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Perinatal development and adult blood pressure

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Abstract

A growing body of evidence supports the concept of fetal programming in cardiovascular disease in man, which asserts that an insult experienced *in utero* exerts a long-term influence on cardiovascular function, leading to disease in adulthood. However, this hypothesis is not universally accepted, hence animal models may be of value in determining potential physiological mechanisms which could explain how fetal undernutrition results in cardiovascular disease in later life. This review describes two major animal models of cardiovascular programming, the *in utero* protein-restricted rat and the cross-fostered spontaneously hypertensive rat. In the former model, moderate maternal protein restriction during pregnancy induces an increase in offspring blood pressure of 20-30 mmHg. This hypertensive effect is mediated, in part, by fetal exposure to excess maternal glucocorticoids as a result of a deficiency in placental 11-ß hydroxysteroid dehydrogenase type 2. Furthermore, nephrogenesis is impaired in this model which, coupled with increased activity of the renin-angiotensin system, could also contribute to the greater blood pressure displayed by these animals. The second model discussed is the cross-fostered spontaneously hypertensive rat. Spontaneously hypertensive rats develop severe hypertension without external intervention; however, their adult blood pressure may be lowered by 20-30 mmHg by cross-fostering pups to a normotensive dam within the first two weeks of lactation. The mechanisms responsible for this antihypertensive effect are less clear, but may also involve altered renal function and down-regulation of the renin-angiotensin system. These two models clearly show that adult blood pressure is influenced by exposure to one of a number of stimuli during critical stages of perinatal development.

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Introduction

The concept of fetal programming in cardiovascular disease is controversial, despite the abundance of epidemiological data supporting this notion. The basic hypothesis suggests that the fetus is programmed *in utero* to develop one of a number of adult diseases, including cardiovascular disease, diabetes mellitus and bronchitis, as a result of some insult which permanently alters its physiological or metabolic processes. The specific nature of this insult is not clear; however, in the case of cardiovascular disease, it seems to involve retardation of fetal growth at a specific stage of development.

One of the earliest studies to describe an association between family history and the risk of ischaemic heart disease in adulthood was reported by Rose (1). This study showed

that, compared with an age- and sex-matched control group, those individuals who suffered from ischaemic heart disease were more likely to have a close relative (parent, uncle, aunt or sibling) who also suffered from ischaemic heart disease. More importantly, they were also twice as likely to have a sibling who had experienced a stillbirth or infant mortality. This association went unnoticed until the first link between early life experience and adult disease was established by Forsdahl (2). He reported that, in a Norwegian population, the current incidence of mortality in middle-age (in 1960) due to arteriosclerotic heart disease or cerebrovascular disease was significantly correlated with the prior infant mortality rates of the same population (in 1896-1925). Thus, individuals who came from areas with high infant mortality rates were much more likely to suffer from cardiovascular disease in adulthood. Furthermore, as infant mortality rates fell due to general improvements in the socioeconomic status of the total population, so too did the adult mortality rates.

Forsdahl's study was followed by a plethora of epidemiological investigations, many of which showed a positive correlation between a mother's blood pressure and that of her offspring, leading to suggestions that maternal factors may be important in determining offspring blood pressure. However, it is the work of Barker and colleagues (3) that has drawn the most attention in the literature recently, by revisiting the association between birth weight and the incidence of cardiovascular disease in adulthood. Following the observation that the geographical distribution of infant death in Britain in the 1900s, which was usually attributed to low birth weight, matched the current pattern of death due to coronary heart disease, a series of epidemiological studies were undertaken to look at mortality and birth weight. These initial studies focused on retrospective data sets from cohorts in Hertfordshire, Preston and Sheffield, England, in which the maternity records were particularly detailed. They showed that in middle-aged men, those with the lowest birth weights and weights at one year had the highest blood pressure and death rates from ischaemic heart disease. Further studies demonstrated that adult blood pressure was related to birth weight, placental weight, length, ponderal index and head circumference; adult blood pressure was greatest in individuals with the lowest birth weights and highest placental weights. This trend was also apparent in children; systolic blood pressure in children aged four years was inversely related to birth weight and positively related to placental weight. See Barker's review (3) for a full discussion of these data.

