A perinatal nitric oxide donor increases renal vascular resistance and ameliorates hypertension and glomerular injury in adult fawn-hooded hypertensive rats

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1Department of Nephrology and Hypertension, University Medical Center, Utrecht, The Netherlands; 2Division of Nephrology and Immunology/Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; and 3Department of Pathology, University Medical Center, Utrecht, The Netherlands

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Koeners MP, Braam B, van der Giezen DM, Goldschmeding R, Joles JA. A perinatal nitric oxide donor increases renal vascular resistance and ameliorates hypertension and glomerular injury in adult fawn-hooded hypertensive rats. Am J Physiol Regul Integr Comp Physiol 294: R1847–R1855, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00073.2008.—Enhancing perinatal nitric oxide (NO) availability can persistently reduce blood pressure in spontaneously hypertensive rats. We hypothesize that this approach can be generalized to other models of genetic hypertension, for instance those associated with renal injury. Perinatal exposure to the NO donor molsidomine was studied in fawn-hooded hypertensive (FHH) rats, a model of mild hypertension, impaired preglomerular resistance, and progressive renal injury. Perinatal molsidomine increased urinary NO metabolite excretion at 8 wk of age, i.e., 4 wk after treatment was stopped (P < 0.05). Systolic blood pressure was persistently reduced after molsidomine (42-wk females: 118 ± 3 vs. 141 ± 5 and 36-wk males: 139 ± 4 vs. 158 ± 4 mmHg; both P < 0.001). Perinatal treatment decreased glomerular filtration rate (P < 0.05) and renal blood flow (P < 0.01) and increased renal vascular resistance (P < 0.05), without affecting filtration fraction, suggesting persistently increased preglomerular resistance. At 4 wk of age, nephriuresis was transiently increased by molsidomine (P < 0.05). Molsidomine decreased glomerulosclerosis (P < 0.05). Renal blood flow correlated positively with glomerulosclerosis in control (P < 0.001) but not in perinatally treated FHH rats. NO dependency of renal vascular resistance was increased by perinatal molsidomine. Perinatal enhancement of NO availability can ameliorate development of hypertension and renal injury in FHH rats. Paradoxically, glomerular protection by perinatal exposure to the NO donor molsidomine may be due to persistently increased preglomerular resistance. The mechanisms by which increased perinatal NO availability can persistently reprogram kidney function and ameliorate hypertension deserve further study. proteinuria; renal hemodynamics; glomerulosclerosis

A large body of experimental and epidemiological studies supports the concept that the intrauterine and early postnatal environment interacts with early development in that it can program metabolic and cardiovascular disease (5, 18, 27). Most of these studies focus on how aberrant perinatal factors increase the occurrence of pathologies in later life. When such factors are superimposed on a specific background of inherited disease, developmental programs will also be affected. Hence, programming can also have long-lasting effects against an abnormal genetic background. Accordingly, advantageous perinatal factors can prevent or correct abnormal development. Because hypertension is associated with decreased nitric oxide (NO) availability (52), it is plausible that factors that support NO availability in the perinatal phase can (re)program development beneficially. This might lead to normalization of blood pressure with increased NO availability. Other potentially effective strategies include preserving nephrogenesis, promoting sufficient renal sodium excretion or correcting renal blood flow (RBF) autoregulation. We have found support for the concept of reprogramming in functional, but not structural, terms.

