Renal baroreceptor-stimulated renin in the eNOS knockout mouse

WILLIAM H. BEIERWALTES, D’ANNA L. POTTER, AND EDWARD G. SHESELY
Hypertension and Vascular Research Division, Henry Ford Hospital, Detroit Medical Campus of Case Western Reserve School of Medicine, Detroit, Michigan 48202

Received 10 May 2001; accepted in final form 3 August 2001

Beierwaltes, William H., D’Anna L. Potter, and Edward G. Shesely. Renal baroreceptor-stimulated renin in the eNOS knockout mouse. Am J Physiol Renal Physiol 51: F59–F64, 2002.—The role of endothelium-derived nitric oxide (NO) in renal baroreceptor stimulation of renin was tested comparing endothelial nitric oxide synthase (eNOS)-deficient mice with C57BL/6J (C57) controls. We measured blood pressure, renal blood flow (RBF), and plasma renin concentration (PRC) in Inactin-anesthetized mice. Blood pressure in eNOS knockout mice was higher than in controls (100 ± 3 vs. 86 ± 1 mmHg, respectively; P < 0.001), but RBF was similar (1.71 ± 0.06 vs. 1.66 ± 0.09 ml·min⁻¹·100 mg kidney wt⁻¹, respectively), so that renal vascular resistance was also higher in the knockouts (59.81 ± 2.07 vs. 51.81 ± 2.66 resistance units, respectively; P < 0.025). PRC was similar (8.24 ± 1.57 in eNOS knockouts vs. 7.10 ± 1.19 ng ANG·I·ml⁻¹·h⁻¹ in C57). NOS inhibition with nitro-l-arginine methyl ester (L-NAME) in C57 controls increased blood pressure (from 85 ± 2 to 106 ± 1 mmHg, P < 0.001) and decreased RBF (from 1.66 ± 0.09 to 1.08 ± 0.02; P < 0.005), but L-NAME had no effect in eNOS knockout mice. When renal perfusion pressure was reduced in C57 controls to 55 mmHg, PRC increased from 6.6 ± 0.9 to 14.5 ± 1.9 μg·ml⁻¹·h⁻¹ (P < 0.025), but this response was blocked by L-NAME. However, in eNOS knockouts, reduced renal perfusion pressure increased PRC from 7.6 ± 1.4 to 15.0 ± 2.8 μg·ml⁻¹·h⁻¹ (P < 0.001). Thus in the chronic absence of eNOS, blood pressure was elevated, but RBF was normal. Additionally, the absence of eNOS did not modify baroreceptor-stimulated renin, suggesting that eNOS-derived NO does not directly mediate this renin-regulating pathway.

Address for reprint requests and other correspondence: W. H. Beierwaltes, Hypertension and Vascular Research Div., 7121 E & R Bldg., Henry Ford Hospital and Health Sciences Ctr., 2799 W. Grand Blvd., Detroit, MI 48202-2689.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajprenal.org 0363-6127/02 $5.00 Copyright © 2002 the American Physiological Society
knockout mice. Contrary to our hypothesis, we found that in the absence of eNOS and without renal vasoconstriction, renal baroreceptor stimulation of renin remains completely intact.

METHODS

To exclude the effect of endothelium-derived NO, we used eNOS knockout mice with the gene for eNOS disrupted by gene targeting (22). The knockout mice have a genetic background that is >99% C57BL/6J (C57) as a result of seven generations of backcrossing to this strain and only one Ren-1c gene. Breeding these animals generates obligate homozygote knockouts. All mice used as breeders are genotyped by PCR to verify that they are homozygous for the eNOS disruption. The normal wild-type controls were C57 mice obtained from Jackson Laboratories (Bar Harbor, ME), which have only the single Ren-1c gene for renin, similar to rats, dogs, and humans. Many other mouse strains have two renin genes, including Ren-1d and Ren-2. Ren-2 contributes additional renin to the circulation and is regulated differently from Ren-1 (7). Breeding and maintenance of this colony and all experimental protocols were approved by the institutional animal care and use committee.

