Prenatal stress in the rat results in increased blood pressure responsiveness to stress and enhanced arterial reactivity to neuropeptide Y in adulthood

Natalia Igosheva[1], Paul D. Taylor[1], Lucilla Poston[1] and Vivette Glover[2]

[1]*Maternal and Fetal Research Unit, Division of Reproduction and Endocrinology, King's College London, St Thomas' Hospital, London SE1 7EH, UK*
[2]*Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 ONN, UK*

We have shown previously that stress in the pregnant rat leads to a heightened cardiovascular response to restraint in adult offspring. The present study was undertaken to explore further the persistent cardiovascular effects of prenatal stress, with a focus on peripheral vascular function. Sprague–Dawley female rats were exposed to restraint/bright light three times daily in the last week of pregnancy. Litters from stressed and control females were cross-fostered to control dams to eliminate possible effects of maternal stress on nursing behaviour. At 120 days, offspring cardiovascular variables were measured by radiotelemetry. Reactivity of mesenteric small arteries was assessed by myography, and responses to electrical field stimulation determined. Resting cardiovascular parameters in prenatally stressed (PS) offspring were similar to controls but PS rats showed a greater increase in systolic blood pressure following restraint stress ($P < 0.05$). Recovery was also prolonged in PS animals compared with controls and was of longer duration in PS females than in PS males ($P < 0.05$). Adult PS females, but not males, also had elevated basal plasma corticosterone levels in comparison with controls ($P < 0.05$). Vascular reactivity to neuropeptide Y ($P < 0.05$) and electrical field stimulation ($P < 0.05$) in mesenteric arteries was also significantly increased in PS animals. Vascular responses to adrenergic agonists as well as endothelial dilator function did not differ between PS and controls. We conclude that prenatal stress during late gestation has long-lasting effects on cardiovascular responsiveness and vascular reactivity to neuropeptide Y in the offspring.

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Corresponding author N. Igosheva: Maternal & Fetal Research Unit, Division of Reproduction and Endocrinology, King's College London, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK. Email: natalia.igosheva@kcl.ac.uk

Numerous studies in man (Barker, 1995; Stein *et al.* 1996) and experimental animals (Armitage *et al.* 2004; Seckl & Meaney, 2004; McMillen & Robinson, 2005) provide strong evidence to support the suggestion that the early life environment is a determinant of the adult phenotype. Two distinct branches of research in this field have evolved: one follows the Barker hypothesis and focuses on nutrition and developmental programming of cardiovascular (CV) and metabolic diseases (Barker, 1995), and the other investigates prenatal stress and its effect on offspring stress responses and behavioural development (Weinstock, 2001; Owen *et al.* 2005; Van den Bergh *et al.* 2005). Despite apparent divergence in animal models between the types of maternal insult imposed during pregnancy (e.g. stress protocols *versus* nutritional challenge), it now seems that there may be more commonality in offspring phenotype than originally recognized.

We have previously shown in rodents that prenatal stress has persistent effects on the CV system of the offspring (Igosheva *et al.* 2004). Exposure of pregnant rats to restraint and bright light during the last week of pregnancy leads to enhanced systolic blood pressure responses (SBP) and blood pressure variability to acute stress, and delayed post-stress recovery. Although the mechanisms of stress-induced hypertension in this model of prenatal stress have not been directly studied, recent reports, using other interventions, have implicated a possible role for altered sympathetic activity in the developmental programming of altered blood pressure responsiveness. The development of the sympathetic nervous system is susceptible to environmental manipulations during early life (Young, 2006) and therefore fetal and neonatal exposures to different stressors may alter sympathetic control of blood pressure later in life. In the rat, prenatal glucocorticoid (GC) exposure (O'Regan *et al.* 2003) and hypoxia in pregnancy (Peyronnet *et al.* 2002), leads to exaggerated blood pressure responsiveness to acute stress in the adult offspring, which is associated with increased

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vascular sensitivity to sympathomimetics (O'Regan *et al.* 2003) and disturbed metabolism in sympathetic ganglia (Peyronnet *et al.* 2002). Increased sympathetic activity has also been linked to offspring CV dysfunction associated with maternal malnutrition (Lesage *et al.* 2002; Fernandez-Twinn *et al.* 2006) and reduced placental blood flow (Sanders *et al.* 2004*b*; Alexander *et al.* 2005). These observations may have relevance to the model of prenatal stress, since decreased food intake (Matthews, 2002) and reduced uterine blood flow (Morishima *et al.* 1979) as well as increased plasma concentration of GCs (Seckl & Meaney, 2004) have been reported in stressed dams. The underlying mechanisms in these apparently divergent models of developmental programming could therefore have some common features.

We have previously reported that stress-induced hypertension in the offspring of stressed dams was not associated with permanent alterations in arterial baroreflex function. The central sympathetic outflow to the heart was also unaltered since resting heart rate, as well as the magnitude of heart rate stress responses, were unaffected in PS offspring (Igosheva *et al.* 2004). Therefore, it is more likely that, if involved, it would be the peripheral rather than central sympathetic pathways that contribute to altered CV function in PS animals.