In previous studies we perinatally supplemented pregnant and lactating spontaneously hypertensive rats (SHR) and offspring up to 4 wk of age with a combination of l-arginine and antioxidants (taurine, vitamins C and E) that resulted in SHR offspring that had persistently lower blood pressure for up to 48 wk of age without an effect on nephron number (32). Perinatal treatment of SHR with the NO donor molsidomine (31) and the arginine precursor citrulline (22) also persistently lowered blood pressure, suggesting that in SHR different maneuvers, which either change redox balance or solely increase NO availability, can persistently reduce blood pressure. However, many mechanistic issues still need to be addressed. Because in SHR GS is absent (13) and there is no elevation of glomerular pressure because preglomerular resistance is high (2), the question remains whether perinatally improving NO availability can protect the kidneys when preglomerular resistance is low. Additionally, how perinatal NO availability can suppress hypertension remains unclear. Underlying mechanisms include enhanced NO availability, improved sodium handling, or altered renal hemodynamics. Therefore, we studied the fawn-hooded hypertensive rat (FHH), a genetic model of hypertension with renal injury characterized by a mild systolic hypertension, progressive proteinuria, and marked focal and segmental glomerulosclerosis (GS), and therefore very different from the SHR model. In FHH van Dokkum et al. (47) observed an impaired control of preglomerular resistance possibly as a result of a low and fixed tension in the afferent arteriole. Because tubuloglomerular feedback is relatively intact (49) this is possibly caused by an impaired myogenic response. This phenomenon might be the underlying mechanism causing GS in FHH rats. Chronic NO synthase inhibition [l-arginine]
arginine methyl ester (L-NAME) leads to accelerated development of GS resulting in renal injury at a younger age (46), revealing partial NO dependency of the adult FHH phenotype.

Because preglomerular resistance is high in SHR (2), perinatal maneuvers supporting NO may well have beneficial effects on blood pressure via decrements of the preglomerular resistance. In the present study we address the challenging issue whether supporting NO availability in a model with a low preglomerular resistance and glomerular injury will also lead to better regulation of renal hemodynamics and consequently less glomerular injury. This would indicate that the consequences of perinatal treatment on the NO system extend beyond a direct vasodilator action on the preglomerular renal vasculature. There is substantial evidence that NO is an important local regulator of tubular reabsorptive function and serves as an important mediator of pressure-induced natriuresis in the kidney (30). Theoretically, perinatally improving NO availability in FHH could only protect the kidney, if a decrease in blood pressure in later life was induced by a postglomerular event that facilitates sodium excretion. We hypothesized that enhancing NO availability in FHH during the perinatal phase ameliorates hypertension and renal injury by persistently increasing NO availability. Hence, we treated FHH dams and their offspring perinatally with molsidomine to directly increase NO.

**METHODS**

**Animals.** FHH were housed at 22°C, humidity 60%, and exposed to a 12-h light-dark cycle. The rats were from our own colony, derived from the original colony at Erasmus University Rotterdam (FHH/EUR) maintained by Dr. A. Provoost. Sentinel animals were housed under the same conditions and regularly monitored for infection by nematodes, pathogenic bacteria, and antibodies for rodent viral pathogens (International Council for Laboratory Animal Science, Nijmegen, The Netherlands). The Utrecht University Board for studies in experimental animals approved the protocol.

**Treatment Protocol.** Adult FHH females and males were mated. Because renal ontogeny is completed at 2 wk (19) we chose a comprehensive window of treatment from day 7 of gestation until pups were 4 wk of age. FHH mothers and their offspring received regular chow (Special Diets Services, Witham, Essex, England) and tap water or regular chow and molsidomine (120 mg/l) in drinking water. Molsidomine was chosen to directly increase perinatal NO availability and because of blood pressure-lowering effects in perinatally treated SHR (31). Molsidomine intake per day at 4 wk was 3 ± 1 mg/100 g body wt. The number of pups and litters and the number of rats per group is indicated in Table 1. At birth large litters were culled to six to eight pups per litter to standardize drug intake during lactation. Depending on litter size we euthanized one or two females and one or two males for another experiment at 2 wk of age. The remaining four pups (ideally 2 females and 2 males) were weaned at 4 wk. All females were followed up to 42 wk with a baseline acute experiment at the end. The males were followed up to 36 wk with either a baseline or an N^2-nitro-l-arginine (L-NNA) acute experiment at the end.

