Renal Injury and Salt-Sensitive Hypertension After Exposure to Catecholamines

Richard J. Johnson, Katherine L. Gordon, Shinichi Suga, Adrian M. Duijvestijn, Karen Griffin, Anil Bidani

Abstract—We investigated whether chronic infusion of phenylephrine could induce structural and functional changes in the kidney of rats with the subsequent development of salt-sensitive hypertension. Rats were infused with phenylephrine (0.15 mmol/kg per day) by minipump, resulting in a moderate increase in systolic blood pressure (BP) (17 to 25 mm Hg) and a marked increase in BP variability as measured by an internal telemetry device. After 8 weeks, the phenylephrine infusion was stopped with the return of BP to normal, and a nephrectomy was performed for histological studies. Glomeruli were largely spared, but focal tubulointerstitial fibrosis was present, with the de novo expression of osteopontin by injured tubules, macrophage and “myofibroblast” accumulation, and focal increases in mRNA for transforming growth factor β by in situ hybridization. Peritubular capillaries at sites of injury had distorted morphology with shrinkage, rounding, and focal rarefaction, and endothelial cell proliferation was also identified. Rats were randomized to a high (8% NaCl or 1.36 mol/kg) or low (0.1% NaCl or 17 mmol/kg) salt diet. After 4 to 8 weeks, phenylephrine-treated rats on a high salt diet developed marked hypertension, which was in contrast with phenylephrine-treated rats placed on a low salt diet or vehicle-treated rats given a high salt diet. Hypertension after phenylephrine exposure correlated with the initial mean systolic BP (r²=0.99) and the degree of BP lability (r²=0.74), and the final glomerular filtration rate (r²=0.58). These studies provide a mechanism by which a markedly elevated sympathetic nervous system can induce salt-dependent hypertension even when the hyperactive sympathetic state is no longer engaged. (Hypertension. 1999;34:151-159.)

Key Words: phenylephrine ■ renal circulation ■ hypertension, episodic ■ sympathetic nervous system

There is ample evidence that a hyperactive sympathetic nervous system (SNS) is present in some forms of human hypertension, especially in early, borderline essential hypertension.1 In addition to the acute effects of SNS activation to raise blood pressure (BP), adrenergic stimulation may induce target organ damage by both hemodynamic and nonhemodynamic mechanisms.2 Adrenergic stimulation can be associated with left ventricular hypertrophy as well as hypertension and stiffening of large arteries. Adrenergic agents have also been shown to stimulate vascular smooth muscle cell proliferation and hypertrophy both in vivo and in vitro.3,4

We have been interested in the potential role of the SNS as a mediator of renal injury. It is known that catecholamine infusions can induce acute elevations in glomerular hydrostatic pressure in association with intense renal vasoconstriction and a fall in renal blood flow (RBF).5,6 Infusions of norepinephrine can also induce microalbuminuria in both normal and diabetic individuals.7 A combination of intrarenal and intravenous norepinephrine in dogs resulted in mild tubulointerstitial disease involving 7% of the renal cortex.8 Acute renal failure thought to be secondary to ischemia can also be induced with larger intrarenal doses of norepinephrine.9

One way to examine the effects of chronic adrenergic stimulation is by administering catecholamines chronically to animals. Interestingly, chronic infusions of catecholamines in rats only cause a modest increase (10 to 20 mm Hg) in mean systolic BP,3,10 which is at a level that is usually not associated with renal damage.11,12 However, continuous infusions of catecholamines markedly increase BP lability (defined as the amplitude of BP fluctuations).3,10

In this article we report that chronic infusion (8 weeks) of phenylephrine (PE) in rats results in microvascular injury and tubulointerstitial fibrosis. Although BP returns to normal after the PE infusion is stopped, persistent hypertension redevelops on a high salt diet. The clinical relevance of this new finding to human hypertension is discussed.