The association between birth weight and the risk of developing cardiovascular disease in adulthood has now been reported in populations from several countries. Indeed, low birth weight has now been correlated with an increased risk of both coronary artery disease and hypertension in diverse populations across the world by many groups. However, there has not been uniform acceptance of these data and the hypothesis that fetal growth retardation increases the risk of developing cardiovascular disease. The main criticism of what has become known as the Barker hypothesis is that confounding variables such as maternal blood pressure, smoking and socio-economic status have not been adequately controlled for in the analyses (4), though Barker vigorously denies this (3). One data set that has yet to be explained by Barker, however, describes the effect of food deprivation during pregnancy in humans during the Dutch famine of 1944-1945. During this severe famine, the calorie intake of mothers had to fall below 1500 cal/day (compared with a normal intake of 2500 cal/day) before fetal growth was retarded. Even then the average decrease in birth weight was only 300 g, which was equivalent to only 0.5 of the population standard deviation (4). These observations are difficult to reconcile

with the fetal malnutrition hypothesis, but, given the extreme widespread nature of the food deprivation, would one expect to see the same effect across the whole population?

Clearly, one way to resolve this debate is to look to animal models of programming for physiological mechanisms that might explain these observations.

Intrauterine models of programmed hypertension

Several fetal undernutrition models have been developed in which low birth weight offspring display high blood pressure in adulthood. Initial studies in the guinea pig showed that severe fetal growth retardation (48-57% reduction in birth weight) induced by ligation of the uterine artery resulted in a 7-mmHg increase in adult blood pressure, though moderate growth retardation was ineffective (5). Similarly, restricting the protein and calorie intake of pregnant rats by 70% resulted in offspring that were 33% lighter than controls and had systolic blood pressure 5-8 mmHg higher in adulthood (6).

More moderate undernutrition, comparable with that which has been shown to lower birth weight in human populations (7) and within the range seen in the United Kingdom (8), has been used by Langley-Evans et al. (9) to induce raised blood pressure in the rat. A low (9%) protein diet during pregnancy resulted in placental enlargement and pups with low to normal birth weights, but with disproportionate growth retardation of the truncal organs, though the brain was unaffected (9). These rats had blood pressure elevations of 20-30 mmHg by 4 weeks of age (10) which persisted into adulthood (11). Maternal undernutrition prior to pregnancy was not required to produce these effects and the period of protein restriction could be limited to a single week during pregnancy (12).

The effect of protein restriction on fetal growth and adult blood pressure has recently been questioned by Tonkiss et al. (13). They have attributed the elevation in blood pressure in these animals to stress caused by the restraint used in the indirect, tail cuff method of blood pressure measurement, rather than to the effect of fetal undernutrition *per se*. Rats born to dams maintained on 6% protein during pregnancy and suckled by foster mothers maintained on a 25% protein diet were implanted with a radiotelemetry transmitter at 96 days and blood pressure was monitored in conscious, freely moving animals over a 24-h cycle. Under these conditions, the rats that had been exposed to protein deficiency *in utero* showed a 4-mmHg increase only in diastolic pressure during the dark phase of the 24-h cycle, by comparison with control animals. This contrasts with the 20-30-mmHg increase in systolic blood pressure, measured by tail cuff plethysmography, reported by Langley-Evans and colleagues (10). When ammonia was used as an olfactory stressor, prenatally malnourished rats showed a greater increase in both systolic and diastolic pressure than control animals on the first, but not subsequent, occasion. Tonkiss et al. (13) therefore suggest that prenatal malnutrition induces pathogenic changes in blood pressure regulatory mechanisms which, when combined with stress, could contribute to hypertension in humans.

Intrauterine programming mechanisms

A number of mechanisms have been proposed to explain how low maternal dietary protein during pregnancy may affect offspring blood pressure, including increased sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis, diminished placental 11-ß hydroxysteroid dehydrogenase (11-ß HSD) activity and altered renal development.