Mice were anesthetized by an intraperitoneal injection of 125 mg/kg body wt of Inactin and placed on a heating pad to maintain a constant body temperature. They were allowed to breathe room air spontaneously. The femoral vein was catheterized with PE-10 tubing for a maintenance infusion of 4 μl/min 0.9% NaCl and for drug delivery. The femoral artery was catheterized with PE-10 tubing attached to a Statham pressure transducer and a Gould recorder for continuous direct monitoring of systemic blood pressure. The pressure transducer was calibrated using a mercury manometer.

A midventral incision was made in the abdominal cavity, and the left renal artery and vein were dissected from the surrounding tissues. Renal venous blood was sampled from a direct line in the left renal vein, using a 25-gauge needle removed from its hub, bent 90°, and inserted into PE-50 tubing attached to a 1-ml syringe. A noncannulating ultrasonic flow probe (Transonic Systems, Ithaca, NY) with an internal circumference of 0.5 mm was placed around the sonic flow probe (Transonic Systems, Ithaca, NY) with an internal circumference of 0.5 mm was placed around the sonicated loop. After surgery, mice received a supplemental bolus of 50–60 mmHg, consistent with the target pressure) were obtained. Then, renal perfusion pressure was restricted the aortic loop to reduce renal perfusion pressure by 10 mmHg and maintained that perfusion for 10 min. The ligature was released, and the perfusion was allowed to return over 15–30 min; the reductions in perfusion were repeated, and the mice were again allowed to recover. After the second set of manipulations, the mice recovered for 30 min and were then given a bolus of L-NAME (10 mg/kg body wt (BW)), which we have previously reported to be the minimal dose needed to induce maximal pressor and renal vasoconstrictor responses (6). Dynamic changes were monitored until the mice stabilized, over 20–30 min after the injection.

In a separate group of mice fitted with the aortic constricting loop, we studied renal hemodynamic responses to sequential decreases in renal perfusion pressure. Changes in RBF as a function of renal perfusion pressure were recorded in eNOS knockout mice (n = 6) and wild-type C57 controls (n = 10) before and after treatment with L-NAME. Once the mice stabilized, we constricted the aortic loop to reduce renal perfusion pressure by ~10 mmHg and maintained that perfusion for ~1 min. RBF and perfusion pressure were monitored continuously. Then, a second 10-mmHg decrease in renal perfusion pressure was produced as above, and additional decreases were applied until the pressure reached 30–40 mmHg. The ligature was released, and the perfusion was allowed to return over ~15–30 min; the reductions in perfusion were repeated, and the mice were again allowed to recover. After the second set of manipulations, the mice recovered for 30 min and were then given a bolus of L-NAME (10 mg/kg BW). The mice were allowed to reach a new baseline over 10–15 min, and then the reductions in renal perfusion pressure were repeated as above.

Renin in response to reduced renal perfusion pressure. Three experimental protocols were used in these studies. First, wild-type C57 controls (n = 6) were prepared as described above. Baseline values for blood pressure, heart rate, RBF, and PRC under control conditions (at normal blood pressure) were obtained. Then, renal perfusion pressure was reduced to ~50–60 mmHg, consistent with the target pressure of previous studies (11, 18), and maintained at this low level of perfusion for 5 min before PRC was collected.

The second protocol was identical to the first, except that the C57 controls were replaced with eNOS knockout mice (n = 14). After baseline control values were obtained, renal
perfusion pressure was decreased and maintained for 5 min before PRC was collected.

In the third protocol, C57 controls (n = 12) were used. Fifteen minutes before the collection period, the mice were given L-NAME (10 mg/kg BW). Once the postdrug baseline had stabilized (10–15 min), control values and PRC were obtained and the protocol was repeated as above. Renal perfusion pressure was decreased and maintained at 50–60 mmHg for 5 min before PRC was collected.