The present study was undertaken to characterize further the effect of maternal physical and emotional stress on the cardiovascular function of the adult offspring, and to determine whether a permanent modification of vascular adrenergic function may contribute.

Methods

Animals and induction of prenatal stress

All animal care guidelines and animal procedures followed were licensed under the UK Home Office Animal (Scientific Procedures) Act 1986. Virgin female Sprague–Dawley rats 120 days old were purchased from Charles River Laboratories (UK). For breeding, the females were caged with mature males and on the day of conception were randomly allocated to control ($n = 10$) and stress ($n = 10$) groups. All dams were maintained under standard conditions with *ad libitum* access to water and rat chow. Maternal food intake and body weight was monitored daily.

Prenatal stress protocol. Restraint stress was performed from embryonic day 15 until day 21. The stress protocol involved placing the pregnant female in a plexiglas restraint tube (19 cm × 6 cm × 9 cm) over which was poised two 100 W flood lights. Three 30 min stress interventions were conducted on each day at 9.00, 13.00 and 16.00 h. Control dams were left undisturbed throughout gestation.

This protocol has been employed in several previous studies including our own and shown to significantly affect cardiovascular (Igosheva *et al.* 2004) and neuroendocrine stress reactivity in adult offspring (Szuran *et al.* 2000; Sternberg & Ridgway, 2003).

Two days post-partum, all offspring were cross-fostered to recently parturient control dams and litters were adjusted to eight pups with equal numbers of males and females. Groups studied were: (1) offspring born to stressed dams and suckled by non-stressed mothers (PS, $n = 20$, 10 offspring of each sex); (2) offspring born to non-stressed dams and suckled by foster non-stressed dams (C, $n = 20$, 10 offspring of each sex). All offspring were weaned at 21 days of age. Food intake and body weight were monitored weekly. Two animals of each sex from any one litter were studied at a given age, and these were randomly chosen to remove any litter effects.

Determination of cardiovascular function by radiotelemetry

At 120 days of age, animals were instrumented with a biocompatible radio-telemetry probe (Data Science International, Arden Hills, MN, USA) and blood pressure (systolic, diastolic and mean pressure), heart rate and activity were measured using the Dataquest IV system (Data Sciences International, Arden Hills, USA) as previously described (Khan *et al.* 2003). The rats were anaesthetized by isofluorane inhalation (4%) and administered preoperative buprenorphine (0.1 mg kg^{-1} subcutaneous, Alstoe Animal Health, York, UK). Following a routine laparotomy, the flexible catheter of the radiotelemetry probe was surgically implanted into the descending abdominal aorta. The depth of anaesthesia was monitored by testing for the pedal pinch reflex. Post-operatively, the animal was placed in a recovery chamber pre-heated to 30°C for 1 h. On recovery, rats were housed in individual cages and collection of data for analysis commenced 1 week after surgery. Variables were recorded over 10 s intervals every 5 min for 1 week. To assess acute responses to restraint stress, CV parameters were also recorded continuously for 30 min after placing the animal in the restraint cylinder and 120 min after returning the animal to its home cage.

Determination of isolated artery function

Small artery function was assessed in the same animals used for telemetric recording of cardiovascular function. Animals were fasted overnight (12 h). On the following morning, animals were killed by a rising concentration of CO_2 between 08.00 and 09.00 h. Organs were quickly removed from all animals, weighed and snap frozen in liquid nitrogen and stored at −80°C. Blood samples

for measurement of corticosterone were obtained by cardiac puncture, and plasma was stored at −70°C before analysis. Third order mesentery arteries were dissected, mounted in physiological salt solution (PSS (mM); NaCl 119, $CaCl_2$ 2.5, KCl 4.7, $MgSO_4$ 7 H_2O 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.025, D-(+)-glucose 6) on a small vessel myograph (Model 610M, Danish Myotechnology, Denmark; Mulvany & Halpern, 1977). Arteries were maintained at 37°C, in PSS gassed with 95% oxygen–5% CO_2 mixture to give a pH of 7.4 and subjected to a standard normalization procedure and run-up protocol as previously described (Mulvany & Halpern, 1977). Vascular function was assessed by cumulative concentration–response curves to noradrenaline (NA, 10^{-8}–10^{-6} M), phenylephrine (PE, 10^{-9}–10^{-5} M), the β_1-adrenoreceptor agonist, dobutamine (10^{-9}–10^{-5} M), acetylcholine (10^{-9}–10^{-5} M) and aqueous nitric oxide (10^{-7}–10^{-4} M). Responses to neuropeptide Y (NPY, 10^{-9}–10^{-6} M) and vasodilators were determined in arteries pre-activated with NA (80% of maximal constriction). Electrical field stimulation (EFS) was performed by passing a stimulating current across specially adapted myograph jaws fitted with platinum electrodes (Danish Myotechnology, Denmark). Arteries were subjected to 0.3 ms pulse, 20 s trains of 40 mA current stimulation using a bipolar current with 3 min between each train (electrical stimulator CS200, Danish Myotechnology, Denmark). The arteries were stimulated at frequencies of 4, 8, 16 and 32 Hz and a frequency–tension response relationship was recorded. In all experiments two arteries from each rat were investigated simultaneously and the results expressed as the average response.

