Prenatal Programming of Hypertension Induces Sympathetic Overactivity in Response to Physical Stress

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Abstract—Small-for-gestational-age infants are known to develop hypertension in adulthood. This prenatal programming of hypertension (PPH) can result from several insults including maternal dietary protein deprivation, uteroplacental insufficiency, and prenatal administration of glucocorticoids. The mechanisms underlying the development of hypertension remain unclear although the sympathetic nervous system has been indirectly implicated. This study was designed to directly measure renal sympathetic nerve activity both at rest and during physical stress in an animal model of PPH. The adult male offspring of rats fed either a 6% (PPH) or 20% protein diet (control) were investigated. Conscious systolic blood pressure measured by tail cuff was significantly higher in PPH compared with control (140±3 versus 128±3 mm Hg; *P*<0.05). Baseline mean arterial pressure, heart rate, and renal sympathetic activity were not different between groups during isoflurane anesthesia or after decerebration. Physical stress was induced in decerebrate animals by activating the exercise pressor reflex during static muscle contraction. Stimulation of the exercise pressor reflex evoked significantly larger changes from baseline in mean arterial pressure (40±7 versus 20±4 mm Hg; *P*<0.05), heart rate (19±3 versus 5±1 bpm; *P*<0.05), and renal sympathetic activity (198±29% versus 68±14%; *P*<0.05) in PPH as compared with control. The data demonstrate that the sympathetic response to physical stress is markedly exaggerated in PPH and may play a significant role in the development of hypertension in adults born small for gestational age. 

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Key Words: prenatal programming ▪ hypertension ▪ diet, protein-restricted ▪ autonomic nervous system ▪ blood pressure ▪ heart rate

It has been demonstrated by Barker and colleagues1–4 that there is an association between small-for-gestational-age infants and the development of hypertension and cardiovascular mortality in later life. However, the cause for the predisposition of low birth weight infants to develop chronically elevated arterial blood pressure (ABP) when they become adults is unknown. To investigate the etiology for the increase in blood pressure in adults who were small for gestational age, studies have attempted to recapitulate common prenatal insults in animals, such as prenatal administration of glucocorticoids, maternal dietary protein deprivation, and uteroplacental insufficiency. In these models, exposure to such insults not only produces offspring that are small for gestational age but also leads to the prenatal programming of hypertension (PPH). As a result, the models can be used to investigate the mechanisms underlying the development of hypertension in adults born of low birth weight.

Investigations using such animal models, as well as studies in humans, have led to a number of hypotheses for the generation and maintenance of hypertension with prenatal programming. Brenner and colleagues5–8 have proposed that intrauterine growth retardation results in a reduction in nephron number that predisposes the development of hypertension in later life. Certainly, human neonates with intrauterine growth retardation have a reduction in nephron number.9–11 In addition, prenatal insults in animals also result in reductions in nephron number.12,13 However, there is a poor correlation between nephron number after prenatal insult and hypertension in adult offspring.14–17 Recent studies have demonstrated that prenatal programming leads to increased renal tubular sodium transport. Indeed, there is an increase in apical sodium transporters and sodium transport in multiple nephron segments that could lead to salt-sensitive hypertension.18–21 An important clue to the etiology of the hypertension with prenatal programming has come from studies showing that renal sympathetic denervation results in the normalization of blood pressure, as well as sodium transporter abundance.21,24,25 Clearly, these findings implicate a role for the sympathetic nervous system in the development of hypertension with prenatal programming. However, to date,
direct assessment of renal sympathetic nerve activity (RSNA) at rest and during physical stress has not been examined with prenatal programming making it difficult to draw definitive conclusions.