RESULTS

Phenotype of the eNOS knockout mice compared with their wild-type controls. Under basal conditions, blood pressure in the eNOS knockout mice was 14 mmHg higher than in the wild-type controls (100 ± 3 vs. 86 ± 1 mmHg, respectively; P < 0.001). The heart rate was also ~100 beats/min greater in the eNOS knockouts (411 ± 18 vs. 306 ± 21 beats/min, P < 0.001). However, RBF was similar in eNOS knockouts and C57 controls (1.71 ± 0.06 vs. 1.66 ± 0.09 ml·min⁻¹·100 mg KGW⁻¹, respectively), so that RVR was also higher in the knockouts (59.81 ± 2.07 vs. 51.81 ± 2.66 RU, respectively; P < 0.025). Surprisingly, despite the elevated renal perfusion pressure in the knockouts, PRC was similar in knockouts and C57 controls (8.24 ± 1.57 vs. 7.10 ± 1.19 ng ANG I·ml⁻¹·h⁻¹, respectively), so that RVR was also higher in the knockouts (51.20 to 98.15 RU, respectively; P < 0.001) (Fig. 1). Heart rate was unchanged by L-NAME treatment (100 mg KW⁻¹, respectively; P > 0.001). The heart rate was 100 beats/min greater in the eNOS knockouts

Renal perfusion pressure and RBF. Changes in RBF as a function of renal perfusion pressure were monitored in C57 and eNOS knockout mice before and after treatment with L-NAME. As shown in Fig. 2, we did not see the typical autoregulation pattern of RBF in these kidneys, as we did not artificially increase renal perfusion pressures. Rather, we found a gradual pressure-dependent decrease in RBF in both strains.

In the C57 mice, basal BP was 83 ± 3 mmHg and RBF was 1.69 ± 0.11 ml·min⁻¹·100 mg KGW⁻¹. Decreases in renal perfusion pressure below 80 mmHg resulted in diminished RBF (Fig. 2). After the recovery period, L-NAME produced a 28 ± 2 mmHg rise in blood pressure (from 79 ± 4 to 108 ± 5 mmHg, P < 0.001) and a 34% decrease in RBF (from 1.51 ± 0.10 to 0.99 ± 0.10 ml·min⁻¹·100 mg KGW⁻¹, P < 0.001). Again, as renal perfusion pressure was reduced, RBF decreased, although at an attenuated rate compared with the decreases seen before NOS inhibition. Notably, in the range of 50–60 mmHg, RBF was still some 30% lower after L-NAME than in controls, and RVR was some 80% greater at this perfusion pressure after L-NAME treatment than before (P < 0.001).

In eNOS knockout mice, basal blood pressure was 98 ± 4 mmHg, and RBF was 1.44 ml·min⁻¹·100 mg KGW⁻¹. RBF declined as renal perfusion pressure was decreased down to 44 mmHg (Fig. 2). After recovery, there was no significant pressor response to L-NAME in the eNOS knockout mice, as resting blood pressure tended to decrease by some 7 ± 3 mmHg and RBF was unchanged. The response to reduced renal perfusion pressure in eNOS knockout mice was no different before and after L-NAME treatment, and, similarly, RBF and RVR in the range of 50–60 mmHg were the same before and after L-NAME (Fig. 2).

Renin in response to reduced renal perfusion pressure. In C57 wild-type controls, when renal perfusion pressure was reduced from 89 ± 2 to 55 ± 1 mmHg, renal venous PRC more than doubled (Fig. 3), going from 6.6 ± 0.9 to 14.5 ± 1.9 μg·ml⁻¹·h⁻¹ (P < 0.025). Similarly, in eNOS knockout mice, when renal perfusion pressure was reduced from 100 ± 3 to 52 ± 2 mmHg, renal venous renin doubled, going from 7.6 ± 1.4 to 15.0 ± 2.8 μg·ml⁻¹·h⁻¹ (P < 0.001). Finally, if C57 mice were first treated with L-NAME, reduction of renal perfusion pressure from 106 ± 1 to 63 ± 2 mmHg did not alter renal venous PRC (from 11.1 ± 1.6 to 8.3 ± 1.6 μg·ml⁻¹·h⁻¹).
DISCUSSION