Corticosterone assay

Plasma corticosterone (CS) was evaluated using a commercial double antibody radioimmunoassay kit (Immunodiagnostic Systems, Boldon, UK).

Statistical analysis

Results are expressed as the mean ± S.E.M. with $P < 0.05$ considered significant. Gestational length, litter size, birth weight, organ weight, body weight and the basal plasma corticosterone concentration were compared between groups by unpaired Student's t test. Radio-telemetric data were expressed as the mean value over 12 h night (active) and day (inactive) periods and analysed by repeated measures analysis of variance (ANOVA). Cardiovascular stress responses were expressed as a percentage change from baseline for both stress and recovery periods and evaluated using mixed design repeated measures ANOVA. Responses to constrictor agonists were expressed as active wall tension (mN mm^{-1}). NPY and dilator responses were expressed as percentage of NE-induced constrictor tone. Concentration–response curves were analysed by fitting individual concentration–response data to a sigmoidal logistic curve using Graphpad Prism version 2.01 (Graphpad Software SanDiego, CA, USA). All statistical analyses were performed using Statistica version 5.0 for Windows (Statsoft Inc., Tulsa, OK, USA).

Results

Effect of prenatal stress on maternal and offspring parameters

Body weight increased during pregnancy in both control and stressed dams ($F = 181$, $P < 0.001$, ANOVA). Restraint stress during the last week of pregnancy had a significant effect on the weight gain of dams ($F = 5.1$; $P < 0.05$, ANOVA) with stressed dams exhibiting a lower

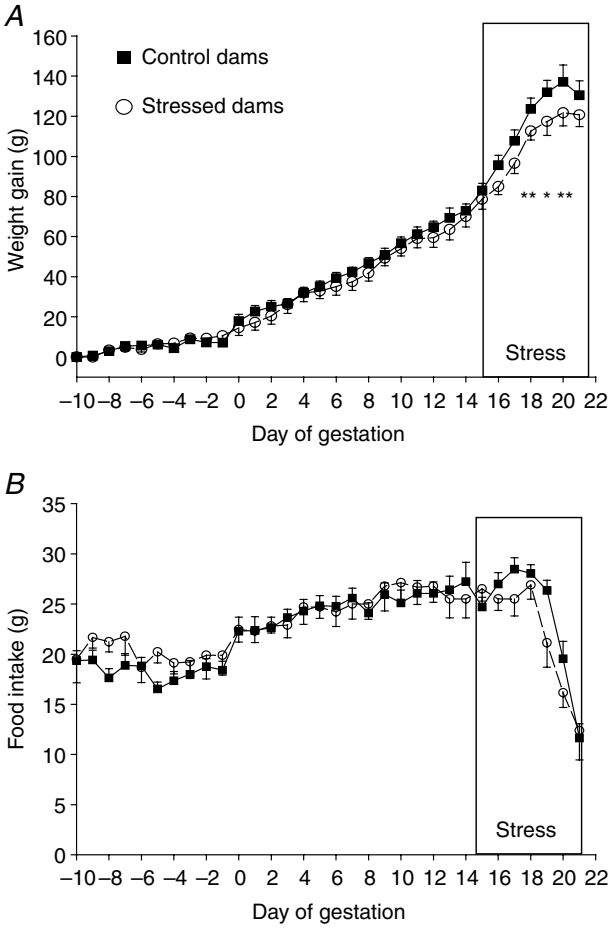

Figure 1. Body weight change and food intake
Body weight change (*A*) and food intake (*B*) in control (■, $n = 10$) and stressed mothers (○, $n = 10$) 10 days before and during pregnancy. *$P < 0.05$ control *versus* stressed dams.

increase in weight from gestation day 16 (GD 16) to GD 21 (Fig. 1*A*). There was a trend for the stressed dams to have a lower food intake than controls but this did not reach statistical significance ($F = 3.8$, $P = 0.07$, by repeated measures ANOVA, Fig. 1*B*).

Prenatal stress had no effect on gestational length (control 22 ± 0.3 days, stressed 21.6 ± 0.5 days), litter size (control 12.5 ± 0.9 pups, stressed 12.6 ± 0.5 pups) and the sex ratios of the litters. At birth the body weights of the offspring born to stressed mothers were not different from those of control mothers (control 7.4 ± 0.6 g *versus* stressed 6.7 ± 0.4 g, $P = 0.34$). Prenatal stress did not affect weight gain in the offspring during both pre- and post-weaning periods.

No significant effect of PS was observed on body and organ weights of the adult offspring at 120 days of age (data not shown).

