

Prenatal dexamethasone 'programmes' hypotension, but stress-induced hypertension in adult offspring

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Abstract

Low birth weight in humans is predictive of hypertension in adult life. Although the mechanisms underlying this link remain unknown, fetal overexposure to glucocorticoids has been implicated. We previously showed that prenatal dexamethasone (DEX) exposure in the rat lowers birth weight and programmes adult hypertension. The current study aimed to further investigate the nature of this hypertension and to elucidate its origins. Unlike previous studies, we assessed offspring blood pressure (BP) with radiotelemetry, which is unaffected by stress artefacts of measurement. We show that prenatal DEX during the last week of pregnancy results in offspring of low birth weight (14% reduction) that have lower basal BP in adulthood (~4–8 mmHg lower); with the commonly expected hypertensive phenotype only being noted when these offspring are

subjected to even mild disturbance or a more severe stressor (up to 30 mmHg higher than controls). Moreover, DEX-treated offspring sustain their stress-induced hypertension for longer. Promotion of systemic catecholamine release (amphetamine) induced a significantly greater rise of BP in the DEX animals (77% increase) over that observed in the vehicle controls. Additionally, we demonstrate that the isolated mesenteric vasculature of DEX-treated offspring display greater sensitivity to noradrenaline and other vasoconstrictors. We therefore conclude that altered sympathetic responses mediate the stress-induced hypertension associated with prenatal DEX programming.

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Introduction

Much evidence suggests that transient adversity in the prenatal environment exerts permanent effects on subsequent physiology and increases disease risk in adult life (Curhan *et al.* 1996b, Fall *et al.* 1998, Law *et al.* 2001, Seckl 2004). A plethora of epidemiological studies have shown that low birth weight, a proposed surrogate for intrauterine adversity, is associated with hypertension (Barker 1990), type 2 diabetes (McCance *et al.* 1994), the metabolic syndrome and deaths from ischemic heart disease in later life (Curhan *et al.* 1996a, Barker 1999). To explain these links, the concept of developmental 'programming' has been advanced, whereby a factor acting during a critical developmental window affects the ontogeny of specifically vulnerable cell targets, permanently altering tissue structure and/or function and hence the eventual risk of disease (Benediktsson *et al.* 1993, Barker 2003, Seckl 2004). However, such environmentally determined developmental effects are widespread in the animal kingdom and often associate with phenotypes which appear to represent a finely balanced transient advantage in a particular environment. Such developmental 'trade-offs' can become disadvantageous late in the lifespan.

Fetal 'programming' may be investigated in a controlled manner using laboratory animals that age relatively rapidly and have similar or identical genetic backgrounds. In rats and many

other mammalian species, fetal exposure to excess glucocorticoids via maternal stress, inhibition of placental 11 β -hydroxysteroid dehydrogenase type 2, the normal 'barrier' to maternal glucocorticoids, or its bypass with non-substrate synthetic glucocorticoids, such as dexamethasone (DEX), commonly used in obstetric practice, all lower birth weight. The adult offspring show permanently elevated glucose and insulin levels and hypertension (Levitt *et al.* 1996, Nyirenda *et al.* 1998, 2001, Langdown *et al.* 2001, Sugden *et al.* 2001). Although programmed increases in the expression of key hepatic gluconeogenic enzymes may later underpin hyperglycaemia (Nyirenda *et al.* 1998, 2001), little is known of the mechanisms by which glucocorticoids 'programme' higher blood pressure (BP) or whether this phenotype carries any advantage.

Prenatal DEX, specifically in the last trimester, affects the development and maturation of specific organs related to BP control and maintenance; notably the developing heart, kidney, vasculature and brain (Torres *et al.* 1997, Ortiz *et al.* 2003, Kreider *et al.* 2005, Roghair *et al.* 2005). Alterations in the activity or responsiveness of the sympathetic nervous system (SNS), either systemically or in one of these specific regions, could provide a potential explanation for the manner in which prenatal glucocorticoids promote adulthood hypertension. Indeed, in humans, increased SNS activity has long been proposed as a mechanism to explain the pathogenesis of

glucocorticoid-induced hypertension (Whitworth *et al.* 1995, Saruta 1996). Consistent with this hypothesis, elevated SNS activity, established *in utero*, links small size at birth with raised BP in adult life (Phillips & Barker 1997). In rats, prenatal DEX alters the development of cardiac and central sympathetic innervation and activity (Slotkin *et al.* 1992, Bian *et al.* 1993). Permanent changes in the pattern and expression of adrenergic receptors (Huff *et al.* 1991, Bian *et al.* 1992) have the potential to alter vascular responsiveness to vasoconstrictors. Similar findings have been reported with DEX administration during pregnancy in sheep (Stein *et al.* 1994, Padbury *et al.* 1995, Tseng *et al.* 1995).

To study more closely diurnal variation and the role of the SNS in adult BP in animals prenatally treated with excess glucocorticoids, we used the DEX-exposed rat model. Radiotelemetry (Anderson *et al.* 1999) enabled the remote recording of BP in conscious, unrestrained rats with minimal stress effects.

Materials and Methods

Animals

Adult Wistar rats (200–250 g; Harlan UK Ltd, Bicester, UK) were maintained under conditions of controlled lighting (lights on 0700–1900 h) and temperature (22 °C) with *ad libitum* access to food (standard rat chow) and water. All experiments were performed according to the UK Animals (Scientific Procedures) Act, 1986.