We found that mice with the eNOS gene deleted had significantly higher blood pressure, but their RBF was not decreased compared with their wild-type controls. Furthermore, despite the elevated renal perfusion pressure, PRC was similar in the two strains. Inhibition of NOS in the controls resulted in a 20-mmHg pressor response and a 35% decrease in RBF such that RVR doubled, whereas there were no apparent changes in the eNOS knockouts in response to NOS inhibition. Renal baroreceptor stimulation of renin in response to lowering renal perfusion pressure was blunted in the controls after pharmacological inhibition of NOS, similar to reports in dogs (6) and rats (16). However, in eNOS knockout mice, the renin response to lowered renal perfusion pressure was identical to that seen in (untreated) wild-type controls, suggesting that the blunted response after L-NAME is not a direct function of endothelium-derived NO but rather is due to renal vasoconstriction attenuating the responsiveness of the renal baroreceptor.

Endothelium-derived NO is a major regulator of vascular resistance, maintaining RBF through its intrinsic dilator tone under normal conditions (3, 13). We confirmed in mice what others have shown in rats (11) and dogs (18), namely, that acute pharmacological inhibition of NOS results in a significant pressor response and marked renal vasoconstriction. In contrast, NOS inhibition in the eNOS knockout mice did not significantly alter either BP or RBF, suggesting that the systemic and hemodynamic changes in response to L-NAME are in fact mediated by the endothelial isoform of NOS. The apparently normal RBF in the eNOS knockouts suggests that despite the absence of endothelium-derived NO, the renal vasculature has somehow chronically adapted to compensate for the absence of NO and maintain normal renal perfusion.

Chronic pharmacological NOS inhibition leads to sustained renal vasoconstriction and significant hypertension (30). Although our eNOS knockout mice were hypertensive compared with the controls (by 10–15 mmHg), their hypertension is not nearly as severe as that seen in animals given chronic L-NAME (30), or even the pressor response we found in normal mice after acute NOS inhibition. In our studies, the absence of any systemic response to L-NAME in the knockouts is somewhat different from a previous report, which actually reported a depressor response to either acute or chronic L-NAME in eNOS knockout mice (10, 14). The reason for these differences is not clear but may be due to the duration of the protocol, or the fact that those mice were hybrids and not as uniformly inbred as our present colony.

Our characterization of the relationship between renal perfusion pressure and RBF in the mouse suggests
that L-NAME treatment reduces RBF at all pressures, similar to reports in rats (6) and dogs (16). Renal perfusion pressures were not artificially elevated to accommodate complete RBF autoregulation studies, but the present data suggest that basal blood pressure in Inactin-anesthetized mice is near the bottom of the range of autoregulation (Fig. 2). Previous studies have suggested that NOS inhibition does not interrupt RBF autoregulation but rather that it persists at a new level characterized by increased RVR and lower RBF (6, 16). Clearly, this is also the case in the L-NAME-treated controls, but it was not apparent in the L-NAME-treated eNOS knockouts, in which L-NAME had no significant effect on RBF or the relationship between renal perfusion pressure and flow. Maintenance of RBF in the absence of eNOS suggests that the response to reduced perfusion is quite different between chronic deletion of eNOS and pharmacological inhibition. It may be that maintenance of RBF is purely myogenic or that some other vasodilator has compensated for the absence of eNOS-derived NO. In any event, NOS inhibition had no effect on the response of RBF to reduced renal perfusion pressure in the eNOS knockouts.

The renal baroreceptor mechanism is a powerful regulatory pathway through which renin is stimulated by subautoregulatory renal perfusion pressures and attenuated by increased, supranormal pressures. Person et al. (18) described how renin stimulation in response to reduced renal perfusion pressure in dogs was decreased by >60% after NOS was inhibited by L-NAME. Knoblich et al. (11) reported similar attenuation of baroreceptor-stimulated plasma renin activity in rats treated with L-NAME. These studies concluded that baroreceptor-stimulated renin could be attenuated by blocking NO synthesis, suggesting that NO serves as a paracrine pathway inversely linking renal perfusion pressure with renin (11). Alternatively, as renal perfusion pressure is increased, both urinary nitrate/nitrite excretion and the apparent activity of NO in the cortex are increased (17). Wilcox (28) has proposed that higher renal perfusion pressure increases vascular shear stress and/or intracellular calcium in endothelial and juxtaglomerular cells, resulting in activation of eNOS and leading to cGMP-mediated inhibition of renin.