The basal plasma corticosterone concentration did not differ between control females and control males. ANOVA analysis of the basal corticosterone concentrations revealed a significant prenatal stress–gender interaction ($F = 6.5$, $P < 0.05$) attributable to a higher concentration in the adult PS female offspring compared with control females (PS, 161 ± 21 ng ml^{-1}, $n = 10$ *versus* C, 107 ± 14 ng ml^{-1}, $n = 10$, $P < 0.05$). The basal plasma CS concentration did not differ between PS and control males (PS, 70 ± 5 ng ml^{-1}, $n = 10$ *versus* C, 82 ± 11 ng ml^{-1}, $n = 10$). Thus, the effect of prenatal stress was gender-specific ($F = 19.3$, $P < 0.001$) with PS females having a significantly higher CS plasma concentration than PS males (PS, females 161 ± 21 ng ml^{-1}, $n = 10$ *versus* PS, males 70 ± 5 ng ml^{-1}, $n = 10$).

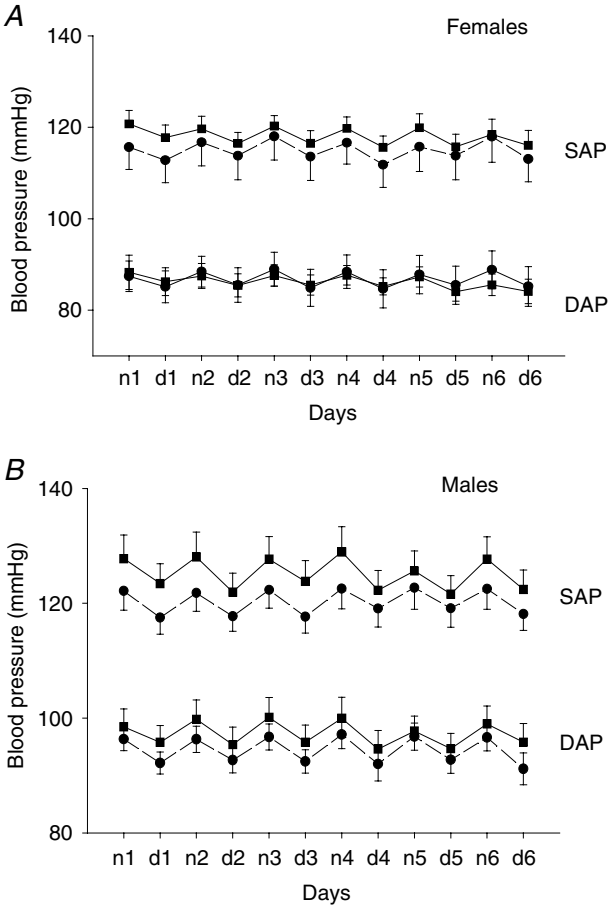

Figure 2. Systolic and diastolic blood pressures
Systolic and diastolic blood pressures in female (*A*) and male (*B*) offspring of control (■, $n = 10$) and stressed dams (●, $n = 10$) at 120 days of age. Values are expressed as the mean ± S.E.M. of 12 h night (n) and day (d) 12 h averages over 6 days (1–6).

Effects of prenatal stress on cardiovascular function

Twenty four hour radiotelemetry monitoring of offspring blood pressure, heart rate and activity. At 120 days of age, basal systolic, diastolic arterial pressure (Fig. 2*A* and *B*) and heart rate did not differ between the groups. The diurnal variation of cardiovascular parameters and motor activity were also found to be similar in PS offspring and controls.

Cardiovascular responses to restraint stress and recovery in control and PS rats. Figure 3*A* and *B* shows changes from baseline in systolic blood pressure (SBP) in PS and control rats during restraint stress and the recovery period. ANOVA analysis revealed a marked difference in SBP stress responses between the groups (prenatal treatment $F = 5$, $P < 0.05$; prenatal treatment–time interaction $F = 3.5$, $P < 0.05$). The magnitude of SBP stress-induced responses was significantly higher in PS rats than in controls. There was no significant effect of gender on SBP stress responsiveness. However, PS females showed a protracted duration of the SBP stress response compared with PS males (30 min *versus* 20 min, respectively). SBP responses during the post-stress period were also modified by prenatal stress ($F = 4.4$, $P < 0.05$, ANOVA). Recovery to baseline SBP after return to the cage was delayed in PS rats in comparison with controls: increased SBP values were sustained for 95 min in PS rats compared with only 45 min in controls. There was a significant interaction of sex and time ($F = 3.3$, $P < 0.05$, ANOVA) in PS group indicating that PS females had higher SBP responses than PS males during recovery ($P < 0.01$, Duncan's *post hoc* test).

As shown in Fig. 3*C* and *D* restraint stress induced a significant but transient increase in diastolic blood pressure (DBP) in all animals ($F = 27$, $P < 0.001$, ANOVA). ANOVA revealed a significant effect of gender ($F = 5$, $P < 0.05$) with male rats having higher DBP stress responses than females regardless of prenatal conditions. There was no effect of prenatal stress on DBP responses during either the restraint stress or recovery period.

No effect of prenatal treatment or gender was found for HR responses during either the stress or the post-stress periods.

Effect of prenatal stress on peripheral vascular function

Constrictor responses of mesenteric arteries to adrenergic agonists and neuropeptide Y (NPY). Baseline diameters (μm) of the arteries were as follows: control females 203 ± 10, PS females 206 ± 7, control males 215 ± 6 and PS males 218 ± 7.