Prenatal treatments

Virgin female rats were housed with male rats. Pregnancy was confirmed by the presence of a vaginal plug, checked every morning. Pregnant females were housed singly, and randomly assigned to one of two treatment groups ($n=9-10$ per group). DEX (100 µg/kg per day, dissolved in 4% ethanol in 0.9% saline, 200 µg/ml; Sigma–Aldrich) was injected (subcutaneously) daily (0900 h) on embryonic days 15–21 inclusive (Nyirenda *et al.* 1998); control animals received vehicle (VEH) injections.

Litters

On the day of birth (postnatal day 1), litters were weighed, sexed and culled to eight pups per litter, retaining equal numbers of male and female pups where possible. Litters were then left undisturbed till weaning (postnatal day 21), apart from routine weekly maintenance. After weaning, male and female pups from each litter were housed in single sex groups of two to four. All rats underwent a minimum of 2 weeks of daily handling prior to being utilised in experiments. Females were tested during oestrus, confirmed by vaginal cytology. Epithelial cells were aspirated from the vagina with 1 ml saline and placed on a glass slide for microscopic inspection. The oestrous cycle was then staged according to the *Journal of Animal Technicians Association*, 12, No. 1. Staging was independently verified by at least one other researcher/animal technician.

Radiotelemetry monitoring of offspring BP, heart rate and activity

BP (systolic BP, SBP; diastolic BP, DBP; mean arterial pressure, MAP and pulse pressure, PP), heart rate (HR), and activity were assessed by radiotelemetry (Dataquest IV, Data Sciences International, St Paul, MN, USA; Anderson *et al.* 1999). Randomly selected littermates (five males and five females, from eight vehicle and eight DEX litters) at 7–8 months were anaesthetised with halothane. A flexible catheter was secured in the abdominal aorta, and the telemetry transmitter was sutured to the abdominal wall. On recovery, rats were housed in individual cages, and each cage was placed over a receiver panel with output to a PC. Each cage and its receiver panel were housed in a specially designed steel cage rack, to prevent radio signal disturbance from neighbouring animals, and the rack was stored in a quiet room. After a 10-day recovery period, haemodynamic measurements were recorded for 10 s every 30 min (Khan *et al.* 2003).

Induction of stress by disturbance, weighing and restraint procedure

Offspring were subjected to a series of graded stressors: i) simple disturbance (a researcher entering their room); ii) being weighed and iii) restraint in a Perspex cylinder for 15 min. To evaluate the stress response to weighing, haemodynamic measurements were recorded prior to and immediately after removal of the rat from its cage and again at 5-min post-weighing, when the animal was replaced. For the restraint procedure, measurements were collected prior to the rat being placed in the cylinder, throughout the 15-min restraint period, and at 15-min post-restraint, on being returned to its cage. A rest period of 2 days was allowed between experiments.

Haemodynamic responses to alterations in catecholaminergic mechanisms

Haemodynamic responses were collected for 15 min following a low dose of *i.p.* D-amphetamine (0.5 mg/kg), to cause systemic catecholamine release.

Vascular responses to noradrenaline, vasopressin and potassium chloride

Pairs of rats (one VEH and one DEX) were anaesthetised with sodium pentobarbitone, and the mesenteric vasculature was catheterised and perfused with Krebs bicarbonate Ringer's solution, and isolated as previously described (McGregor 1965). Perfusion pressure with constant flow rate was measured continuously via an Elcomatic EM 720 transducer, and recorded on a MacLab Chart V3.3.5 program (ADI, Oxford, UK). On alternate days, mesenteric preparations from each treatment group were attached to alternate transducers, to exclude the possibility of any positional artefact. Following a 15-min equilibration period, a brief 'wake-up' bolus of 20 µM noradrenaline (NA; Sigma–Aldrich) was administered (Hadoke *et al.* 2006).

Twelve pairs of mesenteric vasculature preparations from both male and female offspring were tested simultaneously for periods of 3 min with increasing concentrations of agonists in the following order: NA (0.1–20 μM), vasopressin (AVP; 0.5–20 nM; Sigma–Aldrich) and potassium chloride (KCl; 25–125 mM), all agonists were prepared in Ringer's solution. Between tests, the vasculatures were infused with Ringer's solution alone, during which time the perfusion pressure returned to basal values. A recovery period of at least 30 min was allowed between each agonist.

Statistical analyses

BP, HR and activity Basal BP parameters (SBP, DBP, MAP, PP and HR) and activities for each prenatal treatment (VEH or DEX) and gender, are shown in Table 2A (means ± s.d). Data are calculated for each rat as an average over 3 days of telemetry recording. Statistical analysis of the data was by multivariate repeated measures MANOVA (with prenatal treatment, gender and light/dark period as dependent variables, and rat identification number/representative of each litter and time of day as random variables) which was carried out on the raw BP data for each rat and significance values for each dependent variable, together with interactions between variables are shown in Table 2.

Values following drug treatment (amphetamine) were analysed by three-way ANOVA with prenatal treatment, amphetamine treatment and gender as variables, followed by Dunnett's *post hoc* test. Statistical significance was assumed at a value of $P < 0.05$.

Vascular studies All data are mean ± s.e.m. For each mesenteric preparation, the maximal response and the concentration of agonist required for a 50% response (EC₅₀) were determined. Differences in the mean log EC₅₀ and maximal response values between VEH- and DEX-treated offspring mesenteric vasculatures were compared using Student's unpaired *t*-test. Data were further analysed by two-way ANOVA (prenatal treatment × dose of agonist); results were considered significant when $P < 0.05$.