**Chronic follow-up.** Systolic blood pressure (SBP) was measured by tail cuff regularly. The first measurement was performed at 4 wk directly after weaning. Rats were intensively handled and trained to reduce stress as much as possible and warmed with a nearly silent heating ventilator for at least 30 min at 42°C before assessment. Then rats were restrained within a round cylinder while the cuff was applied around the tail base. Measurements were obtained at least three times with the LE 5002 (Lethica Scientific Instruments, Barcelona, Spain) and the quality (e.g., artifacts introduced by movement) of the registration was carefully checked with online Powerlab software. Directly after each SBP measurement rats were placed in metabolism cages without food for 24 h, but with free access to water with 2% glucose, for determination of urinary protein, measured with Coomassie blue. Urine was collected on antibiotics (Sigma, St. Louis, MO; A9909) to prevent formation of NO metabolites and stored at 80°C. At 8, 20, 36, and 42 wk urinary excretion of stable NO metabolites NO2 was measured.

**Acute protocol.** On the day of the experiment, the rats were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg) and placed on a servo-controlled surgical table that maintained body temperature at 37°C. The trachea was intubated with a 16-gauge catheter (Venisystems Abbocath-T, Abbott). A PE-50 catheter was placed in the left jugular vein for infusion of solutions and a second catheter (Venisystems Abbocath-T, Abbott) was placed on a servo-controlled surgical table that maintained body temperature at 37°C. At 4, 8, 20, 36, and 42 wk urinary excretion of stable NO metabolites NO2 was determined by fluorometric quantification of nitrite content as described (4). At 4, 8, 20, 36, and 42 wk urinary excretion of sodium and potassium were determined by flame photometry. At the end of the follow-up we performed acute experiments under anesthesia to determine arterial pressure and kidney function. Subsequently the kidneys were harvested for morphology and morphometry (see below).

**Table 1. Renal characteristics and function**

<table>
<thead>
<tr>
<th></th>
<th>FHH Control</th>
<th>FHH Molsidomine</th>
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<tbody>
<tr>
<td>Litter size (pups/litter)</td>
<td>6.6 ± 0.6</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Females 42 wk final N (pups/litters)</td>
<td>24/10</td>
<td>16/5</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>276 ± 5</td>
<td>274 ± 5</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>1.18 ± 0.02</td>
<td>1.17 ± 0.03</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>113 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>3.31 ± 0.18</td>
<td>2.58 ± 0.10†</td>
</tr>
<tr>
<td>ERPF, ml/min</td>
<td>9.42 ± 0.74</td>
<td>7.55 ± 0.30†</td>
</tr>
<tr>
<td>Hematocrit, vol/vol</td>
<td>0.42 ± 0.01</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>17.23 ± 1.34</td>
<td>12.78 ± 0.65†</td>
</tr>
<tr>
<td>RVR (MAP/ERPF, units)</td>
<td>6.45 ± 0.54</td>
<td>7.76 ± 0.29†</td>
</tr>
<tr>
<td>FF (GFR/RFP)</td>
<td>0.35 ± 0.02</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>FeNa, %</td>
<td>0.066 ± 0.025</td>
<td>0.049 ± 0.014</td>
</tr>
<tr>
<td>FeK, %</td>
<td>12.8 ± 1.4</td>
<td>16.3 ± 1.4</td>
</tr>
<tr>
<td>Males 36 wk final N (pups/litters)</td>
<td>23/10</td>
<td>13/4</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>405 ± 5</td>
<td>395 ± 6</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>1.18 ± 0.03</td>
<td>1.34 ± 0.03‡</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>122 ± 7.2</td>
<td>117 ± 3.3 (P = 0.09)</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>3.34 ± 0.11</td>
<td>3.07 ± 0.14*</td>
</tr>
<tr>
<td>ERPF, ml/min</td>
<td>11.31 ± 0.73</td>
<td>9.11 ± 0.41‡</td>
</tr>
<tr>
<td>Hematocrit, vol/vol</td>
<td>0.47 ± 0.01</td>
<td>0.46 ± 0.01</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>21.16 ± 1.34</td>
<td>16.92 ± 0.79‡</td>
</tr>
<tr>
<td>RVR (MAP/RFP), units</td>
<td>6.13 ± 0.50</td>
<td>7.10 ± 0.40*</td>
</tr>
<tr>
<td>FF (GFR/RFP)</td>
<td>0.34 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>FeNa, %</td>
<td>0.031 ± 0.004</td>
<td>0.055 ± 0.018</td>
</tr>
<tr>
<td>FeK, %</td>
<td>17.7 ± 0.80</td>
<td>15.2 ± 1.1</td>
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Values are means ± SE. FHH molsidomine, fawn-hooded hypertensive rats (FHH) rats perinatally treated with molsidomine; MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; FeNa, fractional excretion of sodium; FeK, fractional excretion of potassium. *P < 0.05 and †P < 0.01 vs. FHH control rats.
intravenously, followed by an infusion of 10 μg·kg⁻¹·min⁻¹ L-NNA (33, 42). This is considered a high dose of a potent inhibitor and therefore we assumed that all NO synthase enzymes were blocked. After equilibration of 30 min a clearance measurement during L-NNA infusion was obtained.

