weight in such infants has been reported as normal (15, 16); however, programming effects of antenatal glucocorticoids are seen in animal models in the absence of reduced birth weight (156). Children exposed to dexamethasone in early pregnancy, because of the risk of CAH, show increased emotionality, unsociability, avoidance and behavioural problems (190).

The human HPA axis also appears to be programmed by the early-life environment. Higher plasma and urinary glucocorticoid levels are found in children and adults who were of low birth weight (191–193). HPA changes precede overt adult disease (194). HPA axis activation is associated with higher blood pressure, insulin resistance, glucose intolerance and hyperlipidaemia (195). The human GR gene promoter has multiple alternate untranslated first exons (Reynolds and Chapman, unpublished observations), analogous to those found in the rat and mouse. Whether these are the subjects of early-life regulation and the molecular mechanisms by which this is achieved remain to be determined, but muscle GR mRNA levels correlate with blood pressure and insulin resistance (196, 197).

Conclusions

Prenatal exposure to glucocorticoids may 'programme' a range of tissue-specific pathophysiologies. The foetus may be exposed to exogenous glucocorticoids, to active steroids of maternal origin or to its own adrenal products. The outcomes in a host of species and models are remarkably consistent, with cardiometabolic and CNS effects predominating. Work on a candidate mechanism, GR gene programming, has illuminated a potential fundamental mechanism underlying this rapidly emerging biology. Such fine-tuning of foetal physiology by the environment is conserved and therefore apparently important. Studies are now making headway in unravelling the underlying processes, a prerequisite for rational treatments for the consequences of adverse perinatal environment.

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