In the sheep, fetuses exposed to undernutrition *in utero* had lower HPA axis activity, lower cortisol and lower blood pressure but, postnatally, they showed an exaggerated

blood pressure response to HPA challenge and had higher arterial pressures. These effects appear to be mediated by changes in hippocampal glucocorticoid receptor gene expression (14) which can either permanently increase or decrease sensitivity to feedback, depending on subtle differences in perinatal experience (15). Further support for a role of glucocorticoids in maintaining high blood pressure following fetal undernutrition comes from the observation that adrenalectomy in rats exposed to a low-protein diet *in utero* lowered blood pressure, and that subsequent replacement of corticosterone increased blood pressure (16).

Transport of maternal glucocorticoids across the placenta appears to be another important factor in determining offspring blood pressure. Glucocorticoids can influence blood pressure in the adult by a variety of mechanisms, including stimulation of hypothalamic receptors (17), increasing sodium and calcium uptake by vascular smooth muscle (18) and potentiation of the actions of angiotensin II (19). In the fetus, maternal glucocorticoid excess may suppress the development of the fetal adrenal glands, by inhibiting ACTH secretion and thus reducing adrenal growth and steroidogenesis, leading to suppression of the HPA axis in adulthood. The fetus is normally protected from the high circulating levels of maternal glucocorticoids by the enzyme 11-ß HSD type 2 which converts corticosterone to 11-dehydrocorticosterone in the rat (cortisol to cortisone in man). Activity of this enzyme positively correlates with birth weight in both the rat (20) and man (21), suggesting that it may play a role in regulating fetal growth and development of cardiovascular disease in adulthood. This hypothesis is supported by the observation that maternal protein restriction during pregnancy in the rat also results in diminished placental 11-ß HSD type 2 activity and increased exposure of the fetus to maternal glucocorticoids, which was associated with increased blood pressure in adulthood (22). A similar effect on offspring blood pressure was observed when maternal 11-ß HSD was inhibited with carbenoxolone in rats fed a normal diet during pregnancy (23). Furthermore, administration of dexamethasone, which is not metabolised by 11-ß HSD and is thus able to cross the placental barrier and exert a glucocorticoid effect on the fetus, to pregnant dams resulted in smaller pups which developed higher blood pressure than control animals, supporting the notion that impaired maternal glucocorticoid metabolism influences intrauterine programming (20).

One aspect of fetal development that has so far received less attention is that of the fetal kidney. It is well established that the kidney plays a major role in the long-term regulation of blood pressure in the adult, and preliminary evidence now suggests that it too may be programmed *in utero* with long-term consequences for blood pressure. In common with placental 11-ß HSD activity and fetal HPA axis sensitivity, the fetal kidney may be affected by the maternal diet during pregnancy. Maternal isocaloric protein restriction reduced kidney weight in the neonatal rat, suggesting that protein limitation has an organ-specific effect on renal development (24). In particular moderate protein restriction was associated with a reduction in the number of mature glomeruli in the rat at birth, which persisted for the first 2-4 postnatal weeks even if the pups were fed a normal diet (24). A reduction in the number of nephrons or a reduction in the filtration surface area of the glomerulus would result in a reduction in sodium excretion, leading to increased systemic pressure, glomerular capillary hypertension and thus glomerular sclerosis, which would perpetuate the situation.

In addition to the effect on glomerular development, maternal protein restriction also influences fetal renin-angiotensin system (RAS) activity. In human fetus with intrauterine growth retardation, renin-con-

taining cells were found throughout the renal cortex at 40 weeks of gestation, compared with the normal fetal kidney in which renin was localised in the glomeruli of outer cortical nephrons by this stage (25). In the rat, renin and angiotensin converting enzyme (ACE) activities were elevated in animals exposed to a low-protein diet *in utero*, and brief (2 weeks) administration of an ACE inhibitor lowered blood pressure in both neonates (2 week) and young adults (10 week) to levels comparable with those observed in rats exposed to a normal protein diet *in utero* (26). Although these effects were obtained using very high doses of captopril (100-182 mg day^{-1} kg^{-1}) which, in the case of the neonatal animals, was administered at a stage of development when ACE inhibition can cause persistent, irreversible histopathological abnormalities in the kidney (27), these observations suggest that the neonate's RAS may have an important influence on the kidney's subsequent ability to regulate blood pressure.