Perfusion-fixation. The right kidney was harvested, blotted dry, weighed, snap frozen, and stored at −80°C. Subsequently left kidney preservation was done as described by Black et al. (7). Heparin sodium (1 unit heparin/g body wt), to prevent clotting, and papaverine hydrochloride (1.2 mg/rat), to dilate the vasculature, were administered via the femoral artery. After ∼3 min the abdominal aorta was exposed and tied off above the left renal artery. Then, via a 21-gauge needle, the kidney was perfused in situ with saline before fixation with 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4) at a pressure of 10 mmHg above measured MAP for ∼3 min. The left kidney was then excised, decapsulated, and placed in 4% buffered formaldehyde.

Morphology. In each rat, 50 glomeruli were scored for the presence of sclerotic lesions, i.e., segmental glomerular scarring, obliteration of glomerular capillaries, mesangial matrix expansion, and adhesion formation between tuft and Bowman’s capsule (45). We differentiated full sclerotic and partial sclerotic glomeruli. The extent of glomerular damage was expressed either as percentage of glomeruli exhibiting one or more of these features minus full sclerotic glomeruli (partial sclerosis), or as percentage of glomeruli exhibiting full sclerotic lesions (full sclerosis) and additionally the sum of these two (sum). Average glomerular tuft diameter of these 50 glomeruli was also determined. Glomerular and tubulointerstitial ED-1-antigen-positive monocytes/macrophages were determined as described (4).

Calculations and statistics. Values are expressed as means ± SE. Data were compared with unpaired t-test, one-way ANOVA, and two-way ANOVA for repeated measurements where appropriate. The Student-Newman-Keuls test was used as a post hoc test (P < 0.05).

RESULTS

Development. All litters were carried to full gestation. Litter size was not affected by prenatal molsidomine (Table 1). Although perinatal molsidomine did not significantly influence body weight at any age (Supplemental Figure S1), terminal left kidney weight was decreased in males (P < 0.01).

NOx and electrolyte excretion. Because the FHH is an outbred strain, different control strains have been used in previous studies, for example Brown Norway (26), Wistar (40), Sprague-Dawley (20), and fawn-hooded normotensive rat (FHL) (47, 50). Compared with previously published data from normotensive Wistar-Kyoto (32), control FHH rats displayed nearly 50% less excretion of stable NO metabolites (NOx) at 8 wk, suggesting impaired NO availability in FHH. Perinatally treated groups showed increased NOx excretion at 8 wk of age (Fig. 1), suggesting a programming phenomenon. Although at 20 wk there were no differences between groups, at 42 wk molsidomine-treated females displayed a significantly higher NOx excretion. Both perinatally treated females and males showed increased natriuresis at 4 wk (Fig. 2). From 8 wk onward differences in natriuresis were not present. Kaliuresis was similar in all groups (not shown). Food intake at 4 wk of age was not different between groups (0.20 ± 0.01 and 0.18 ± 0.02 g·day⁻¹·g body wt⁻¹ for control and molsidomine-treated rats, respectively).

SBP. Perinatal molsidomine reduced SBP at 4 wk in females and males, i.e., at the end of treatment (P < 0.001, Fig. 3). Perinatal molsidomine in both females and males had a pronounced long-term anti-hypertensive effect resulting in a persistently reduced SBP (all P < 0.001).