Results

Gestational weight gain, birth phenotype and catch-up growth

DEX administration throughout the final week of gestation resulted in a significant reduction in maternal weight gain, with no differences noted in the length of gestation, litter size, ratio of male to female pups born or pup viability (Table 1). Consistent with previous studies, DEX caused a significant reduction in the birth weight of offspring, which was observed to the same degree in both male and female pups (O'Regan *et al.* 2004). By weaning (postnatal day 21) and throughout adult life, the body weights of male and female offspring, treated with VEH or DEX were similar, that is, full

Table 1 Maternal weight before treatment, maternal weight gain during the last week of pregnancy (days 15–21), gestation length, litter size, male:female ratio and group, male, and female offspring weight at birth, postnatal day 21, and at 7 months of age (i.e. adulthood). Results are mean ± s.e.m.

	Maternal weight before treatment (g)	Maternal weight gain (g)	Gestation length (d)	Litter size	Male:female ratio	Birth weight (g)			Postnatal weight Day 21			Weight at 7 months		
						Group	Male	Female	Group	Male (g)	Female	Group	Male (g)	Female
VEH	365 ± 14 (n=8)	97 ± 5	22 ± 0	13 ± 2.0	1.1 ± 0.1	6.6 ± 0.1 (n=99)	6.6 ± 0.9 (n=55)	6.5 ± 0.8 (n=44)	55.7 ± 1.1 (n=52)	56.4 ± 1.7 (n=26)	57.6 ± 1.2 (n=26)	525 ± 13 (n=20)	690 ± 12 (n=10)	360 ± 15 (n=10)
DEX	363 ± 6 (n=9)	58 ± 5*	22 ± 0	12 ± 1.8	1.2 ± 0.2	5.7 ± 0.1† (n=108)	5.6 ± 0.8† (n=56)	5.7 ± 0.7† (n=52)	52.7 ± 1.4 (n=56)	54 ± 2.1 (n=29)	55.2 ± 1.2 (n=52)	507 ± 19.5 (n=20)	653 ± 25 (n=10)	353 ± 13 (n=10)

* $P < 0.05$, † $P < 0.01$ compared with vehicle group.

catch-up growth had occurred (Table 1). Kidney size was not apparently altered by prenatal glucocorticoid treatment in either males (VEH: 0.005 ± 0.0002 g/g bwt, $n=7$ and DEX: 0.0051 ± 0.00014 g/g bwt, $n=5$) or females (VEH: 0.00535 ± 0.00011 g/g bwt, $n=5$ and DEX: 0.00495 ± 0.000177 g/g <0.001 bwt, $n=6$).

Radiotelemetric monitoring of offspring BP, HR and activity under basal conditions

BP, HR and activity showed the expected sex variations and circadian variation in male and female offspring of both the prenatal treatment groups with the highest BP (MAP, SBP and DBP), HR and activity levels in the dark phase and lowest values during the light phase (Table 2 and Figs 1 and 2). Contrary to previous reports using indirect (tail cuff) and

direct but stressful (carotid cannulation) methods to assess BP, both male and female DEX offspring showed basal hypotension with lower SBP, DBP, PP and HR compared with VEH rats (Figs 1 and 2 and Table 2), which was not dependent on whether the measurements were taken in the active, dark phase or the quiescent, light phase. However, there was a significant interaction between BP parameters and gender (Table 2), greater effects of prenatal treatment (PNT) being apparent in the males.

It was also observed that the DEX rats were less active than the VEH controls (Table 2), this effect was greater in the females compared with the males (interaction of PNT and gender, $P<0.001$) and was observed mostly in the active, dark phase of the 24 h (interaction of PNT and day/night phase, $P<0.001$). The reduced activity of the DEX rats may contribute to the observed hypotension.

Table 2 (A) Blood pressure parameters measured under basal conditions in adult offspring of VEH- and dexamethasone (DEX)-treated dams. (B) Table of P values resulting from MANOVA of telemetry data in A. Dependent variables are prenatal treatment (VEH or DEX), gender and day or night measurements. Results are mean \pm s.d. (number of observations), from all data collected over 72-h period for each rat