The uterine environment and genetic hypertension

If *in utero* fetal programming is responsible, at least in part, for the development of cardiovascular diseases such as hypertension in man, it is reasonable to expect that it may also contribute to the development of hypertension in experimental, genetic models. This notion is supported by the observation that in the most commonly used genetic model of human essential hypertension, the spontaneously hypertensive rat (SHR), the uterine environment differs from that of control, normotensive Wistar Kyoto (WKY) rats. Amniotic fluid volume was found to be lower at days 15-18 of gestation in the SHR compared with WKY rats, but continued to increase in the later stages of pregnancy, rather than diminish as was the case in the WKY, such that fluid volumes were significantly higher prior to term. Low amniotic fluid volume is found in women with pre-eclampsia or essential hypertension (28). Amniotic fluid sodium concentration was comparable between the strains, but potassium was lower in the amniotic fluid of SHR dams. These differences were associated with reduced fetal growth during the last 2-3 days of gestation, when fetal weight typically doubles, and with larger placentae (29). The placentae of SHR dams were also found to have larger haemorrhagic regions of the decidua basalis at day 15 (30). This may reflect the increase in maternal blood pressure that occurs at this stage of pregnancy; however, similar haemorrhagic necrosis has been reported in rats treated with dexamethasone (31), suggesting that placental glucocorticoid concentrations may be elevated in the SHR. These differences in fetal and placental size are also associated with a reduction in placental amino acid transport and an increase in glucose transport, as determined by amino-isobutyric acid and 3-O-methyl glucose transport, respectively (32).

However, despite these differences between the uterine environments of SHR and WKY rats and the resultant reduction in SHR fetal growth, exposure to the SHR uterus does not appear to have a long-term effect on blood pressure. In elaborate studies by two groups (33,34), SHR embryos have been transferred to control WKY dams within 5 days of conception to assess the effect of the uterine environment on blood pressure. In Gray's study (34), SHR embryos transferred to WKY dams had similar blood pressure to those which developed during a normal pregnancy in an SHR dam at both one day and 16 weeks of age. The reciprocally transferred WKY embryos also had comparable blood pressures with normal WKY rats. Azar et al. (33) found that exposure to the WKY uterus was the major determinant in reducing blood pressure in SHR pups up to the age of 7 days compared with those which developed in an SHR uterus, but by 120 days, embryo strain accounted for 60% of the variability in blood

pressure. Thus, the intrauterine environment does not appear to exert a significant influence on the adult blood pressure of the SHR.

Postnatal programming

The major focus of epidemiological studies in the field of cardiovascular programming has been on the uterine environment. However, there is growing evidence from animal studies that the postnatal environment may be of equal importance in determining adult blood pressure. Since its development in the 1950s, the SHR has been considered to be primarily a genetic model of hypertension; however, there have been a number of studies which have shown that environmental stimuli can influence the severity of hypertension expressed. Exposure of the young SHR to factors such as social isolation (35) and tactile stimulation (36) can alter the degree of hypertension in the adult SHR. However, the most compelling evidence that environmental factors can influence the severity of hypertension comes from the work carried out by Cierpial and McCarty (37) and DiNicolantonio (38).