Baseline renal hemodynamics. Perinatal molsidomine reduced MAP, significantly in females (P < 0.01; Table 1) and marginally in males (P = 0.09). Thus qualitative differences in blood pressure found with the tail cuff were confirmed by direct intra-arterial measurement. Perinatal molsidomine decreased glomerular filtration rate (GFR) significantly (males P < 0.01; females P < 0.05). RBF [=effective renal plasma flow (ERPF)/1 − hematocrit] was also markedly decreased (P < 0.01). Consequently renal vascular resistance (RVR = MAP/RBF) was significantly increased (females P < 0.01; males P < 0.05). There were no consistent effects on filtration fraction (FF = GFR/ERPF) and no differences in fractional excretion of sodium or potassium (Table 1). Correction of renal clearance data by two-kidney weight did not affect differences substantially in females (Supplemental Table S1). However, in males, where left kidney weight was lower in the perinatally treated group, differences in corrected data were not significant.

Proteinuria and morphology. Protein excretion in FHH females was very low until 28 wk; thereafter excretion levels rose quite rapidly to more than 100 mg/day (Fig. 4A). Perinatal molsidomine in females had no significant effect on protein
excretion. Levels of protein excretion in FHH males started to rise earlier than in females, i.e., at 12 wk (Fig. 4B). Perinatal molsidomine diminished the development of proteinuria in male FHH (36 wk: 99 ± 7 mg/day in molsidomine vs. 129 ± 9 mg/day in control, P < 0.001). Perinatal molsidomine treatment reduced GS: the sum of partially sclerotic and full sclerotic glomeruli was significantly lower in female (Fig. 5A) as was the relative number of full sclerotic glomeruli in male (Fig. 5B). Representative glomeruli are shown in Fig. 5C. In males perinatal molsidomine decreased glomerular ED-1-positive monocytes/macrophages significantly (Fig. 6A) and tubulointerstitial ED-1-positive cells (supplemental Fig. S2). In femalesglomerular diameter was markedly decreased by perinatally molsidomine (P < 0.001; Fig. 6B). RBF was positively correlated with GS in FHH control rats (P < 0.001; Fig. 7), suggesting a low preglomerular resistance. Compared with FHH controls the perinatally treated group displayed values in or much closer to the gray area that represents RBF values of 48-wk-old normotensive Wistar-Kyoto and Sprague-Dawley rats measured in our laboratory. Moreover, there was no correlation between RBF and GS in the molsidomine group, suggesting a persistent increase of preglomerular resistance.

**Vasoreactivity.** Aspecific NO synthase inhibition with L-NNA increased MAP to a similar extent in all male groups. However, RVR increased significantly more in both perinatally treated groups compared with control FHH (7.36 ± 0.58 units in molsidomine vs. 3.97 ± 0.60 units in control, P < 0.05; Fig. 8).

**DISCUSSION**

The aim of the present study was to investigate whether perinatal treatment supporting NO availability could persistently suppress hypertension and renal injury in adult FHH rats. Perinatal molsidomine was previously found to be successful in ameliorating hypertension in SHR (24), a totally different genetic model of hypertension without renal injury. Supporting perinatal NO availability in FHH with a NO donor indeed ameliorated the subsequent development of both hypertension and GS. Additionally, compared with untreated FHH of the same sex, we found temporary increases in sodium and NO...
metabolite excretion, an increase in RVR, an increase in NO dependency of RVR, and decreased GFR. Interestingly, this suggests that a decrease in blood pressure in later life was induced by facilitated sodium excretion in the presence of increased preglomerular resistance and not by a vasodilator effect of NO on the preglomerular renal vasculature. Evidently, perinatal enhancement of NO availability, previously documented to be successful in SHR (32), can have beneficial effects on blood pressure, renal injury, and preglomerular resistance in a totally different rat model of genetic hypertension. These beneficial effects of a perinatal NO donor in different models of genetic hypertension merit further mechanistic study.