Methods

Experimental Protocol

Studies were designed to examine the acute and postinfusion effects of PE on BP and renal structure in the rat. For these studies, BP was measured in unrestrained and unanesthetized rats with the use of radiotelemetry, with BP readings obtained continuously at 10-minute intervals.13 BP was obtained over a 7- to 14-day period after the...
installation of the radio transmitters, after which the rats received PE (30 to 35 mg/kg per day or 0.15 mmol/kg per day SQ) continuously by minipump for 8 weeks. A control group received only vehicle. At the end of 8 weeks, the PE infusions were stopped and the pumps were removed. The rats underwent removal of 1 kidney for histological studies and after 1 to 2 weeks were placed on either a high salt (8% NaCl or 1.36 mol/kg) or low salt (0.1% NaCl or 17 mmol/kg) diet for an additional 4 to 8 weeks. The control rats also underwent nephrectomy and were then placed on a high salt diet for the second 6 to 8 weeks. In addition to histological studies, serum (for creatinine) and urine (collected overnight in metabolic cages) were obtained before the PE infusion, 2 weeks after the PE infusion was started, 2 weeks after the PE infusion was stopped, and at the end of the study. Finally, at the time of death both RBF (transmural) and glomerular filtration rates (GFR) (by inulin clearances) were measured. The study was performed in accordance with guidelines for the humane use of laboratory animals by the National Institutes of Health.

**PE Infusions**

PE (Sigma Chemical) was constituted in 0.2% ascorbate in Ringer’s lactate and infused by subcutaneous minipump (Alzet, model 2002, Alza Corp) at a rate of 0.15 mmol/kg per day in Sprague-Dawley rats (male; weight, 225 to 300 g; Charles River). Pumps were replaced at 30 days.

**Continuous BP Measurements**

Rats were anesthetized with pentobarbital sodium and underwent laparotomy with the placement of a BP sensor catheter in the aorta above the renal arteries with fixation of the radiofrequency transmitter to the peritoneum (model TA11PA-C40, Data Sciences). After surgery, rats were housed in individual plastic cages, where the signals from the pressure sensor were converted, temperature compensated, and sent through the radio transmitter to the telemetry receiver placed under the cage. The receiver was connected to a BCM-100 consolidation matrix, which entered the information into the Dataquest IV acquisition system, which recorded and displayed the BP measurement every 10 minutes for the duration of the study.

From the recordings, mean systolic BP could be determined for any given day. In addition, BP variability (amplitude of BP fluctuations) was calculated as the SD for mean systolic BP measurements, and the severity of labile hypertension was defined as mean systolic BP ±1 SD.

**Immunohistological Studies**

Renal biopsies were collected in methyl Carnoy’s fixative, processed, paraffin-embedded, sectioned (4 μm), and immunostained by indirect immunoperoxidase staining with the following primary antibodies: ED-1 (Serotec), a monoclonal IgG1 to rat macrophages, monocytes, and dendritic cells; ß-smooth muscle actin (4 mm²) at ≥75%. For each biopsy, the entire cortical and juxtamedullary regions were evaluated under low power (×100), and a mean score per biopsy was calculated. In addition, the percentage of glomeruli with juxtaglomerular renin immunostaining was determined in each biopsy. Previous studies from our group have shown that this measurement correlates semiquantitatively with tissue renin content.

**Renal Functional Studies**

At the end of the study, rats were anesthetized with intravenous pentobarbital and surgically prepared for GFR measurements by inulin clearance. The femoral vein was canulated with polyethylene tubing (PE-50), and a priming dose of inulin was administered, followed by a continuous infusion of 150 mmol/L NaCl containing inulin to maintain a plasma inulin concentration of 0.1 mmol/L (50 mg/dL). The contralateral femoral vein and ureter were then canulated, followed by a 150-mmol/L NaCl bolus equal in volume to 1% of the body weight. This was then followed by an infusion of 0.055 mL/min for the replacement of surgical and ongoing fluid losses. Two 20-minute clearances of inulin were obtained, with the midpoint used for each urine collection. Inulin concentrations were measured spectrophotometrically by the diphenylamine method, and the GFR was calculated with the use of standard formulas. RBF was measured with a 1.0-mm Transonic R series flow probe that was placed around the renal artery. Serum creatinines were measured by autoanalyzer. Urinary protein was measured by sulfosalicylic acid precipitation.

**Statistical Analysis**

Comparison between groups was made by the Mann-Whitney test, Student’s t test, or ANOVA with Fisher’s correction when indicated.

