Glucocorticoids and fetal programming

J. R. Seckl\textsuperscript{1}, M. J. Nyirenda, B. R. Walker and K. E. Chapman

Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, U.K.

Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, U.K.

Glucocorticoids

Glucocorticoid hormones (cortisol in humans and most mammals, corticosterone in rats, mice and most lower vertebrates) are produced by the adrenal glands, predominantly under the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Receptors for glucocorticoids are members of the nuclear hormone receptor superfamily and, when activated by their hormone ligands, regulate (positively or negatively) the transcription of target genes, either by directly binding to palindromic DNA sequences or indirectly via interactions with other transcription factors, such as AP1 and the cyclic AMP response element binding protein, CREB.

Glucocorticoids play a series of key roles in physiology, maintaining fuel metabolism under basal conditions and underpinning many of the body’s adjustments to stressful stimulation. During prenatal and early postnatal mammalian development, glucocorticoid levels are generally low, although towards term they rise in many species and effectively signal the onset of late fetal tissue maturation in preparation for extra-uterine existence. Key targets for such late effects include the lung (surfactant secretion), gut, liver and brain. Indeed, when premature labour threatens, synthetic glucocorticoids, such as dexamethasone, are exploited to accelerate maturation of the lung and gut; such therapy efficaciously reduces the incidence of postnatal respiratory distress syndromes. But are all effects of prenatal glucocorticoids uniformly beneficial?

From the earliest therapeutic exploitation of glucocorticoids in pregnancy, it became apparent that they caused reductions in birthweight. This was observed in experimental animals, including non-human primates, and in humans [1–5]. However, the potential significance of birthweight reduction for later pathophysiology has only recently begun to be appreciated.

Early life origins of adult disease

Recently, a series of epidemiological studies have shown that low weight at birth is associated with an increased risk of adult cardiovascular and metabolic disorders. Exploiting the long-term maintenance of obstetric records in some British hospitals, a group in Southampton uncovered a close geographic correlation between heart disease mortality and parameters of the early life environment, notably birthweight and early postnatal growth [6,7]. Subsequent studies in the U.K., elsewhere in Europe, Asia, Australia, the Caribbean and the U.S.A. have confirmed these findings and shown that low birthweight or thinness at birth strongly predicts the subsequent occurrence of hypertension, hyperlipidaemia, insulin resistance, Type 2 diabetes mellitus and death from ischaemic heart disease in adult life [8–13]. Indeed, the smaller of twins at birth has higher blood pressure in later life [14], greatly complicating any interpretation of data from this ‘simple’ paradigm to dissect the ‘genes × environment’ equation. Classical adult ‘lifestyle’ risk factors (smoking, alcohol consumption, exercise, nutrition, obesity, social class) are additive to the influence of early life [8,9]. Importantly, the relationships are apparently continuous and represent birthweights within the normal range, rather than severe intrauterine growth retardation, multiple or very premature babies [8,10,12]. Postnatal ‘catch-up’ growth is also a risk factor for subsequent hypertension [8,14], ischaemic heart disease [15] and insulin resistance [11], suggesting that smallness at birth due to environmental influences restraining intrauterine growth is of particular importance, rather than genetic smallness per se. Whether postnatal catch-up growth is an independent risk factor for adult disorders remains to be established.

These ‘early life’ associations appear to be important predictors of later disease. In the Preston study, a small baby with a large placenta had a relative risk of adult hypertension three times that of a large baby with a normal placenta [16]. In a study of more than 22000 American men, babies who weighed less than 5.5 lbs at birth had a relative risk of adult hypertension of 1.26 and of Type 2 diabetes of 1.75 compared with babies of average birthweight. Similarly, the relative risk

Abbreviations used: HPA, hypothalamic–pituitary–adrenal; 11β-HSD-2, 11β-hydroxysteroid dehydrogenase type 2.

\textsuperscript{1}To whom correspondence should be addressed.
of hypertension in light normal babies was 1.43 in 71,000 American female nurses [10,11].

To explain these findings the idea of early life physiological ‘programming’ or ‘imprinting’ has been proposed [9,17]. Such programming has been documented in a variety of systems and reflects the ability of a factor during a sensitive period or ‘window’ of development to exert organizational effects that persist throughout life. Programming agents might include growth factors, hormones and nutrients. These factors may produce adaptations which permanently alter adult metabolism and responses in a direction optimizing survival under continued conditions of malnutrition, stress or other deprivation, but such responses might be detrimental when the later environment is ‘unexpectedly’ less challenging.

**Glucocorticoids and programming**

Long-term organizational effects are typically found with hormones, particularly steroids. A good example is the neonatal action of androgens to programme the expression of steroid metabolizing enzymes in the liver, as well as the development of sexually dimorphic structures in the brain and sexual behaviour [18]. These effects can only be exerted during a specific perinatal period, but then persist throughout life, largely irrespective of any subsequent sex steroid manipulations.