NO has been implicated in many physiological mechanisms that regulate both acute and long-term control of kidney function and thus blood pressure. A brief perinatal increase in NO availability might persistently change NO bioavailability and reprogram sodium handling or renal hemodynamics. Perinatal molsidomine initially increased urinary NOx and sodium excretion in both females and males, suggesting effective NO delivery to the kidney. After cessation of treatment NOx excretion remained higher than in controls but this difference disap-

![Fig. 4.](image)

**Fig. 4.** Protein excretion in control female and male FHH (○, 24 females and 23 males) and FHH perinatally treated with molsidomine (■, 26 females and 13 males). *P* < 0.05 and †P < 0.01 vs. FHH control.

![Fig. 5.](image)

**Fig. 5.** Full, partial, and sum glomerulosclerosis (GS) incidences in female and male control FHH (open bars, 10 females and 12 males; A) and FHH perinatally treated with molsidomine (solid bars, 11 females and 12 males; B). Representative examples are shown of a normal (C), partially sclerotic (D), and fully sclerotic (E) glomerulus. *P < 0.05, †P < 0.01 vs. FHH control.
peared over time in males and became smaller in females. Hence, temporarily increased NO availability conceivably ameliorated the abnormalities in the developing FHH kidney that underlie full-blown hypertension in adult life.

From the first measurement at 4 wk, SBP was persistently lower in perinatally treated rats compared with controls. Although molsidomine can act as a peroxynitrite donor in vitro (41), others have shown that because of the relatively low oxygen concentration molsidomine will behave as a NO donor in vivo (12). Concordant with the reduction of blood pressure, proteinuria was suppressed and significantly lower in perinatally treated males 9 mo after cessation of treatment, but not in females. In contrast, molsidomine females had the lowest blood pressure, indicating dissociation between blood pressure and proteinuria. Thus in FHH blood pressure and proteinuria seem to be dissociated and are perhaps reprogrammed via different pathways. Possibly this is related to distinct effects on the different quantitative trait loci (RF-1 to RF-5) that are recognized in this model (43, 44).

Although end-stage renal disease was not yet present at 42 wk in female FHH and 36 wk in male FHH (10 and 25% fully sclerotic glomeruli, respectively), renal damage was clearly reduced by perinatal molsidomine. RBF was positively correlated with sclerosis. Possibly this illustrates a low preglomerular resistance in FHH resulting in glomerular hyperperfusion, and consequently glomerular hypertension and injury. This correlation was abrogated by perinatal treatment, suggesting a reduction of glomerular hypertension toward normotensive values. Rodriguez-Iturbe et al. (36) proposed that inflammation is associated with, and has a major role in, the pathogenesis of hypertension. In line with this view we observed a decrease in ED-1-positive cells accompanying the antihypertensive effects of perinatal treatment.

NO is an important local regulator of tubular reabsorptive function and serves as a modulator of pressure natriuresis in the kidney. In the setting of salt-sensitive hypertension for instance it has been proposed that endothelin (ET)-1 influences NO synthase via ET-B receptors (30). It has been shown in FHH rats that the pulmonary vessels respond with enhanced constriction to ET (6), suggesting a shift in expression of ET receptors from ET-A to ET-B. However, the renal ET system has yet to be studied in FHH rats. Effects of molsidomine on blood pressure in adult SHR are inconsistent. In adult male SHR, molsidomine given for 1 wk at a similar dose as in our study increased blood pressure (14); however, in other studies effects of 1–6 wk of molsidomine on blood pressure were not significant (23, 37). Shorter exposure times consistently induce pronounced blood pressure reduction (15, 34, 38). Interestingly, despite the increase in blood pressure in adult male SHR, molsidomine decreased both GFR and RPF and increased RVR (14), just as we observed in FHH in the present study. Natriuresis was significantly increased at 4 wk at the end of perinatal treatment in females and males. Note that weaning at 4 wk was directly followed by a tail-cuff pressure measurement, after which the rats were directly placed in the metabolism cage for urine collection. Presumably there will still have been molsidomine in the gut. Thus the increased natriuresis may well be a direct effect of the NO donor. Moreover, it is clear that the increase in natriuresis in molsidomine-treated rats at 4 wk must have been transient. How long this negative sodium balance lasted is currently unknown, but it was no longer present at 8 wk, and we did not detect weight differences at 4 or 6 wk, so
it must have had a relatively short duration. There was also no persistent effect on natriuresis during the acute experiments, i.e., at the end of the follow-up. Taken together these observations are suggestive for the appealing hypothesis that perinatal improvement of NO availability increases the capacity to excrete sodium and thereby improves blood pressure regulation in FHH. The pathogenesis of hypertension in FHH is unknown; however, it seems logical to implicate a postglomerular phenomenon. A decrease in blood pressure could well be due to actions on the tubules directly, or indirectly, by influencing the release of a natriuretic factor. An increase in solute delivery to the macula densa would be expected to initially reduce GFR via the tubuloglomerular feedback mechanism, which appears to be intact in FHH (49). The resulting reduction in solute delivery to the macula densa would then restore distal delivery.