There were no gender differences in NPY-induced vasoconstriction and so data from females and males were pooled for further analysis. Whilst the sensitivity to NPY was not significantly different among the groups (EC_{50}: C: -7.7 ± 0.2, PS -7.9 ± 0.2), PS offspring showed enhanced reactivity to NPY across the entire concentration range compared with controls ($F = 4.5, P < 0.05$, ANOVA, Fig. 4*A*)

There were no differences in dose–response curves to either noradrenaline or phenylephrine between the groups (Fig. 4*B* and *C*).

Electrical field stimulation (EFS) induced a frequency-dependent vasoconstriction in all rats ($F = 23$, $P < 0.001$; ANOVA; Fig. 4*D*). Gender did not have a significant effect on EFS-induced vasoconstriction and so data from females and males were pooled for further analysis. ANOVA revealed a significant effect of prenatal stress ($F = 5.7$, $P < 0.05$, ANOVA) and a significant prenatal conditions–frequency interaction ($F = 5$, $P < 0.01$, ANOVA). *Post hoc* comparisons

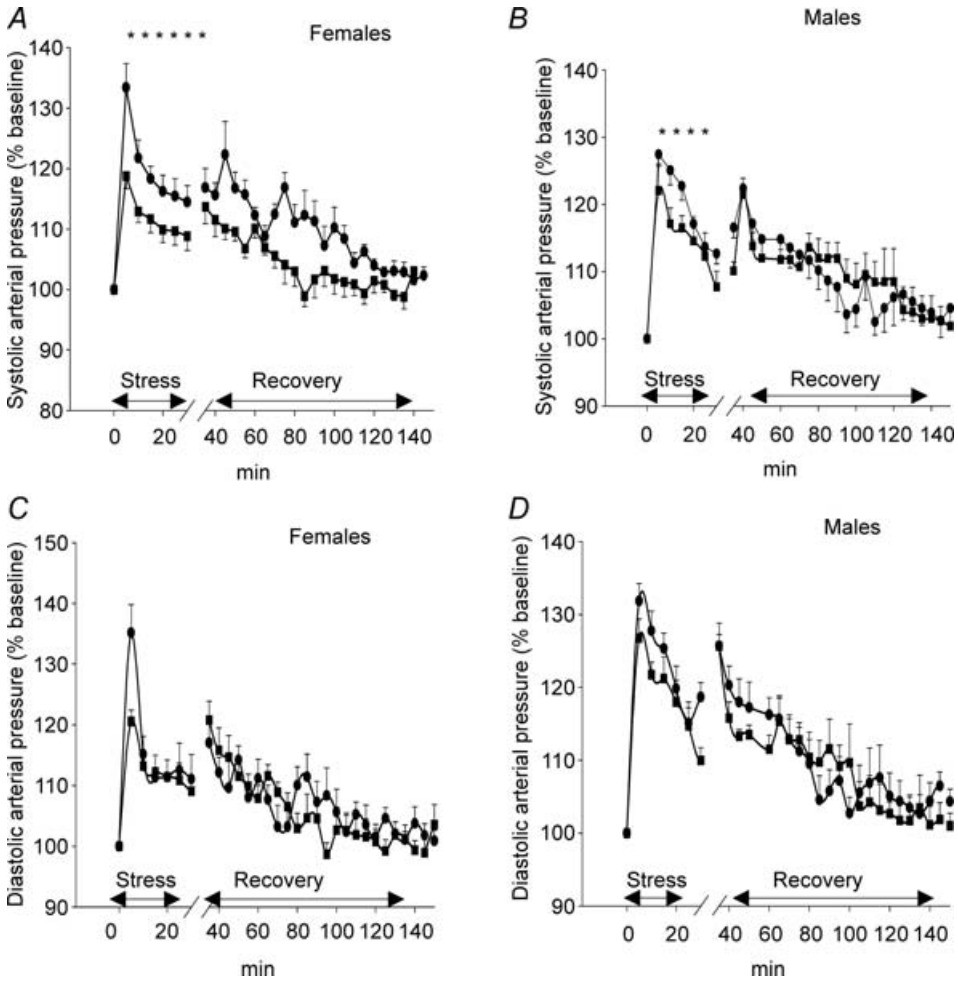

Figure 3. Changes in systolic and diastolic blood pressure
Changes in systolic (*A* and *B*) and diastolic blood (*C* and *D*) pressure during 30 min of restraint and for 120 min following return to the home cage in the adult offspring of control (■, $n = 10$) and stressed dams (●, $n = 10$). Data are expressed as the mean ± S.E.M. PS rats showed a greater increase in systolic arterial pressure (SAP) following restraint stress and extended SAP responses during recovery compared with controls (95 min *versus* 45 min). Diastolic arterial pressure (DAP) responses during stress and recovery did not differ between the groups. *$P < 0.05$ PS *versus* controls (repeated measures ANOVA).

confirmed that contractions induced by 16 Hz and 32 Hz EFS were significantly enhanced in arteries from PS offspring compared with controls.