The purpose of this study was to directly assess RSNA at rest and in response to physical stress in both control and PPH adult rats. The physical stressors chosen for this purpose were experimental activation of the exercise pressor reflex (EPR) and its individual functional components, the muscle mechanoreflex and metaboreflex. The EPR is an important source of neural input to the brainstem during exercise contributing to the control of the cardiovascular system.26 In this reflex, contraction-induced sensory signals are generated by stimulation of group III (predominantly mechanically sensitive A-δ fibers associated with the mechanoreflex) and IV (primarily chemically sensitive C fibers associated with the metaboreflex) skeletal muscle afferent neurons, which reflexively increase ABP and heart rate (HR).27 EPR-induced alterations in cardiovascular hemodynamics are primarily mediated by increasing efferent sympathetic activity.28 Thus, activating the EPR (or its individual components) is a reliable means by which to assess RSNA responsiveness. It was hypothesized that prenatal programming induces enhanced levels of basal RSNA and produces exaggerated increases in RSNA during selective activation of the EPR, mechanoreflex, and metaboreflex. To test these hypotheses, RSNA was measured at rest and during activation of the EPR (via electrically induced static muscle contraction), the muscle mechanoreflex (via passive muscle stretch), and the muscle metaboreflex (via intra-arterial capsaicin administration in the hindlimb) in control and PPH (produced by maternal dietary protein deprivation during the last half of pregnancy).

Materials and Methods
For a complete description of the Materials and Methods see the online-only Data Supplement.

Animals
Pregnant Sprague Dawley rats were fed either a low 6% protein diet to produce PPH offspring (Teklad 6% Protein Diet) or an isocaloric control diet (Teklad 20% Protein Diet) from day 12 of gestation until birth.12,29 All of the mothers were fed the control diet after delivery. Experiments were performed in male, age-matched (16-20 weeks) control (n=13) and PPH (n=14) rats. The procedures outlined were approved by the institutional animal care and use committee of the University of Texas Southwestern Medical Center at Dallas. All of the studies were conducted in accordance with the US Department of Health and Human Services National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Protocols
Stimulation of the EPR
The EPR was stimulated in control and PPH animals by contracting the gastrocnemius and soleus muscles of the right hindlimb for 30 s via electrical stimulation of the isolated L3 and L5 ventral roots. Constant current stimulation was used at a 3-times motor threshold (ie, the minimum current required to produce a muscle twitch) with a pulse duration of 0.1 ms at 40 Hz.

Stimulation of the Muscle Mechanoreflex
To selectively activate the mechanically sensitive component of the EPR, hindlimb muscles were passively stretched in control and PPH animals using a calibrated 9.5-mm rack and pinion system (Harvard Apparatus).

Stimulation of the Muscle Metaboreflex
Selective activation of chemically sensitive afferent fibers innervating skeletal muscle was achieved by administering capsaicin into the arterial supply of the hindlimb (0.3 and 1.0 μg/100 μL). Capsaicin was injected into the right common iliac artery, whereas the reversible ligature placed around the right common iliac vein was occluded for 2 minutes.

Statistical Analyses
All of the values are expressed as means±SEM. Data were analyzed using unpaired t tests. The significance level was set at P<0.05.

Results
Characterization of the Pregnant Rats and Offspring
To quantitate the food and water intake of pregnant rats, some pregnant rats were placed in metabolic cages starting on day 12 of pregnancy. After a day of acclimatization, average daily food and water consumption and weight gain were recorded. The results are shown in Table 1. The PPH mothers ate more food (6% protein chow) and had a tendency to drink less water than the control mothers. The weight gain, however, was comparable. The 1-day-old offspring of the mothers fed a 20% protein diet weighed more than the offspring of mothers fed a 6% protein diet (6.3±0.1 g versus 5.4±0.2 g; P<0.001).

Characterization of the Prenatal Programming of Hypertension
Morphometric characteristics and baseline hemodynamics are summarized in Table 2. Morphometric characteristics were not different between groups. Systolic blood pressure in the conscious state was significantly higher in PPH than control animals, whereas baseline cardiovascular and sympathetic parameters were not different between groups during administration of inhalant anesthesia or after decerebration.