Several features of fetal glucocorticoid over-exposure suggest a potential role in early life programming. First, exogenous glucocorticoids retard fetal growth [1–4]. Fetal cortisol levels are increased in human intrauterine growth retardation [19]. Cortisol also affects placental size, the effect being dependent upon the dose and timing of exposure [20]. Secondly, prenatal glucocorticoids alter the rate of maturation of organs such as the lung [21], heart [22], kidney [23] and gut. Some of these effects are transient whereas others persist throughout life. Perinatal glucocorticoids or stress programme specific effects in the brain [24–26], the immune system [27] and kidney [23]. Thirdly, intracellular glucocorticoid receptors are expressed in most fetal tissues from mid gestation [28–30]. Expression of higher affinity mineralocorticoid receptors is also seen in some tissues from mid gestation [31]. Fourthly, the major systems affected by ‘early life’ programming are glucocorticoid targets. Thus, glucocorticoids increase blood pressure and blood glucose levels in adults [32]. These systems are sensitive in early life since cortisol also elevates fetal blood pressure when infused directly in utero in sheep [33] and at birth in humans [34]. Such effects have multiple mechanisms. For example, glucocorticoids affect blood pressure via direct vasoconstrictor potentiating effects on the vasculature, regulation of biosynthesis of key mediators such as catecholamines, angiotensinogen and nitric oxide and actions on the central nervous system. Fifthly, prenatal glucocorticoid exposure permanently elevates offspring blood pressure and blood glucose levels. The treatment of pregnant rats with modest doses of dexamethasone, a synthetic glucocorticoid, reduces birthweight and elevates blood pressure in the adult offspring [35]. Even short-term glucocorticoid exposure in the last trimester increases blood pressure in adult life in rats [36]. These effects are not merely related to differences in adult weight, since weight differences normalize by weaning at 3 weeks of age. Last trimester administration of dexamethasone also ‘programmes’ permanent hyperglycaemia and, particularly, hyperinsulinaemia in the adult offspring [37], modelling the insulin resistance or ‘metabolic syndrome’ phenotype which is a key feature of the human ‘fetal origins’ observations.

**Feto-placental 11β-hydroxysteroid dehydrogenase type 2**

Although glucocorticoids cross the placenta, the fetus has much lower levels of physiological glucocorticoids than the mother [38]. This gradient is thought to be achieved by placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), which catalyses the rapid metabolism of the active physiological glucocorticoids, cortisol and corticosterone, to inert 11-keto forms (cortisone and 11-dehydrocorticosterone respectively) [39,40]. This placental enzymic barrier is quite efficient and ensures that most, but not all [41], maternal cortisol is inactivated on reaching the fetal blood, such that the majority of cortisol in the human fetal circulation at term is derived from the fetal adrenals [38]. In contrast, dexamethasone, a poor substrate for 11β-HSD-2 [42], readily crosses the placenta.

The efficiency of placental 11β-HSD-2 near term varies considerably in both rats and humans [35,43]. It has therefore been hypothesized that a relative deficiency of placental
11β-HSD-2, by allowing increased access of maternal glucocorticoids to the fetus, may retard growth and programme responses leading to later disease [17]. Indeed in rats, lower placental 11β-HSD activity, and presumably greater fetal exposure to maternal glucocorticoids, is seen in the smallest fetuses with the largest placentas. Similar associations between birthweight and placental 11β-HSD-2 have been mooted in humans [43], although not all studies have confirmed this [44]. Moreover, markers of fetal exposure to glucocorticoids, such as fetal osteocalcin, a glucocorticoid-sensitive osteoblast gene product which does not cross the placenta, also correlate with placental 11β-HSD-2 function [45]. Deleterious mutations of the 11β-HSD-2 gene in humans are associated with low birthweight [46], suggesting that lack of 11β-HSD-2 in the fetus and/or placenta (but not the unaffected heterozygous mother) causes growth retardation.

Inhibition of 11β-HSD by treatment of pregnant rats with carbenoxolone exerts similar effects to dexamethasone, reducing birthweight and programming hypertension and hyperglycaemia in the adult offspring [47,48]. These effects of carbenoxolone require maternal glucocorticoids (are lost with maternal adrenalectomy), suggesting the action is upon 11β-HSD-2 rather than other processes. Also, the effects of carbenoxolone upon the fetus and adult offspring do not associate with alterations in maternal blood pressure, electrolytes or glucose levels, perhaps suggesting that the placenta may be the key locus of action. Nevertheless, many fetal tissues also highly express 11β-HSD-2 and clearly carbenoxolone might be acting directly at the level of the fetus as well as the placenta. The role of fetal 11β-HSD-2 in determining glucocorticoid access to developing fetal tissues remains unclear, but many organs are exquisitely sensitive to glucocorticoids, which exert potent effects upon cellular differentiation and development. In particular, glucocorticoids alter the trajectory of organ maturation, most notably accelerating late development of the lung and gut to facilitate adjustment to extra-uterine life, while apparently slowing aspects of brain and renal maturation. The potential importance of the tissue- and development-specific expressions of 11β-HSD-2 in regulating such glucocorticoid effects is obvious, but evidence for such actions has yet to be provided.