Hemodynamic forces have a significant regulatory effect on eNOS. Vascular shear stress (2, 8, 25) and cyclic strain (1, 2, 8) stimulate NO synthesis and up-regulate eNOS, and these should correlate positively with renal perfusion pressure. Thus it might be expected that renal perfusion below the range of RBF autoregulation should be accompanied by diminished endothelium-derived NO production. However, it has also been reported that acetylcholine enhances pressure-dependent renin secretion (15). The increase in renin secretion produced by decreasing renal perfusion pressure through renal baroreceptors, presumably mediated by decreased afferent arteriolar distension, is blunted by NOS inhibition in conscious dogs (18) and also in the isolated, perfused kidney (12). In rats, a similarly diminished renin response to reduced renal perfusion during chronic NOS inhibition could be reversed by a NO donor at levels that induced severe hypotension (11). These observations have led to a proposed NO-mediated pathway involved in renin stimulation in response to low renal perfusion. However, accompanying the inhibition of NOS was a doubling of RVR, suggesting significant constriction of the renal resistance vessels. If the renal baroreceptor mechanism involves changes in tension in the afferent arteriolar wall at the level of the juxtaglomerular cells in response to altered renal perfusion pressure (24, 29), then the severe renal vasoconstriction may severely dampen the vascular response to pressure. Thus the decrease in renin seen after L-NAME treatment may be a function of dynamic changes in the response mechanism secondary to NOS inhibition rather than any direct effect of NO on renin. When we used pharmacological inhibition of NOS in mice, we found similar results, in that baroreceptor stimulation of renin was completely eliminated by L-NAME treatment. However, if we eliminated eNOS without the massive renal vasoconstriction seen in response to L-NAME, baroreceptor-stimulated renin in the eNOS knockouts remained completely intact.

Despite the observation that increased renal perfusion (and resulting shear stress) which stimulated eNOS also retarded renin secretion, Knoblich et al. (11) proposed that paradoxical stimulation of renin by NO at low perfusion pressures could be the result of either 1) stimulation of NO by vascular wall tension and transmural pressure (9) or 2) an increase in renin caused by stimulation of the macula densa pathway. NO derived from neuronal NOS (nNOS) in the macula densa has been shown to be part of a renin-stimulating cascade (4, 5). However, if measurements of plasma renin activity in response to reduced renal perfusion pressure were repeated using a selective nNOS inhibitor (leaving eNOS intact), there was no renal vasoconstriction and both RVR and the increase in renin secretion rate with reduced renal perfusion pressure were identical before and after NOS inhibition (4). These data were interpreted to suggest that NOS in the macula densa does not contribute to acute renal baroreceptor regulation of renin. Wagner et al. (27) reported that L-NAME given to sodium-restricted mice on a converting enzyme inhibitor (to enhance basal renin expression) diminished renal renin mRNA in controls and nNOS knockouts but had no effect in eNOS knockouts. Similar to our findings, they concluded that eNOS is not essential for the (up- or down-) regulation of renin expression (27).

In summary, we have shown that, despite the chronic absence of eNOS due to gene deletion, RBF adapted to a normal level, in contrast to the significant vasoconstriction seen in the L-NAME-treated animals. With reduced renal perfusion pressure, the RBF response was identical in the knockouts and their wild-type controls. Furthermore, L-NAME did not induce any pressor response or renal vasoconstriction, suggesting that the full hemodynamic response to L-NAME in dogs, rats, and wild-type mice was mediated by the
eNOS isoform. Finally, although l-NAME inhibition of NOS eliminated baroreceptor-stimulated renin, genetic deletion of eNOS had no effect, suggesting that eNOS-derived NO does not directly mediate this renin-regulating pathway.

This work was supported in part by National Heart, Lung, and Blood Institute Grant HL-28982.

REFERENCES