Sympathetically Mediated Cardiovascular Response to EPR Activation
Representative tracings from control and PPH animals during electrically induced static muscle contraction, a maneuver known to preferentially activate the EPR, are shown in Figure 1. In response to the same amount of tension development, activation of the EPR elicited significantly larger elevations in mean arterial pressure (MAP), HR, and RSNA in PPH as compared with controls (Figure 2A). As depicted in Figure 1, these exaggerated changes in MAP in PPH resulted from enhanced increases in both systolic and diastolic pressures. Likewise, the integrated HR and RSNA responses were significantly greater in PPH than in controls.
Baseline signal/noise ratio for RSNA
HR, beats/min
MAP, mm

After decerebration n=11 n=10
Baseline signal/noise ratio for RSNA

Discussion

The significant findings of the current investigation were that PPH did not exhibit alterations in baseline RSNA (contrary to our original hypothesis), and PPH did display markedly exaggerated increases in RSNA, ABP, and HR in response to physical stress (in concurrence with our original hypothesis). The latter finding supports a role for the sympathetic nervous system in the development of hypertension with prenatal programming and is in agreement with a growing body of evidence, albeit predominately indirect, that there is an alteration in sympathetic nerve activity in human adults born small for gestational age. For example, although an imperfect assessment of sympathetic tone, resting HR is higher in adults that were of low birth weight compared with controls. Furthermore, the increases in blood pressure in response to psychological stresses such as public speaking were greater in offspring of mothers exposed to the Dutch famine during their first trimester of pregnancy than unexposed control subjects. Direct measurement of sympathetic activity has been reported in adults who were small for gestational age and compared with those who were of normal weight at birth. Although one study showed an increase in resting sympathetic nerve activity in adults who were small for gestational age, another did not. However, the latter study found a significant increase over basal sympathetic nerve activity in response to the stress of breath holding in those who were born of low birth weight compared with controls. These latter findings are comparable to our study where we demonstrated similar sympathetic nerve activity at baseline but an augmented RSNA response to physical stress.

The current study demonstrating that there is an enhanced RSNA response to physical stress in rats that were the offspring of mothers fed a low-protein diet is consistent with previous studies examining the effect of renal denervation on blood pressure in this animal model. In the previous studies, blood pressure was elevated in adult rats exposed to uteroplacental insufficiency on blood pressure in this animal model. In the previous studies, blood pressure was elevated in adult rats exposed to uteroplacental insufficiency or prenatal exposure of glucocorticoids. The offspring of mothers exposed to uteroplacental insufficiency had greater renal renepinephrine content than controls at 6 weeks of age. Renal renepinephrine content was also greater in offspring whose mothers were administered prenatal dexamethasone than controls when studied at 3 weeks of age but not at 8 weeks of age. Although denervation did not affect blood pressure of control rats, blood pressure normalized to control levels in offspring of mothers that had a prenatal insult. These renal renepinephrine content studies and our investigation that need to be resolved. In the current study, baseline RSNA was comparable in both groups.
of animals under anesthesia, as well as after decerebration. An augmented increase in sympathetic activity in PPH was only noted in response to activation of the EPR, mechanoreflex, and metaboreflex. It remains unknown whether the conscious PPH rat would exhibit enhanced basal sympathetic activity and an exaggerated increase in RSNA in response to physical stress. In addition, baseline blood pressures significantly declined as compared with the conscious state in both control and PPH groups when the animals were placed on isoflurane anesthesia or decerebrated. One possibility for these reductions in pressure was the performance of the extensive surgery requisite for EPR testing. Another was the use of the precollicular decerebrate technique. For example, it has been suggested that portions of the hypothalamus (e.g., paraventricular nucleus) contribute to generating the elevated baseline SNA characteristic of hypertension.\textsuperscript{34,35} Removal of such areas within the hypothalamus by precollicular decerebration may have abrogated baseline elevations in RSNA in PPH animals and produced the reductions in baseline ABP as compared with the conscious state. It has been proposed that using a midcollicular decerebrate procedure may be preferable when investigating EPR function,\textsuperscript{36,37} although use of this technique would likewise remove the hypothalamus. These technical limitations must be taken into account when interpreting the results of the current study. Resolving these inconsistencies will only be answered by directly measuring blood pressure and sympathetic nerve activity in conscious unanesthetized rats, the latter of which is currently a technically difficult experimental endeavor.