Correcting the afferent arteriolar function can potentially suppress glomerular hyperperfusion and hence ultimately prevent glomerular hypertension and injury. With our perinatal treatments we significantly decreased RBF and GFR and increased RVR without any consistent effect on FF. In addition the decrease in glomerular diameter in molsidomine females might indicate a decrease in glomerular pressure. This suggests a reduction of the glomerular hypertension via increased preglomerular resistance, although a change in postglomerular resistance cannot be excluded (8). Taken together, this is not a proof for but is compatible with increased afferent arteriolar resistance resulting in a partially suppressed FHH adult phenotype with respect to glomerular injury. This is quite the opposite of the response to arginine plus antioxidants that we observed in SHR where RVR was decreased by perinatal treatment (21). It remains unclear how in early life to exogenous NO, a well-known vasodilator, can increase preglomerular resistance in adult life many months later. Nevertheless, perinatally increasing NO availability can prevent the development of abnormal RVR, which, depending on genetic background, is destined to be either high or low. Note that RBF was calculated from ERPF and could be affected by changes in PAH extraction. Although it is well known that during renal ischemia PAH extraction is reduced, and therefore ERPF grossly underestimated (9, 10, 29), this does not appear to be the case in essential hypertension (35). Because renal plasma flow was high in control FHH (with the most injury) and fell within the normal range in perinatally treated FHH (Fig. 7), we do not expect PAH extraction to be a confounder in our study.

Despite a similar increase in MAP, perinatal molsidomine enhanced the renal response to L-NNA, resulting in significantly more increase in RVR. This indicates that perinatal molsidomine persistently increase the NO dependency of renal resistance, again pleading for persistent functional and/or structural alteration in the renal microvasculature due to perinatal treatment. The nature of this alteration is unclear but the baseline increase in RVR, together with unchanged FF, points toward an as yet unidentified preglomerular constrictor. Alternatively, this alteration could be due to a correction of the impaired myogenic response (24), for instance by more myosin light chain phosphorylation. The stronger increase in RVR after L-NNA suggests that mobilization of this putative constrictor is (partially) offset by increased NO generation. Probably, this reset constrictor-dilator balance is not specific for the current modulation of renal hemodynamics.

The rate of progression of renal disease is generally higher in men than in women (11, 39). Similarly, we observed that FHH males develop proteinuria and GS sooner than females. It has been found that estradiol inhibits and androgen promotes the progression of renal injury (3, 16, 39, 48) and that in females NO release is greater (51). In multiple studies of developmental plasticity, in different species including humans, sex differences have been observed (1, 17, 25, 28). We also observed sex differences after perinatal molsidomine in FHH, possibly indicating interaction between steroid-dependent or Y-chromosome genes and the RF loci. Recent chromosomal mapping data in male and female FHH implicate steroid-dependent rather than Y-chromosome genes (24).
This suggests that in early life NO affects multiple pathways (merular resistance), and enhanced intrarenal NO dependency. Paradoxical increase of RVR (possibly by increased preglo-uria. We found an early increase in sodium excretion, a molsidomine exposure. However, this remains to be shown.

Perspectives and significance. Perinatal treatment of FHH with molsidomine, presumably by enhancing NO availability, ameliorated the development of hypertension, GS, and proteinuria. We found an early increase in sodium excretion, a paradoxical increase of RVR (possibly by increased preglo-

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PERINATAL NITRIC OXIDE REDUCES RENAL INJURY IN FHH

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