(A) Telemetry data

	VEH ♂	DEX ♂	VEH ♀	DEX ♀
Systolic (mmHg)				
Av daily BP	144.5 \pm 10.6 (750)	140.2 \pm 15.2 (717)	138.3 \pm 10.4 (450)	129.7 \pm 12.7 (749)
Av BP in light phase	141.7 \pm 9.5 (390)	138.3 \pm 16.2 (386)	135.2 \pm 10.2 (234)	126.6 \pm 11.8 (390)
Av BP in dark phase	147.6 \pm 11.0 (360)	142.5 \pm 13.7 (331)	141.6 \pm 9.5 (216)	133.1 \pm 12.8 (359)
Diastolic (mmHg)				
Av daily BP	106.1 \pm 9.8 (750)	103.3 \pm 12.8 (717)	101.5 \pm 8.9 (450)	99.4 \pm 13.0 (749)
Av BP in light phase	103.4 \pm 8.6 (390)	101.6 \pm 13.4 (386)	99.4 \pm 8.4 (234)	96.3 \pm 12.0 (390)
Av BP in dark phase	109.0 \pm 10.2 (360)	105.3 \pm 11.4 (331)	103.8 \pm 8.9 (216)	102.7 \pm 13.3 (360)
MAP (mmHg)				
Av daily BP	118.9 \pm 10.6 (750)	115.6 \pm 15.5 (717)	113.8 \pm 9.2 (450)	109.4 \pm 4.7 (749)
Av BP in light phase	116.1 \pm 8.7 (390)	113.9 \pm 14.4 (386)	111.3 \pm 8.9 (234)	106.4 \pm 11.7 (390)
Av BP in dark phase	121.9 \pm 10.3 (360)	117.7 \pm 12.1 (331)	116.4 \pm 8.9 (216)	112.7 \pm 12.9 (359)
Pulse pressure (mmHg)				
Av daily PP	38.5 \pm 3.9 (750)	36.9 \pm 3.7 (717)	36.8 \pm 4.0 (450)	30.4 \pm 5.9 (749)
Av PP in light phase	38.3 \pm 3.7 (390)	36.7 \pm 3.8 (386)	35.8 \pm 3.8 (234)	30.3 \pm 5.1 (390)
Av PP in dark phase	38.7 \pm 4.2 (360)	37.2 \pm 3.6 (331)	37.8 \pm 4.08 (216)	30.4 \pm 5.9 (359)
Heart Rate (beats/min)				
Av daily HR	339.3 \pm 60.4 (750)	349.2 \pm 62.3 (717)	366.9 \pm 64.2 (450)	366.0 \pm 62.2 (750)
Av HR in light phase	306.7 \pm 44.1 (390)	323.1 \pm 53.3 (386)	335.7 \pm 54.8 (234)	332.1 \pm 48.4 (390)
Av HR in dark phase	374.6 \pm 55.7 (360)	379.6 \pm 58.4 (331)	400.7 \pm 56.0 (216)	398.7 \pm 56.5 (359)
Activity (arbitrary units)				
Av daily activity	2.88 \pm 2.81 (745)	2.51 \pm 2.50 (745)	4.85 \pm 4.38 (447)	3.50 \pm 3.74 (750)
Av activity in light phase	1.41 \pm 1.73 (385)	1.29 \pm 1.58 (385)	2.53 \pm 2.90 (231)	1.70 \pm 2.25 (385)
Av activity in dark phase	4.46 \pm 2.88 (360)	3.82 \pm 2.64 (360)	7.32 \pm 4.34 (216)	5.42 \pm 4.05 (360)

(B) P values resulting from MANOVA of telemetry data

Parameter	PNT	Day/night	Gender	Interaction PNT \times gender	Interaction PNT \times day/night phase
SBP	0.001	0.001	0.001	0.003	0.467
DBP	0.001	0.001	0.001	0.008	0.847
MAP	0.001	0.001	0.001	0.489	0.894
PP	0.001	0.001	0.001	0.001	0.017
HR	0.038	0.001	0.001	0.002	0.236
Activity	0.001	0.001	0.001	0.001	0.001

Bold numbers indicate significance ($P<0.05$) of dependent variables and interactions of these variables. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate.

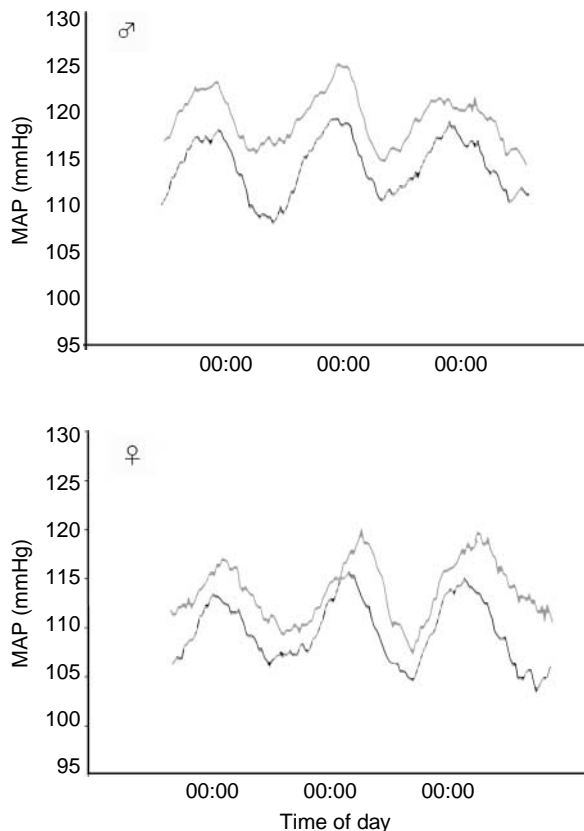


Figure 1 Basal mean arterial blood pressure (MAP) in male and female offspring exposed to VEH (grey) or DEX (black) during the last week of gestation. Data are expressed as 6-h rolling averages over 3 days for each treatment group. $n=4-5$ per group.