When SHR pups were cross-fostered at birth to either normotensive WKY (37) or Sprague-Dawley dams (38) the SHR pups developed significantly lower blood pressure at maturity than those suckled by SHR dams. We (39,40) and others (41) have subsequently confirmed that cross-fostering SHR pups to WKY dams consistently lowers adult blood pressure by 20-30 mmHg. In the reverse experiment, cross-fostered normotensive WKY or Sprague-Dawley pups suckled by SHR dams did not develop elevated blood pressure at maturity (37,38). As with intrauterine models of hypertension, there appears to be a critical period during which fostering is effective. Cross-fostering was found to be effective if the SHR neonate was suckled by a WKY surrogate for postnatal days 1-7, 1-14, 1-21 or 8-21, but adult blood pressure was not lowered when pups were fostered during postnatal days 15-21 (42). These data show that exposure to a normotensive dam for as little as one week is effective in lowering adult blood pressure, but that this exposure must occur within the first two postnatal weeks. This critical period coincides with the pup's complete dependency on its mother for food, as solid food is not ingested until postnatal day 15 (43).

Initial attempts to identify the nature of the hypertensive stimulus provided by the SHR dam focused on behavioural differences between nursing SHR and WKY dams. SHR dams rearing their natural litters spend more time in nursing postures (pups attached to teats, dam either lying over pups or with arched back and legs extended) and in general contact with their pups than WKY dams both during the light (40,44,45) and dark phases (44). This increased nursing behaviour has been positively correlated with offspring adult blood pressure (45), which has led to the suggestion that nursing may stimulate long-term changes in the cardiovascular system. One possible mechanism could involve the rapid increase in blood pressure upon milk ingestion observed in several species including humans (46) and rats (47). Developing rat pups normally respond to milk ejection with a rapid, 30-50% increase in blood pressure (47); this response is significantly elevated in SHR pups (48). Since the SHR dam displays nursing behaviour more frequently than the WKY dam (44,45) she may also deliver more milk to her offspring, triggering this reflex increase in blood pressure more frequently, which could facilitate vascular damage.

Though attractive, this hypothesis is not supported by our subsequent study of the maternal behaviour of foster dams (40). WKY dams rearing SHR foster litters increased their nursing behaviour, whereas SHR dams rearing WKY foster litters reduced the time spent in nursing postures, suggesting that the pup strain may influence the behaviour of

the dam. Indeed, pup strain accounted for a greater proportion of the variance in nursing behaviour in the ANOVA than dam strain in our study. This notion has been confirmed in other studies in which differences in maternal behaviour of SHR and WKY dams were largely attributed to the characteristics of the pups (45,49). The influence of pup strain on dam behaviour has been explored further in a single pup cross-fostering study, in which a single SHR pup was fostered to a WKY dam nursing a litter of WKY pups (50). In this paradigm, the influence of the SHR pup on the dam's behaviour is minimised, such that it should experience maternal behaviour similar to that experienced by naturally reared WKY pups. Under these conditions, adult blood pressure was reduced and body weight at weaning was increased to the same extent as SHRs reared in complete foster litters of 8 pups. Taken together, these observations suggest that maternal behaviour does not account for the blood pressure-lowering effect of cross-fostering.

The other likely candidate mechanism for the protective effect of cross-fostering on SHR blood pressure is a difference in the dam's milk. As cross-fostering is only effective during the period when the pups are feeding exclusively on maternal milk, it is tempting to speculate that the hypertensive trigger may be found in the milk of the SHR dam. In order to begin to address this question, we measured SHR and WKY dams' milk electrolyte concentrations throughout lactation and found that, in the case of sodium and potassium, there were no differences between the strains. The calcium concentration of SHR milk was lower than that of the WKY, but this difference did not become apparent until the third week of lactation (39). Our data contrast with some of those previously reported for sodium in particular (41,51), however, these discrepancies can be explained by methodological differences. In earlier studies milk collection methods have involved long (>6 h) separation of the dam from her litter to allow milk to accumulate in the mammary glands (51), or repeated milking of the same dam at different stages of lactation (41). We have demonstrated that both of these procedures can alter the electrolyte content of the milk (52), so we used a short separation (<2 h), single milking protocol to obtain milk which more closely reflected that ingested by suckling pups. Under these conditions, the electrolyte content of SHR and WKY dams' milk did not differ significantly over the first 2 postnatal weeks.