Endothelium-dependent relaxation. Intrauterine stress did not alter vasodilator responses to either acetylcholine or dobutamine in mesenteric arteries (Fig. 5*A* and *B*).

Endothelium-independent relaxation. Vasodilator responses to nitric oxide were similar in PS and control rats. (Fig. 5*C*).

Discussion

This study has confirmed our previous findings of exaggerated SBP responses to stress in the offspring of rat dams subjected to stress in pregnancy (Igosheva *et al.* 2004) and provides new insight into underlying mechanisms. We report an association between prenatal stress and an enhanced constrictor response to NPY and also to perivascular nerve stimulation of mesenteric arteries. PS females demonstrated greater prolongation of SBP both during stress and recovery and higher basal plasma corticosterone concentration than PS males. Since all offspring were fostered to non-stressed dams, these abnormalities could not have been due to altered maternal nursing behaviour (Champagne & Meaney, 2006) but must have arisen as a direct physiological consequence of maternal stress in late pregnancy.

In our previous study (Igosheva *et al.* 2004) arterial blood pressure in the adult PS rats was measured directly using the indwelling fluid-filled catheter method which allows continuous recording of haemodynamic parameters in the conscious animal. However, this method requires that the rat is semi-restrained and potentially stressed. Moreover, catheter patency limitations prevent long-term measurements (Butz & Davisson, 2001). Radiotelemetry has the benefit of long-term assessment of cardiovascular parameters in unrestrained animals in a stress-free environment, and thus has considerable advantage over methods hitherto used in studies of fetal programming (Bertram & Hanson, 2001). The use of telemetry in the present study enabled us to undertake more accurate and reliable testing of cardiovascular function and confirm our previous findings of altered cardiovascular function in PS animals. As in our previous study, prenatal stress did not affect resting haemodynamic parameters in adulthood. Other recent studies of the developmental programming of

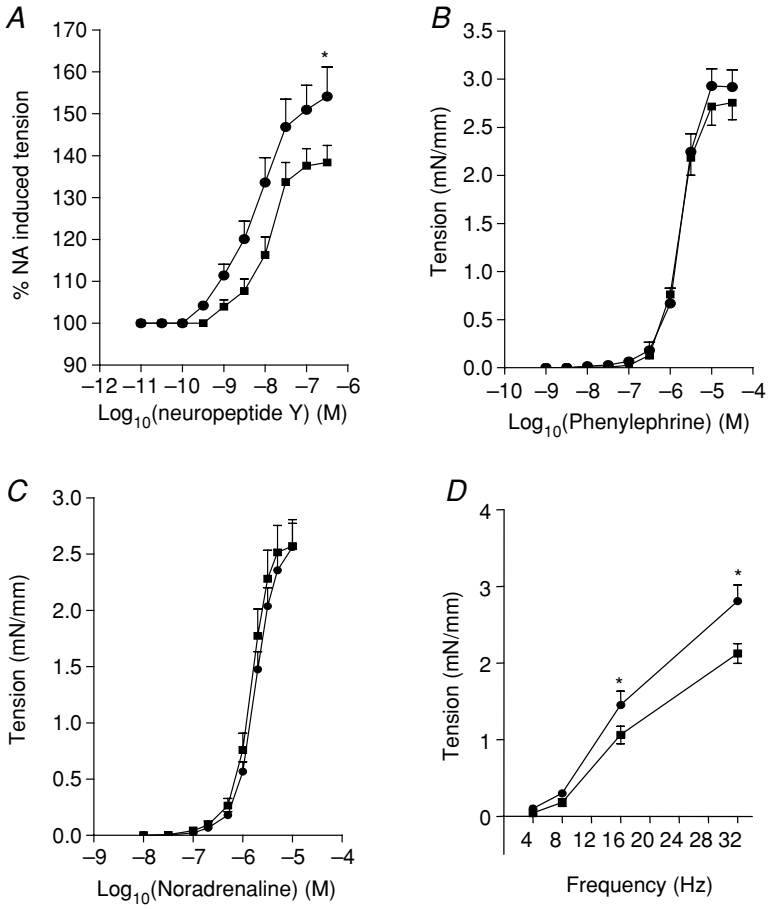

Figure 4. Contractile responses
Contractile responses to neuropeptide Y (*A*), phenylephrine (*B*), noradrenaline (*C*) and electrical field stimulation (*D*) in small mesenteric arteries from control (■, $n = 10$) and prenatally stressed (●, $n = 10$) rats. Responses to neuropeptide Y were determined on the arteries pre-activated with NA (80% of maximal constriction) and expressed as percentage of NA-induced tension. Significance was assessed by ANOVA: $^*P < 0.05$ controls *versus* PS animals. The level of NA-induced pre-constrictor tension (mN mm^{-1}) were not different between experimental groups: control females 2.1 ± 0.2 (S.E.M.), PS females 2.06 ± 0.26, control males 2.14 ± 0.35, PS males 2.18 ± 0.13.