It has been well documented that, in hypertension, the cardiovascular response to exercise is abnormally heightened and characterized by exaggerated increases in ABP, HR, and vascular resistance.\textsuperscript{38–42} To this end, our laboratory has demonstrated previously that selective activation of the EPR elicits greater increases in MAP, HR, and RSNA in spontaneously hypertensive rats, a model of essential hypertension, compared with normotensive rats.\textsuperscript{43–45} These findings provide evidence that the exaggerated cardiovascular response to exercise in hypertension is mediated, in part, by a dysfunctional EPR. There are differences between our findings in the spontaneously hypertensive rat and PPH rats compared with their respective controls. Although the blood pressure was not different under anesthesia in the PPH compared with the control group, it was >50 mm Hg higher in the SHR compared with their respective controls. In addition, the normalized heart weight was not different between PPH and control groups unlike previous studies in spontaneously hypertensive rats, which exhibit significant cardiac hypertrophy.\textsuperscript{43–45} Despite the possibility of PPH being a milder form of hypertension than that in the SHR model, the magnitude of the EPR overactivity was comparable.\textsuperscript{43–45} Collectively, these studies suggest that the EPR contributes significantly to the abnormally exaggerated

![Figure 1](http://hyper.ahajournals.org/)

Figure 1. Cardiovascular and sympathetic responses to exercise pressor reflex (EPR) activation in representative control and prenatal programming of hypertension (PPH) animals. The contraction-induced increases in arterial blood pressure (ABP), heart rate (HR), and renal sympathetic nerve activity (RSNA) were larger in PPH vs control.
sympathetically mediated cardiovascular response to exercise in multiple forms of hypertension. Given that the accentuated cardiovascular response to physical activity is associated with elevated risks for myocardial ischemia, myocardial infarction, cardiac arrest, and stroke during or immediately after a bout of exercise, it is clinically important to determine the mechanisms underlying EPR dysfunction in each etiology of the disease.

There are several viable possibilities for the enhanced cardiovascular and sympathetic responses demonstrated during activation of the EPR in this study. Because prenatal programming by maternal dietary protein deprivation results in a reduction in nephron number, it is possible that renal dysfunction or injury contributes to EPR overactivity in PPH. Renal sympathetic afferents can be activated by minor injury resulting in an increase in central sympathetic nerve activity and hypertension, which is prevented by renal denervation. In patients with chronic kidney disease, there is an increase in muscle sympathetic nerve activity, which normalizes after bilateral nephrectomy. Thus, it is possible that the sympathetic overactivity may be initiated by renal afferents. However, prenatal programming can also have a primary effect on the brain, which may result in increased sympathetic nerve activity when animals are put under stress. The relative roles of the kidney and central nervous system in mediating EPR overactivity with prenatal programming will have to be resolved in future studies.

**Perspectives**

The mechanisms underlying the development of hypertension in adults born small for gestational age are now being elucidated using animal models. This study has implicated a role for the sympathetic nervous system in the generation of hypertension in adult rats born small for gestational age. The applicability of these findings to humans born small for gestational age is unclear and requires translational investigation. Although these data do not support the contention that a chronic basal elevation in sympathetic activity contributes to the development of hypertension under resting conditions, it shows that there are markedly exaggerated elevations in sympathetic nerve activity in response to physical stress, which may have a significant impact in mediating the hypertension because of prenatal insults. Future studies will focus on telemetric measurements of sympathetic nerve activity and blood pressure at rest, during physical activity and with environmental stress to determine...
whether these environmental perturbations cause parallel changes in sympathetic nerve activity and blood pressure in rats that had a prenatal insult. Telemetric measurements of blood pressure and sympathetic nerve activity may also provide insight into the best therapeutic regimen for the treatment of hypertension seen with prenatal programming.