Despite the lack of difference in electrolyte concentration, SHR pups do experience differences in electrolyte intake as a result of lower milk production by the SHR dam. We (40) and others (53) have shown that naturally reared SHR pups ingest significantly less milk over the first 7-10 days of lactation. This reduction in milk intake is not the result of an inability of SHR pups to suckle adequately and stimulate a milk ejection reflex, as SHR pups suckling a WKY dam ingested more milk than their naturally reared counterparts (40). Rather, it appears that SHR dams secrete less milk during the early stages of lactation than WKY dams. Milk yield by SHR dams at postnatal day 6 was approximately 50% lower than the yield of WKY dams. Accordingly, SHR pups ingested all of the available milk during a test feed period, whereas WKY pups ceased feeding before the milk supply was exhausted, but not before ingesting more milk than the SHR pups (40). The consequence of this difference in milk intake is that SHR pups ingested significantly less sodium and calcium over the first 2 postnatal weeks compared with both WKY pups and cross-fostered SHR (39).

Postnatal programming mechanisms

Less is known about the potential mechanisms which could account for the blood pressure-lowering effect of cross-fostering in the

SHR. It seems likely that milk composition and also volume are of importance, but the precise constituents which might influence blood pressure in the young SHR are unknown. We have shown that SHR pups receive significantly less sodium than WKY pups and their calculated sodium balance over the first 2 critical weeks of lactation is some 40% lower at a stage when both the cardiovascular and renal systems of the rat are still developing. The importance of this observation is not yet clear, but it is interesting to note that the activity of the renal RAS in the SHR is greatly increased at this age.

Renal renin activity in both SHR and WKY neonates increases rapidly during the later stages of gestation and the early postnatal period, which is in accord with a reported increase in renal AT_1 mRNA levels at this stage (54). However, SHR pups have significantly higher concentrations of renal renin than WKY pups from birth until the beginning of the third postnatal week (55), as well as increased expression of angiotensinogen mRNA (56) and renal AT_1 receptor density (57). The consequence of this up-regulation in renal RAS activity in the SHR pup may be gross changes in renal haemodynamics. Harrap and Doyle (58) have carried out a number of detailed studies which have revealed that the elevated renal renin concentration of the SHR is linked to increased renal vascular resistance and thus to a reduced renal blood flow and glomerular filtration rate (GFR) (59). These renal abnormalities have been genetically linked to the development of high blood pressure in the SHR (60) and brief ACE inhibition, which permanently lowers blood pressure in the SHR (60), also permanently lowers renal vascular resistance (59,60). Furthermore, we have recently shown that cross-fostering reduces renal tubular sensitivity to angiotensin II in the young SHR. In an anaesthetised preparation, a non-pressor dose of angiotensin II reduced GFR and fractional sodium excretion in the naturally reared SHR, whereas sodium handling was unaltered in the cross-fostered SHR (Gouldsborough I and Ashton N, unpublished results). These data suggest that the RAS plays an important role in the development of hypertension in the SHR and that a reduction in its activity may contribute to the blood pressure-lowering effect of cross-fostering.

Conclusion

Animal models have clearly shown that cardiovascular programming occurs during critical phases of perinatal development. Despite their focus on different stages of development, the *in utero* protein-restricted rat and the cross-fostered SHR share some common features. In both models renal function is altered as a result of changes in renin-angiotensin system activity. In the protein-restricted rat exposure to maternal glucocorticoid excess *in utero* results in organ-specific growth retardation with specific inhibition of nephrogenesis and an ACE inhibitor reversible increase in blood pressure. In the naturally reared SHR renin-angiotensin system activity is increased coincident with sodium deficiency and adult blood pressure may also be permanently reduced by an ACE inhibitor. Cross-fostering SHR pups, which restores sodium balance during the period when renin-angiotensin system activity is elevated, lowers adult blood pressure to the same degree as ACE inhibition and restores the kidney's ability to handle sodium when challenged with angiotensin II. These models offer an opportunity to explore the phenomenon of cardiovascular programming in detail and may provide valuable insight into the mechanisms involved in the initiation of hypertension in man.

Acknowledgments

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