Haemodynamic and activity responses to graded stress

In utero exposure to DEX has been shown to cause a lifelong elevation in basal BP when measured by tail-cuff plethysmography and carotid cannulation. An exaggerated stress response to these measurement techniques could account for apparent hypertension. As shown in Fig. 3 (A, B and E), male, prenatal DEX-treated offspring were exquisitely sensitive to stress, displaying graded hypertensive responses when disturbed by a researcher entering their room and when restrained. Even weighing elicited a hypersensitive response in DEX-treated offspring (change in SBP; VEH 10 ± 2 mmHg and DEX 19 ± 3 mmHg; $P < 0.05$). Moreover, DEX-treated offspring start with a reduced MAP compared with vehicle controls (Fig. 3A and B) and maintain their stress-induced hypertension for longer once released from restraint (Fig. 3E), and after weighing (change in SBP 5-min post-weighing; VEH 7 ± 5 mmHg and DEX 23 ± 5 mmHg; $P < 0.05$). DEX-treated offspring further displayed significantly greater increases in HR in response to weighing (VEH 117 ± 17 bpm and DEX 156 ± 25 bpm; $P < 0.05$), and restraint (VEH 86 ± 11 bpm and DEX 136 ± 19 bpm; $P < 0.05$). The greater tachycardia on disturbance of prenatal DEX offspring was not

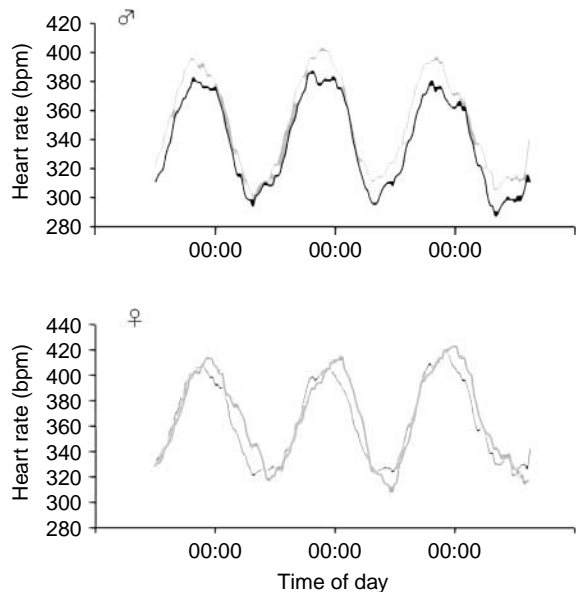


Figure 2 Basal heart rate patterns in male and female offspring exposed to VEH (grey) or DEX (black) during the last week of gestation. Data are expressed as 6-h rolling averages over 3 days. $n=4-5$ per group.

associated with changes in activity (weighing: VEH 5.2 ± 1 AU, DEX 5 ± 1 AU and restraint: VEH 4.8 ± 1.2 AU, DEX 4.6 ± 1.8 AU). Although there was a significant response in BP to stress in all females, there was no significant difference between the VEH- and DEX-treated offspring in response to disturbance or restraint stress but the recovery period after stress shows DEX females take longer to return basal levels of MAP following restraint (Fig. 3C–E).

Prenatal DEX causes exaggerated hypertensive responses to catecholaminergic stimulation

To assess the role of the SNS in mediating this stress-induced hypertension, we administered a low dose of D-amphetamine, to induce catecholamine release. While the DEX-treated offspring showed lower basal MAPs prior to amphetamine administration (Fig. 4A and B), they showed significantly greater increases in MAP and HR when treated with the drug compared with controls (amphetamine treatment $F(1,20) = 21.54$, $P = 0.0002$; prenatal treatment $F(1,20) = 13.19$, $P = 0.0017$; Fig. 4A–C). When the data were separated into male and female responses, it was demonstrated that there was no indication of a sex-specific effect ($F(1,20) = 0.52$, $P = 0.48$; Fig. 4C).

Responsiveness of mesenteric vasculature to NA, AVP and KCL

To examine further the basis for increased responsivity to catecholaminergic stimulation in DEX-exposed offspring, resistance (mesenteric) vessels were perfused *ex vivo* (Fig. 5A–C). Basal perfusion pressures were similar in the two groups of rats. Infusion of all agonists caused a concentration-