CV function have also shown no change in basal blood pressure in the adult offspring of rat dams subjected to undernutrition (Holemans *et al.* 1999), hypoxia (Peyronnet *et al.* 2002) or uterine artery ligation (Jansson & Lambert, 1999). However, elevated basal blood pressure has been reported in adult offspring of rats prenatally exposed to high dose of synthetic GC (Levitt *et al.* 1996) and this may suggest a different mechanistic pathway (O'Regan *et al.* 2004). In common with the present study, it has been shown in a number of animal models that offspring exposed prenatally to an adverse environment only demonstrate altered CV function when challenged (Li *et al.* 2003; Molnar *et al.* 2003; Louey & Thornburg, 2005). The results of this study confirm and extend our previous findings (Igosheva *et al.* 2004) on this 'silent programming' of cardiovascular function in the prenatal stress protocol. Thus, PS animals showed no changes in basal CV parameters compared with controls, but significant elevation in SBP only when challenged by acute restraint stress. This is similar to the enhanced blood pressure responsiveness to stress in the offspring of rat dams subjected to protein undernutrition (Tonkiss *et al.* 1998) or hypoxia (Peyronnet *et al.* 2002). Our data also show a sex-specific effect of prenatal stress on blood pressure responsiveness to stress. Consistent with our previous findings, PS females had more prolonged SBP responses to restraint stress than PS males and showed delayed recovery. Greater female susceptibility to programming of CV function has also been observed in offspring of rat dams fed a high fat diet (Khan *et al.* 2003; Khan, 2004) and those exposed to synthetic GCs prenatally (O'Regan *et al.* 2004). The present experiments also demonstrate the sex-specific effects of prenatal stress on basal plasma corticosterone concentration. Adult PS females, but not males, have higher basal plasma corticosterone levels in comparison with controls. Elevated basal plasma corticosterone concentration in female rats born to PS dams has been reported in a number of studies (Weinstock *et al.* 1992; McCormick *et al.* 1995; Ward *et al.* 2000) consistant with adrenal hypertrophy (Ward *et al.* 2000). Others have shown that prenatal stress differentially affects adult hippocampal corticosteroid receptor density, with permanently decreased numbers of hippocampal glucocorticoid receptors and mineralocorticoid receptors in prenatally stressed females (Koehl *et al.* 1999; Szuran *et al.* 2000), whereas no differences in hippocampal glucocorticoid binding was apparent in male offspring (Szuran *et al.* 2000); this provides a potential explanation in the present study. It is also possible that prenatal stress could affect the level of corticostereone binding globulin in females but not in males (McCormick *et al.* 1995).

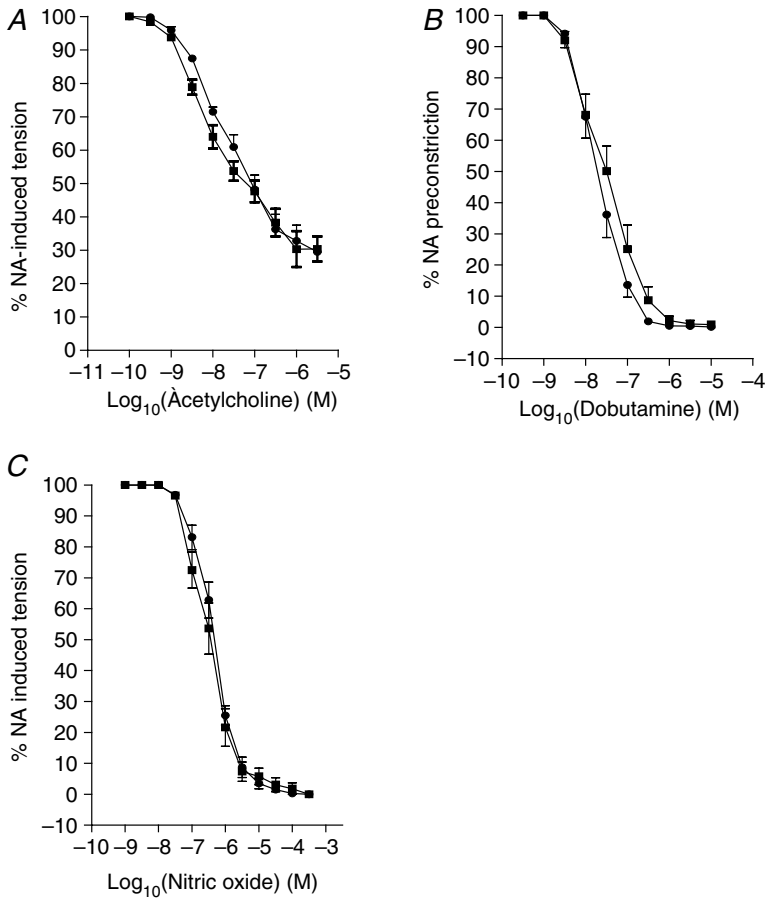

Figure 5. Relaxing responses
Relaxing responses to acetylcholine (*A*), dobutamine (*B*) and nitric oxide (*C*) in small mesenteric arteries from control (■, $n = 10$) and prenatally stressed (●, $n = 10$) offspring rats.

