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Gestational hypertension in atrial natriuretic peptide knockout mice and the developmental origins of salt-sensitivity and cardiac hypertrophy

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ABSTRACT

Objective: To determine the effect of gestational hypertension on the developmental origins of blood pressure (BP), altered kidney gene expression, salt-sensitivity and cardiac hypertrophy (CH) in adult offspring.
Methods: Female mice lacking atrial natriuretic peptide (ANP −/−) were used as a model of gestational hypertension. Heterozygous ANP +/− offspring was bred from crossing either ANP +/+ females with ANP −/− males yielding ANP +/−^{WT} offspring, or from ANP −/− females with ANP +/+ males yielding ANP +/−^{KO} offspring. Maternal BP during pregnancy was measured using radiotelemetry. At 14 weeks of age, offspring BP, gene and protein expression were measured in the kidney with real-time quantitative PCR, receptor binding assay and ELISA.
Results: ANP +/−^{KO} offspring exhibited normal BP at 14 weeks of age, but displayed significant CH ($P < 0.001$) as compared to ANP +/−^{WT} offspring. ANP +/−^{KO} offspring exhibited significantly increased gene expression of natriuretic peptide receptor A (NPR-A) ($P < 0.001$) and radioligand binding studies demonstrated significantly reduced NPR-C binding ($P = 0.01$) in the kidney. Treatment with high salt diet increased BP ($P < 0.01$) and caused LV hypertrophy ($P < 0.001$) and interstitial myocardial fibrosis only in ANP +/−^{WT} and not ANP +/−^{KO} offspring, suggesting gestational hypertension programs the offspring to show resistance to salt-induced hypertension and LV remodeling. Our data demonstrate that altered maternal environments can determine the salt-sensitive phenotype of offspring.

1. Introduction

The natriuretic peptide system (NPS) is a key regulator of cardiovascular tissue dynamics and homeostasis [1,2]. Mice lacking atrial natriuretic peptide (ANP) exhibit salt-sensitive hypertension [3] and left ventricular (LV) hypertrophy independent of elevated arterial blood pressure (BP) [4]. Physiologic actions of ANP occur via generation of cyclic cGMP [5], which promotes natriuresis and diuresis [5,6]. We have previously reported that heterozygous ANP +/− mice with a single copy of the *Nppa* gene display normal BP and LV mass on normal salt (NS) diet, but when treated with a high salt (HS) diet develop hypertension and LV hypertrophy [3,7,8].

The NPS has important roles in pregnancy and fetal development. Prior studies have shown that ANP increases during pregnancy [9–11], possibly secondary to hypervolemia and increased preload. The natriuretic effect of ANP during pregnancy is also blunted due to elevated renal phosphodiesterase 5 (PDE5) activity [12], thereby contributing to volume expansion during pregnancy. ANP antagonizes vasoconstriction of the maternal and fetal placental vasculature [13–15], and ANP is synthesized *de novo* in the human placenta [16,17]. Mice lacking ANP and corin (the convertase that cleaves proANP to produce functional ANP) exhibit gestational hypertension [18,19], and ANP receptors are reduced in human placenta from growth restricted fetuses [20]. These studies suggest a key role for maternal ANP in regulating blood supply to the fetus [21], and highlight a possible role for ANP in regulating maternal and placental hemodynamics.

Abbreviations: ANP, atrial natriuretic peptide; BP, blood pressure; BNP, B-type natriuretic peptide; CV, cardiovascular; CVD, cardiovascular disease; eNOS, endothelial nitric oxide synthase; EIA, enzyme immunoassay; HS, high salt; IUGR, intrauterine growth restriction; LV, left ventricle; NS, normal salt; *Nppa*, natriuretic peptide precursor A gene; *Npr1*, natriuretic peptide receptor 1 gene; *Npr3*, natriuretic peptide receptor 3 gene; NPS, natriuretic peptide system; NPR-A, natriuretic peptide receptor A; NPR-C, natriuretic peptide receptor C; PDE, phosphodiesterase; RIA, radioimmunoassay.

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It is now well-established that fetal exposure to environmental stressors in utero can result in adaptive changes in fetal growth and development of the cardiovascular (CV) system, which may lead to increased risk of cardiovascular disease (CVD) in adulthood [22–28]. This suggests that exposure to gestational hypertension may determine the CV phenotype of the fetus. We have established a new approach to study the developmental origins of CVD due to gestational hypertension using ANP−/− mice, and thus provided direct evidence for a transgenerational effect of maternal gestational hypertension on the risk of developing cardio-renal dysfunction in offspring. Our objective was to determine the effect of gestational hypertension, secondary to maternal ANP gene disruption, on the development of hypertension, kidney NPS gene expression, salt-sensitivity and LV hypertrophy in heterozygous adult offspring. We hypothesized that the absence of maternal ANP and consequent gestational hypertension would result in adaptive changes in fetal cardiac growth and blood pressure (BP) regulation independent of fetal ANP genotype.

2. Methods

2.1. Animal model and genotyping

Experimental protocols pertaining to the use of mice in this study have been approved by the Animal Care Committee of Queen's University in accordance with the guidelines of the Canadian Council on Animal Care. All mice were bred and cared for in the Animal Care Facility at Queen's University. The ANP gene-disrupted mouse (ANP−/−, C57BJ/6 × 129 background) was originally developed by John et al. [3]. To assess the influence of maternal ANP genotype on the development of salt-sensitivity in offspring we obtained heterozygous ANP+/− offspring in the following manner: crossing ANP+/+ females with ANP−/− males to produce ANP+/−^{WT} offspring (heterozygotes from wild-type dams), or, crossing ANP−/− females with ANP+/+ males to produce ANP+/−^{KO} offspring (heterozygotes from knockout dams) (see Fig. 1 for details). All mice were weaned between 19 and 21 days of age, and 2 mm tail biopsies were used for extraction of genomic DNA for genotyping. The genotype of each mouse was determined using multiplex PCR as previously described [29].