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**Disclosures**

None.

**References**


Novelty and Significance

What Is New?

- The mechanisms underlying the development of hypertension in adults born small for gestational age because of maternal dietary protein deprivation remain largely undetermined. For the first time, the present study directly measures sympathetic nerve activity at rest and during physical stress in an animal model of prenatally programmed hypertension (induced by maternal dietary protein restriction) to assess the role of the sympathetic nervous system in the generation of chronic high blood pressure in adulthood.

What Is Relevant?

- Baseline renal sympathetic activity in adult hypertensive rats subjected to prenatal programming is not different compared with normal healthy adult rats. In contrast, the sympathetic response to physical stress is markedly exaggerated in prenatally programmed hypertensive rats.

Summary

This investigation has implicated a role for the sympathetic nervous system in the generation of hypertension in adults born of small birth weight because of dietary restriction during gestation. Although this study does not support the contention that chronic basal elevations in sympathetic activity contribute to the development of hypertension under these conditions, it does suggest that acute, markedly augmented elevations in sympathetic activity in response to physical stress may have a significant impact.
SUPPLEMENTARY MATERIALS AND METHODS

Animals

Males were studied to lessen variability and because they are more severely affected by prenatal programming than females. Animals were housed in standard rodent cages on 12-h light-dark cycles and were given food and water ad libitum. To compare the food and water intake of pregnant rats on the low protein and control diet some pregnant rats were placed in metabolic cages starting at day 12 of pregnancy for 1 week. After 24 hours of acclimatization, the rats were weighed daily and food and water intake was recorded every 24 hours.

Measurement of Blood Pressure in Conscious Animals

Systolic blood pressure and HR was measured at 12 weeks of age by tail cuff using a Visitech Systems BP-2000 Series II blood pressure analysis system (Apex, NC). Animals were acclimated to the measurement procedure and trained for four days by being placed in a restraining chamber and inflating the blood pressure cuff several times. Blood pressure and HR was measured on the fifth day. The person obtaining the blood pressure and HR measurements was blinded to the experimental group to which rats belonged.

Acute Surgical Procedures

As previously described, prior to EPR, mechanoreflex and metaboreflex testing rats were anesthetized with isoflurane gas (2–4% in 100% oxygen) and intubated for mechanical ventilation (Model 683, Harvard). Fluid-filled catheters were placed in the right jugular vein for the administration of solutions and left common carotid artery for the measurement of ABP (MLT0380/D, ADInstruments). Needle electrodes were placed on the back of the animal to obtain electrocardiograph recordings and HR measurements (Animal Bio Amp, ADInstruments). To stabilize fluid balance and maintain baseline ABP, a solution (2ml 1M NaHCO3 and 10 ml 5%
dextrose in 38 ml Ringer Solution) was continuously administrated through the jugular vein (3–5ml h⁻¹ kg⁻¹). Dexamethasone (0.2 mg) was given intramuscularly to minimize edema. Body temperature was maintained within normal ranges throughout the experiment. The renal nerve was exposed and attached to a pair of stainless steel wire electrodes (Bioflex wire AS633, Cooner Wire, CA) through a left flank incision. To insulate and fix the tissue in place, the nerve and electrodes were covered with silicone glue (Kwik-Sil, World Precision Instruments, Sarasota, FL). To quantify RSNA, the pre-amplified nerve signal was band-pass filtered at 150–1000 Hz (Neuro Amp EX, ADInstruments) then full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz. To allow preferential activation of the EPR, a laminectomy exposing the lower lumber portions of the spinal cord (L₂–L₆) was performed. The L₄ and L₅ ventral roots were carefully isolated and sectioned. The gastrocnemius and soleus muscle of the right hindlimb were isolated. The calcaneal bone of the right hindlimb was cut and the Achilles’ tendon connected to a force transducer for the measurement of muscle tension (FT-10, Grass Instruments). The latter procedure likewise allowed for manipulation of the muscle mechanoreflex. To administer capsaicin into the arterial supply of the right leg during metaboreflex activation, a catheter (PE-10, polyethylene tubing) was placed in the left common iliac artery and its tip advanced to the bifurcation of the abdominal aorta. A reversible vascular occluder was placed around the common iliac vein. Animals were held in a stereotaxic head unit (Kopf Instruments), and a pre-collicular decerebration was performed. Immediately following the decerebrate procedure, gas anesthesia was discontinued. A minimum recovery period of 1.25 h was employed after decerebration before beginning any experimental protocol. This period of recovery has been shown to be sufficient to dissipate the effects of isoflurane anesthesia in the rat model used⁵,⁸.