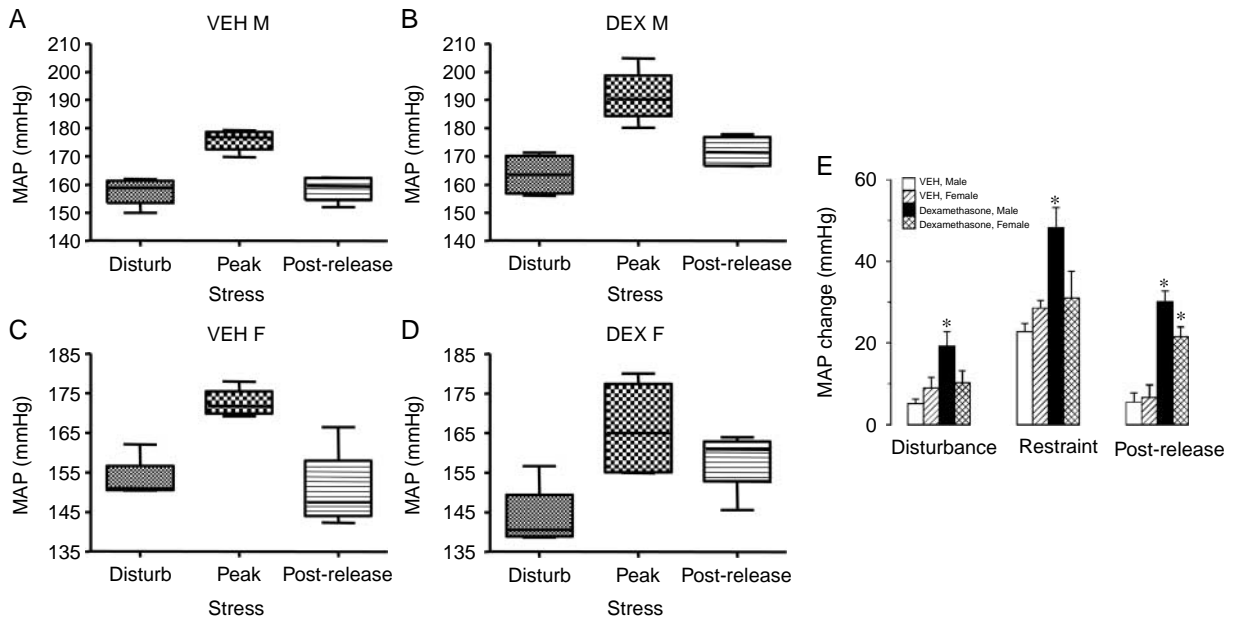


Figure 3 Box-Whisker plots depicting MAP in response to disturbance, restraint and at 15-min post-restraint ($n=4-5$ per group) in (A) male offspring from VEH-treated dams, (B) male offspring from DEX-treated dams, (C) female offspring from VEH-treated dams and (E) female offspring from DEX-treated dams, (E) shows stress-induced MAP changes in prenatal VEH- and DEX-treated offspring, following disturbance, restraint and at 15-min post-restraint (mean \pm s.e.m., $n=4-5$ per group). * $P<0.05$ compared with vehicle group.

dependent increase in perfusion pressures, with plateaux reached in both groups at $5 \mu\text{M}$ for NA and 10 nM for AVP. However, tissues from DEX-treated male offspring were significantly more responsive (the curves from DEX-treated offspring were significantly different from VEH controls; Fig. 5) and the V_{max} (indicative of number of receptors) was higher in the DEX group in response to NA in both sexes (male VEH: $305 \pm 13 \text{ mmHg}$; male DEX: $360 \pm 4 \text{ mmHg}$; $P=0.004$; t -test. Female VEH: $292 \pm 5 \text{ mmHg}$; female DEX: $345 \pm 7 \text{ mmHg}$; $P=0.001$).

Female offspring

Several reports suggest that prenatal 'programming' phenotypes are sexually dimorphic, so the perfusion studies were

repeated in females. Similar basal perfusion pressures were noted in the two prenatal treatment groups. NA caused a concentration-dependent increase in perfusion pressures, with plateaux reached at $2 \mu\text{M}$ in both treatment groups (Fig. 5D). However, the mesenteric vasculatures from DEX-treated offspring were significantly more responsive to NA, (as analysed by two-way ANOVA), even though the maximal contraction (VEH $344 \pm 40 \text{ mmHg}$ versus DEX $373 \pm 30 \text{ mmHg}$; $P=0.16$) and EC_{50} (vehicle $0.43 \pm 0.02 \mu\text{M}$ versus DEX $0.39 \pm 0.03 \mu\text{M}$; $P=0.16$) values were similar for both treatment groups. DEX treatment did not affect the pattern of responses to either AVP or KCl. Maximal responses in both groups were achieved at 5 nM AVP and 125 mM KCl (data not shown).

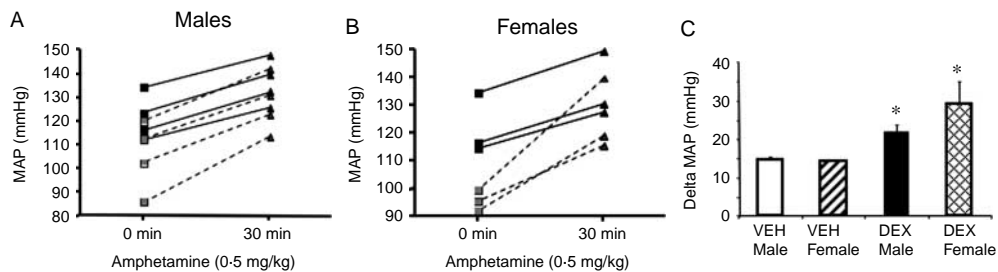


Figure 4 Line plots indicating MAP from individual rats 0 and 30 min following amphetamine (0.5 mg/kg) in (A) male and (B) female offspring from either VEH (black symbols with solid line) or DEX (grey symbols with dotted line) dams. (C) Δ -MAP response to amphetamine (0.5 mg/kg ; i.p.), in male and female offspring of VEH- and DEX-treated dams. Results are mean \pm s.e.m., three-way ANOVA (prenatal treatment \times gender \times postnatal drug), $n=7$ (4 δ and 3 ϕ); * $P<0.05$ compared with vehicle group.