2.2. Radiotelemetry recording of gestational blood pressure in ANP+/+ and ANP−/− females

Radiotelemetry recordings using TA11PA-C10 radio transmitters (Data Sciences International, St. Paul, MN, USA) were performed as described previously [30] in separate groups of ANP+/+ (N = 4) and ANP−/− (N = 6) females. At day ten after surgical implantation of radio transducers, continuous, 24-hour data collection began using the Dataquest ART Acquisition System (Data Sciences International, version 4.1). Data were collected for 30 s every 4 min. Females were selected for estrus and paired with genetically matched males for timed breeding. Detection of a copulation plug was dated gestational day (GD) 0, and males were removed. Mated females were recorded until birth.

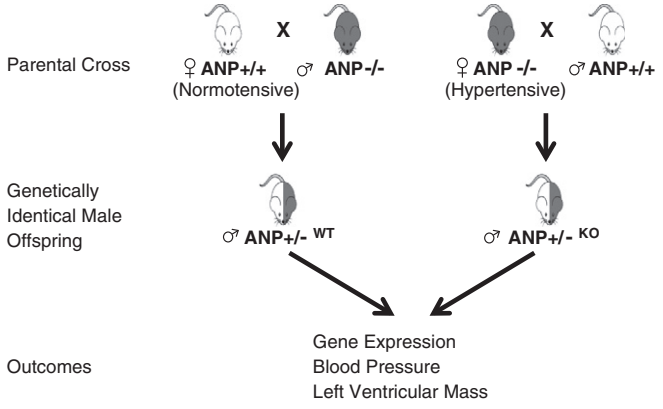

Fig. 1. Generation of genetically identical ANP+/− mice from dams that are normotensive or hypertensive during pregnancy. Mice that were born to normotensive and hypertensive dams are designated as ANP+/−^{WT} and ANP+/−^{KO} mice, respectively.

2.3. Dietary salt treatment and non-invasive tail-cuff blood pressure measurements

In a separate experiment, nine week old male ANP+/−^{WT} and ANP+/−^{KO} mice were fed either NS chow (0.8% NaCl) or HS chow (8.0% NaCl, LabDiet 5001®, Brentwood, MO, USA) for a total of 5 weeks (N = 4–5). Tap water was provided ad libitum. At the beginning of the third week of salt treatment, mice underwent a two week training period for blood pressure (BP) measurements three times per week using the CODA non-invasive tail-cuff system (Kent Scientific, Connecticut, USA), according to manufacturer's instructions and the work of others [31]. Following the training period BP measurements were recorded and a mean value from two separate days was calculated. Salt treatment study was repeated twice, with at least 6 male offspring per group each time across different litters.

In order to confirm that salt treatment was effective, separate groups of ANP+/−^{WT} (N = 3) and ANP+/−^{KO} (N = 3) mice were used to collect urine from each mouse in metabolic cages and analyzed for urinary Na^+ excretion. A total of 12 mice were studied and data were compared between NS chow and HS chow.

2.4. Organ excision, RNA isolation and real-time quantitative PCR

In a separate experiment, male mice were euthanized and kidneys excised as described previously [32]. Hematocrit was measured in newborn pups using heparinized capillary tubes and a capillary centrifuge. Organ weight from ANP+/−^{WT} (N = 6) and ANP+/−^{KO} (N = 6) offspring was normalized to tibia length measured using digital calipers; both tibiae were isolated following incubation of the lower limbs in 200 mM NaOH at 60 °C overnight to remove muscle and connective tissue. RNA was isolated and reverse transcription was performed as described previously [32]. Real-time quantitative PCR was performed with the LightCycler® 480 Real-Time PCR System (LC-480; Roche Applied Science, Laval, Quebec, Canada) using the standard curve method for each gene, with *Gapdh* as a reference gene. Cycling parameters consisted of a 95 °C denaturation step for 5 min followed by 40–50 cycles at 95 °C for 15 s, 62 °C for 20 s and 72 °C for 20 s. Primer sets were designed from published NIH GenBank sequences (NCBI, Bethesda, MD, USA) using Primer Design 2.01 software (Scientific and Educational Software, Cary, North Carolina). Sequences and efficiencies of primer sets are shown in Table 1. The listed primers had an efficiency of 1.8 or higher. PCR products from each primer set were resolved on 1%

Table 1
Sequences and efficiencies of primer sets used for real-time qPCR.

Gene	Primer sequence	Primer efficiency
Gapdh	S: GCATCGAAGGTGGAAGAG AS: ACCACAGTCCATGCCATC	1.826
Nppa	S: CAAGAACCTGCTAGACCACC AS: AGCTGTTGCAGCCTAGTCC	1.900
Npr1	S: GGTTCGTTCCTATTGGCTC AS: CCACCATCTCCATCCTCTC	1.911
Npr3	S: CAGCAGACTTGGAACAGGA AS: CCATTAGCAAGCCAGCAC	1.942
Ets1	S: GACTTGCCTGCCTTGATG AS: CTTCCTGTGAAGTGAACGTG	1.865
P300	S: CCACCAACACATCTGCCT AS: CTGGCCTTCAATGCTCAC	1.935
Pde5a	S: TGTATGAAGCGGTGGAGAG AS: GTACCACAGCAGCCGATAA	2.177