**Stimulation of the EPR**

Muscles were initially stretched to 70–100 g of tension prior to each perturbation. Both the mechanically and metabolically sensitive components of the EPR are stimulated concomitantly by contracting hindlimb skeletal muscle in this manner⁹.

**Stimulation of the Muscle Mechanoreflex**

To evoke a mechanical stimulus similar to that elicited during muscle contraction, care was taken to generate the same pattern and magnitude of muscle tension developed during static contractions. Muscles were initially stretched to 70–100 g of tension prior to each perturbation. Passively stretching hindlimb skeletal muscle does not appreciably increase muscle metabolism and, therefore, is commonly employed to selectively activate the mechanically sensitive component of the EPR¹⁰-¹².

**Stimulation of the Muscle Metaboreflex**

This maneuver essentially isolated the circulation of the hindlimb preventing capsaicin from entering the general circulation. The capsaicin receptor, transient receptor potential vanilloid 1 (TRPv1), is
primarily associated with Group IV afferent fibers in skeletal muscle although the receptor has also
been identified in a small number of Group III neurons\textsuperscript{13,14}. As such, stimulation of these receptors
predominatey activates neurons known to mediate metaboreflex activity and is commonly used for
this purpose\textsuperscript{11,15,16}.

**Experimental protocols**

Of the 27 rats in which conscious tail cuff measurements were obtained, 11 control and 10 PPH
animals completed contraction, stretch and capsaicin (0.3 μg/100 μL) protocols. In addition, 6
animals in each group received an additional capsaicin injection at a higher dose (1.0 μg/100 μL).
The order in which the protocols were conducted was randomized and all trials were separated by a
minimum of 10 minutes. At the conclusion of all experiments, an intravenous infusion of
hexamethonium bromide (60 mg kg\textsuperscript{-1}) abolished RSNA signals indicating that they were recorded
from postganglionic renal sympathetic fibers. RSNA background noise was determined over a 30
min period after the insentient decerebrated animal was humanely killed by intravenous injection of
saturated potassium chloride (4 M, 2 ml/kg). In all animals, the heart and lungs were excised and
weighed *post mortem*. Additionally, the tibia was harvested, weighed, and measured.

**Data Acquisition**

ABP, HR, RSNA and muscle tension were recorded with data acquisition software (LabChart,
ADInstruments) and stored in a computer hard drive. Baseline values for mean arterial pressure
(MAP, mmHg), HR (bpm) and tension (kg) were determined by evaluating 30 s of recorded data
before a muscle contraction, stretch or capsaicin administration. The peak response of each variable
was defined as the greatest change from baseline elicited by muscle contraction, stretch or capsaicin
administration. To quantify RSNA responses, basal measurements were obtained by taking the mean
value of 30 s of baseline data immediately prior to the maneuver. This mean was considered 100% of
basal RSNA. Subsequently, relative changes in RSNA (%) from this baseline were evaluated.
Integrated MAP (mmHg s), HR (bpm s), RSNA (% s), and tension (kg s) were also calculated during
EPR and mechanoreflex testing by integrating each response during the contraction or stretch period,
respectively.

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