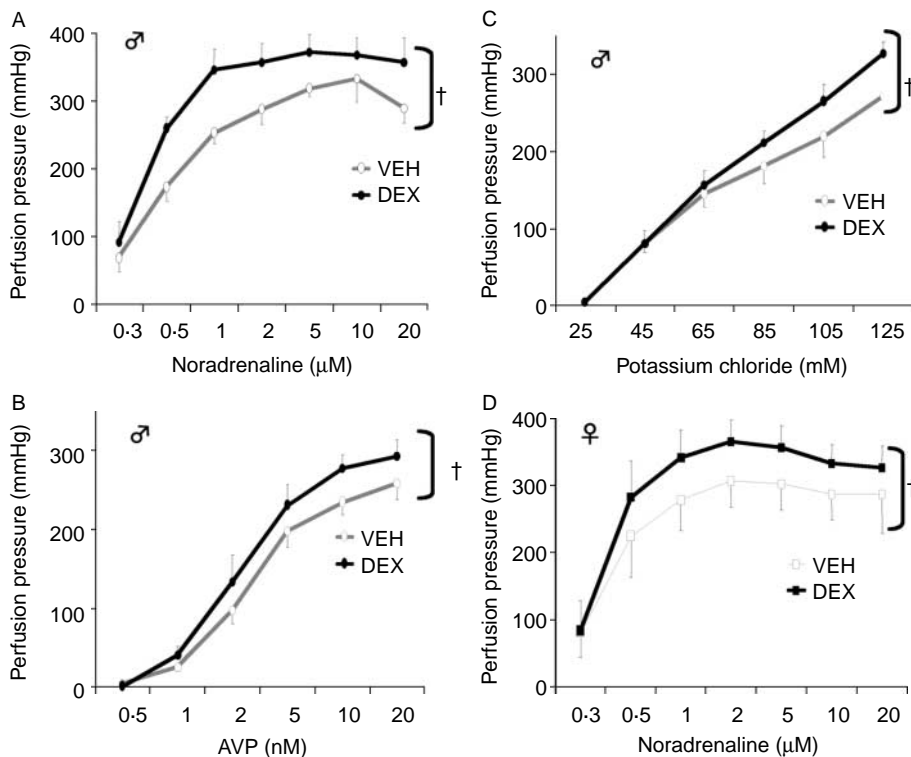


Figure 5 Male offspring dose–response curves to (A) noradrenaline, (B) vasopressin, (C) potassium chloride and (D) female offspring dose–response curve to noradrenaline, in the isolated mesenteric vasculature from VEH- and DEX-treated rats. Results are mean \pm s.e.m., $n=6$ per group. $^{\dagger}P<0.01$ compared with vehicle group.

Discussion

The striking novel finding of this study is that prenatal DEX results in lower basal BP in the male and female adult offspring; with the commonly expected hypertensive phenotype only being noted when these offspring are subjected to a stressor, after which they exhibit a prolonged and excessive hypertensive response. These exaggerated hypertensive responses are mediated by alterations in the responsivity of the SNS, being further exaggerated by promotion of catecholamine release. Additionally, DEX-treated offspring display greater sensitivity to catecholamines, and only in males other vasoconstrictors, in resistance (mesenteric) vasculature.

Unexpectedly, over many days of recording undisturbed rats in their home cages, prenatal DEX-exposed adult rats showed lower BP than controls. This contrasts with both tail cuff plethysmography and direct carotid cannulation data in this model (Levitt *et al.* 1996) and in other models of 'fetal programming' (Woodall *et al.* 1996, Langley-Evans 1997, Woods 2006) most of which have generally employed techniques which involve stressful preheating of the tail and/or physical restraint or short-term recovery from anaesthesia. Indeed, several studies have documented by telemetry the stress-induced hypertension observed following restraint or using the

tail-cuff protocol (Gross & Luft 2003, Whitesall *et al.* 2004, D'Angelo *et al.* 2005, Kurtz *et al.* 2005), confirming the telemetry technique as the 'gold standard' for BP recording. In our study, radiotelemetric measurements were recorded 1 week after implantation of intra-arterial cannulae to allow complete recovery from established effects of surgery. Previous studies have used telemetry to determine BP in other models of fetal programming following maternal undernutrition (Tonkiss *et al.* 1998, Khan *et al.* 2003, Fernandez-Twinn *et al.* 2006) or uterine artery ligation (Jansson & Lambert 1999). BP differences by telemetry have generally been slight, with normotension or at most mild hypertension, compared with more overt hypertension using indirect techniques. The quite clear-cut lower basal BP seen here in males and females in both active and quiescent phases may therefore reflect specific effects of prenatal glucocorticoid excess compared with other manipulations, even though it is acknowledged that glucocorticoid programming is combined with an effect on food intake and maternal weight (present data; Levitt *et al.* 1996, Welberg *et al.* 2001, Woods 2006). However, short-term measurements of BP, even over a few hours, failed to attribute statistical significance between the prenatally treated groups in this study, emphasizing the statistical power of the telemetric BP measurements, but highlighting potential differences between studies and analysis of the data.

The hypotension observed in the DEX rats may in part be attributable to decreased activity in DEX rats compared with VEH. However, activity differences are greater in the female DEX rats (compared with female VEH rats), while BP parameters of hypotension are greater in the male DEX rats indicating that several parameters may be altered to mediate the BP changes and that these mechanisms may be sex specific.

Merely entering the room in which the rats were housed was sufficient to drive hypertension selectively in male, prenatal DEX-exposed offspring. When further subjected to a moderate stressor (weighing) and the more severe restraint stress the hypertensive responses of the male DEX-treated offspring were significantly augmented, suggesting increased sensitivity to environmental stress. An increased reactivity to stressful environmental stimuli may lead to established hypertension through frequent transient BP elevations (Folkow *et al.* 1978). Whether or not the prenatal DEX-exposed offspring will eventually become permanently hypertensive warrants further study, as true life consists of a constant series of 'stressful situations' that have to be dealt with, and this is not mimicked in undisturbed laboratory animals. Indeed, the DEX-treated offspring maintained their hypertension long after the removal of each stressor. Interestingly, offspring of malnourished dams display a greater hypertensive response to stress (ammonia odour) echoing the clear stress-responsive hypertension shown here in prenatal glucocorticoid programming (Tonkiss *et al.* 1998). It is therefore tempting to speculate that prenatal glucocorticoid programming of exaggerated cardiovascular responses to stress may be the fundamental change in these models, with permanent hypertension a secondary phenomenon dependent upon the postnatal environment and its challenges. This would parallel the emerging concepts of amplification of the low birth weight baby metabolic phenotype by postnatal caloric excess (Ozanne & Nicholas Hales 2005). Moreover, lower basal BP but enhanced responses to stress align perhaps better with concepts of adaptive advantages to the predicted (stressful) postnatal environment of a stressed pregnancy than merely constant hypertension, which has few apparent benefits.