Although vascular function has been investigated in nutritional (Taylor *et al.* 2004; Khan *et al.* 2005) and hypoxic (Ruijtenbeek *et al.* 2002; Sanders *et al.* 2004*a*) models of developmental programming of CV function, this is, to our knowledge, the first report of the effects of maternal stress on the peripheral vascular responses of the offspring. Here we show that PS resulted in permanent alterations in agonist-induced reactivity of offspring mesenteric arteries confined to enhanced contractile responses to NPY.

We have also shown that PS increased neurogenic contractions induced by EFS in a frequency-dependent manner. The high-frequency stimulations (16 and 32 Hz), mimicking neurotransmission at high level of sympathetic nerve activity (Han *et al.* 1998*b*), evoked higher contractions in arteries from PS offspring compared with controls whereas low-frequency stimulations had similar effects on mesenteric arteries from control and PS animals.

EFS-induced vascular contractions arise from activation of perivascular sympathetic nerves, the major associated neurotransmitters being NA and NPY (Donoso *et al.* 1997). As vasoconstrictor responses to exogenous NA and phenylephrine did not differ between the groups this suggests that adrenergic sensitivity of mesenteric arteries is unaltered in PS offspring and is unlikely to contribute to the enhanced constrictor response to high-frequency EFS. Furthermore, NPY is known to be preferentially released by high-frequency stimulation and/or during stimulation that leads to a higher degree of sympathetic nerve activity (Chronwall & Zukowska, 2004). Therefore, it is plausible that the enhanced vasoconstriction upon intense EFS in the PS rats is predominantly mediated by activation of NPY neurotransmission. Whilst this is a likely explanation other mechanisms cannot be ruled out including altered neuronal reuptake of NA, or density of perivascular sympathetic nerves or of presynaptic α_2-adrenoreceptor. Vascular smooth muscle is also innervated by calcitonin gene-related peptide (CGRP)-containing vasodilator sensory–motor nerve fibres and it is possible that reduced CGRP-induced vasodilatation could play a role.

However, the enhanced sensitivity of mesenteric arteries from PS animals to NPY is supportive of a central role for the NPY constrictor pathway in the 'programmed' vascular anomalies observed. Although not determined here, alterations in peripheral NPY Y_1 receptor density could contribute as these receptors are implicated in stress-induced mesenteric vasoconstriction in rats as assessed *in vivo* by Doppler blood flow probes (Zukowska-Grojec *et al.* 1996), in elevation of the pressor response to acute stress (Han *et al.* 1998*a*; Carrasco & Van de Kar, 2003) and in the pathogenesis of hypertension (Westfall, 2006). In studies of developmental programming, alterations in the NPY-dependent pathway have been implicated in sheep models of vascular dysfunction induced by prenatal acute hypoxaemia (Fletcher *et al.* 2003), maternal undernutrition (Warnes *et al.* 1998) and synthetic GC exposure (Fletcher *et al.* 2000). Neuropeptide Y binding to NPY Y_1 receptors activates different signalling pathways that regulate constriction of vascular smooth muscle. One of these pathways is linked to inhibition of adenylyl cyclase (Prieto *et al.* 2000). Since in the present study constrictor responses to NPY were determined on the arteries pre-activated with NA, differences in arterial reactivity to NPY between PS and control animals may probably be ascribed to differences in β-adrenoreceptor-mediated adenylyl cyclase activation. As vascular responses to the β-adrenergic agonist dobutamine did not differ between control and PS animals it is unlikely that increased β-adrenoreceptor-mediated adenylyl cyclase activation could contribute to the enhanced constrictor response to NPY in PS animals. Up-regulation of peripheral NPY Y_1 receptors is therefore a plausible explanation for the enhanced peripheral vasoconstrictor responses to exogenous NPY and EFS-induced stimulation in PS animals. Alternatively changes in activity or expression of dipeptidil-peptidase IV could occur, resulting in alteration in NPY breakdown and NPY receptor binding. This requires interrogation in future studies in which determination of receptor density coupled with the use of dipeptidil-peptidase IV and NPY Y_1 receptor antagonists would enable the mechanisms to be unravelled.

In summary, this study provides new evidence for the hypothesis that maternal stress in pregnancy can program enhanced cardiovascular responsiveness to stress. The increased vascular contractile responses to NPY occurred in conjunction with enhanced blood pressure responsiveness to stress in PS offspring. We propose that maternal stress in pregnancy may affect the NPY pathway and that the persistently elevated vascular sensitivity to NPY may account for the stress-induced systemic hypertension in the rat model of prenatal stress. Future studies will test this relationship in a different cohort of animals. Subsequent investigations should also include detailed evaluation of mechanisms of NPY-induced vasoconstriction using diverse pharmacological tools. Further understanding of how prenatal stress can influence central and peripheral mechanisms implicated in the regulation of cardiovascular responses to stress may provide insight into the risk factors that determine susceptibility to cardiovascular diseases in later life.

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