Dietary protein restriction during rat pregnancy selectively attenuates 11β-HSD-2, but not other enzymes in the placenta [49]. Thus glucocorticoid exposure may be a common mechanism linking maternal environmental factors with fetal growth and programming. Indeed, in the maternal protein restriction model, offspring hypertension can be prevented by giving the pregnant dam (and her offspring) glucocorticoid synthesis inhibitors, and can be recreated by concurrent ‘replacement’ of corticosterone [50].

**Tissue mechanisms of fetal programming**

Prenatal glucocorticoid exposure affects glucose–insulin homeostasis in the adult offspring. Glucocorticoids regulate several important hepatic enzymes of carbohydrate and fat metabolism, such as phosphoenolpyruvate carboxykinase, the rate-limiting step in gluconeogenesis. Glucocorticoids also attenuate insulin sensitivity, and regulate adipocyte distribution and function. Prenatal glucocorticoid administration programmes increased phosphoenolpyruvate carboxykinase gene transcription and hence enzyme activity [37]. Prenatal dexamethasone also permanently increases hepatic glucocorticoid receptor gene expression. This appears to be of functional importance since such offspring are more sensitive to the hyperglycaemic effects of corticosterone. Thus elevated glucocorticoid receptor gene expression may underpin the programming of phosphoenolpyruvate carboxykinase, a gene prominently regulated by glucocorticoids [37]. Certainly, increased hepatic glucocorticoid receptor expression temporally precedes the increase in phosphoenolpyruvate carboxykinase in this model.

Prenatal dexamethasone also permanently affects glucocorticoid receptor and indeed mineralocorticoid receptor mRNA expression in the adult hippocampus, a region of the brain involved in glucocorticoid negative feedback upon the HPA axis, as well as in cognitive function. However, in this tissue, glucocorticoid receptor mRNA is reduced by prenatal dexamethasone exposure. This apparently reduces sensitivity to feedback, producing increases in basal plasma corticosterone levels in adulthood [36]. Of course, elevated glucocorticoid levels might contribute directly to hypertension and hyperglycaemia [35]. Importantly, in men in their 60s, plasma cortisol levels correlate negatively with
birthweight six decades earlier [51], implying that early life programming of the HPA axis occurs in humans as it does in rodents.

The detailed molecular mechanisms whereby glucocorticoid receptor transcripts are permanently increased in the liver but reduced in the hippocampus by a single prenatal event remain undefined. However, the existence of multiple tissue-specific alternate (untranslated) first exons/promoters of the glucocorticoid receptor gene may allow such tissue-specific effects upon the same receptor gene. Unravelling such issues is the next key step in understanding the biology of early life events and their effects upon human pathophysiology.

Work in the authors’ laboratory was supported by a programme grant from the Wellcome Trust (J.R.S.), Senior Clinical Research Fellowships from the British Heart Foundation (B.R.W.) and Wellcome Trust and project grants from the World Health Organisation (M.J.N.) and project grants from the Wellcome Trust, BHF and SHERT.
The Cre/loxP system – a versatile tool to study glucocorticoid signalling in mice

H. M. Reichardt, C. Kellendonk, F. Tronche and G. Schütz
Division of Molecular Biology of the Cell I, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Introduction
During the last decade, the development of gene targeting opened up completely new perspectives in the analysis of gene function. It became possible to engineer genes in vivo using homologous recombination in murine embryonic stem cells [1], leading to the generation of a plethora of knock-out mouse strains (http://BioMedNet.com/ DNA sequence can be recombined by Cre, and if they have the same orientation the intervening sequence is excised. Because Cre does not require any cofactors it is also possible to use the Cre/loxP system in eukaryotic cells [4], which allows mammalian genes to be manipulated in vivo. To this end, the gene of interest has to be modified with loxP sites such that they flank the region to be targeted. By appropriate expression of Cre, recombination and removal of the intervening sequence is achieved [2] such engineering of genes can be performed in cells as well as in mice, ultimately resulting in the generation of mice carrying point mutations [5], cell-type-specific mutations. In this paper we discuss some applications of the Cre/loxP system as exemplified by the analysis of glucocorticoid receptor function.

Abbreviations used: GR, glucocorticoid receptor; GRE, glucocorticoid response element; PGKneo, phosphoglycerol kinase-neomycin phosphotransferase; HPA, hypothalamic–pituitary–adrenal; CRF, corticotropin releasing factor.

1To whom correspondence should be addressed.

Received 23 September 1998

The Cre/loxP system

Cre recombinase derived from the P1 phage is a member of the λ-integrase superfamily [3]. As a site-specific recombinase, Cre recognizes a 34-bp sequence named loxP, consisting of two inverted repeats of 16 bp each and a directional spacer of 8 bp. Any two loxP sites present in a DNA sequence can be recombined by Cre, and if they have the same orientation the intervening sequence is excised. Because Cre does not require any cofactors it is also possible to use the Cre/loxP system in eukaryotic cells [4], which allows mammalian genes to be manipulated in vivo. To this end, the gene of interest has to be modified with loxP sites such that they flank the region to be targeted. By appropriate expression of Cre, recombination and removal of the intervening sequence is achieved [2]. Such engineering of genes can be performed in cells as well as in mice, ultimately resulting in the generation of mice carrying point mutations [5], cell-type-