DEX-treated offspring had higher HR with stress, a marker, although indirect, of increased sympathetic drive. The cardiodynamic responses to catecholamine release with amphetamine (or catecholamine depletion with reserpine, O'Regan unpublished results) strongly support this notion of a more responsive SNS in DEX-programmed adult rats. Amphetamine caused an exaggerated increase in BP in DEX-treated offspring compared with controls and reserpine abolished BP differences between DEX and VEH groups basally and in response to stress. The SNS participates in several stress responses, even in fetal life (McMillen *et al.* 2001) and therefore prenatal exposure to various stressors might affect the development of sympathetic innervation or its regulation. Epidemiological evidence of HR variability in infants demonstrates a positive correlation of this index of sympathetic activity with low birth weight, suggesting that decreased fetal growth is associated with a dysfunctional autonomic nervous

system which may play a role in programming of BP (Massin *et al.* 2001). Indeed in rats, the offspring of prenatal DEX-treated dams have increased NA turnover in central brain regions concerned with BP control (Slotkin *et al.* 1992), however, cardiac β -adrenergic responses are blunted in low protein diet-induced-programmed rats (Fernandez-Twinn *et al.* 2006). In distinct prenatal rat models, gestational nicotine impairs the maturation of both central and peripheral catecholaminergic pathways (Navarro *et al.* 1990) and hypoxia alters development of sympathetic centres involved in BP regulation (Peyronnet *et al.* 2002), effects which persist into adulthood. Similarly, ligation of the umbilical artery alters SNS activity in newborn sheep (Oyama *et al.* 1992) and adult female rat offspring (Jansson & Lambert 1999). Taken together, these data indicate that diverse challenges *in utero* alter the development and function of the adult SNS. A recent study of low birth weight adult humans proposed that their lower resting muscle sympathetic nerve activities were a function of altered SNS development (Weitz *et al.* 2003).

Sex effects on BP regulation in prenatal-programming models have been shown in a few studies (Kind *et al.* 2002, O'Regan *et al.* 2004), but not others (Langley-Evans *et al.* 1996, Woods & Weeks 2005), although the hypertension may be generated by sex-specific mechanisms (McMullen & Langley-Evans 2005a,b). Indeed in humans, females have been shown to have more efficient regulation of SNS pathways, making them less susceptible to hypertension (Hinojosa-Laborde *et al.* 1999). However, in this study, we show similar effects on BP in males and females, but the magnitude of hypotension and a greater stress-induced hypertension is observed in males. The response to catecholamine release was similar in both sexes. Therefore, further studies will be required to tease out the mechanisms underpinning the sex-specific responsivity in this programming model.

Whatever the changes in SNS drive, DEX-treated offspring showed greater *in vitro* mesenteric pressor responses to NA in both male and females suggesting increased tissue sensitivity. There were also greater pressor responses to AVP and a depolarizing potassium solution in males only. Similar results were obtained in a study looking at female offspring from mothers treated with DEX throughout pregnancy (Hadoke *et al.* 2006). The *in vitro* perfusion preparation used in these studies was separated from other *in vivo* systems (e.g. circulating angiotensin II, endothelin), suggesting that a change in receptor binding or alterations in the production of local vasorelaxants/constrictors underlies the increased vascular sensitivity. Glucocorticoids may regulate the synthesis of vasoactive compounds, such as prostaglandins or nitric oxide, which in turn modulate peripheral vascular reactivity. Indeed, inhibition of nitric oxide synthesis (Wallerath *et al.* 1999) and decreased levels of both prostaglandin and its mediator adenylate cyclase (Handa *et al.* 1984) have been demonstrated in rats with adult DEX-induced hypertension. Similarly, increased mesenteric vasculature sensitivity to NA has been observed in rats treated as adults with low doses of DEX (Russo *et al.* 1990). Whether the effects are due to the known modest elevations of

corticosterone in DEX-programmed rats (Levitt *et al.* 1996, Welberg *et al.* 2001) or reflect primary developmental effects on the mesenteric vasculature that persist into adult life remains uncertain. The gender differences in mesenteric vasculature responses of DEX-treated offspring to both AVP and KCl are intriguing, and could possibly reflect changes in vascular reactivity during the reproductive cycle (Dalle Lucca *et al.* 2000), and the effect of sex steroids on vascular smooth muscle sensitivity (Garcia-Villalon *et al.* 1996). Interestingly, gender has previously been demonstrated to have no effect on the sensitivity to NA in both the rat tail artery and aorta (Li & Duckles 1994, Fulton & Stallone 2002).

The present study provides novel and exciting information regarding the nature and mechanisms of glucocorticoid-induced alterations in postnatal BP. As the prenatal glucocorticoid exposure models extend our understanding of the fetal origins phenomena, it is clear that there is a need for replication and extended studies in humans. Only then, will we be better equipped to understand both the immediate and long-term consequences of antenatal glucocorticoid treatment.

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