**Results**

**PE Infusion Results in Severely Labile Hypertension**

The infusion of PE resulted in a modest (17 to 25 mm Hg) increase in mean systolic BP and a marked increase in BP variability (Table 1). Representative BP tracings (measured by continuous telemetry) in rats receiving PE and in a control rat are shown in Figure 1. Although the mean systolic BP was modestly elevated, rats receiving PE exhibited large spontaneous fluctuations in systolic BP, with frequent readings >180 mm Hg (Table 1, Figure 1).
Renal Structural and Functional Changes Are Induced With PE Infusion
At the end of 8 weeks of PE infusion, rats underwent nephrectomy for histological studies. Light microscopy demonstrated focal renal injury in rats receiving PE that was primarily in a striped pattern radiating into the cortex from the outer medulla (Figure 2). Glomeruli were relatively well preserved, although occasional glomeruli showed glomerular collapse consistent with ischemia. Afferent arterioles and interlobular arteries also showed some thickening with occasional hyalinosis. Juxtaglomerular renin content tended to be higher than in control rats but did not reach significance (Table 2). The most dramatic findings related to the tubulointerstitium. Many tubules were atrophic or dilated, and there was an influx of mononuclear cells into the interstitium, with widening of the interstitial space (Figure 2A, Table 2). In addition, there was marked upregulation of the macrophage adhesive protein osteopontin by both proximal and distal tubules in areas of injury (Figure 2B). An infiltration of macrophages (ED-1 cells) was also present near the sites of osteopontin expression (Figure 2C). Interstitial cells expressing α-smooth muscle actin, consistent with a “myofibroblast” phenotype, were also present (Figure 2D). There was also increased expression of TGF-β mRNA noted by in situ hybridization in the areas of striped fibrosis (Figure 2E and 2F), and this was associated with interstitial deposition of type IV collagen (Figure 2G). Control rats displayed minimal abnormalities.

The integrity of the microvasculature (glomerular and peritubular capillaries) was assessed by immunostaining for the endothelial cell–specific marker RECA-1. Whereas control rats had a normal, lacy pattern of capillaries encircling the tubules, PE-infused rats demonstrated focal areas of altered capillary morphology, with shrinking and rounding of capillaries and focal rarefaction (Figure 3A and 3B). Capillary endothelial cell proliferation (identified by RECA-1 and PCNA double staining) was also focally observed in the capillaries in or near the sites of injury (Figure 3C), whereas it was practically absent in control kidney tissue.

The infusion of PE was also associated with a modest rise in the serum creatinine and a slight increase in urinary protein excretion (Table 1).

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Figure 1. PE infusion induces severe labile hypertension in rats. Shown is a BP tracing from a control rat that received vehicle followed by a high salt diet (A), a rat that received PE followed by a low salt diet (B), and a rat that received PE followed by a high salt diet (C).
Figure 2. Structural changes induced with PE infusion. Rats receiving PE developed striped tubulointerstitial injury, with occasional arteriolar thickening and hyalinosis (inset) (A) (periodic acid–Schiff, magnification ×100). PE infusion was also associated with induction of osteopontin in injured distal and proximal tubular cells (B) (magnification ×50); with an infiltration of ED-1+ macrophages (C) (magnification ×400); with the de novo expression of α-smooth muscle actin by a population of interstitial cells (D) (magnification ×50); with an increase in expression of TGF-β mRNA in areas of fibrosis, as noted by light-field (E) (methyl green and eosin, magnification ×100) and dark-field (F) (magnification ×100) microscopy; and by the interstitial deposition of type IV collagen (G) (magnification ×50).
subsequently placed on a high salt (8% NaCl) or low salt (0.1% NaCl) diet within 1 to 2 weeks after discontinuation of the PE infusion. Systolic BP steadily rose to hypertensive levels in the rats on a high salt diet that had previously received PE. In contrast, systolic BP increased slightly but still remained within the normotensive range in PE-treated rats that were placed on a low salt diet, as well as in control rats that had received vehicle and then were placed on a high salt diet (Table 1).

Rats were maintained on the high and low salt diets for up to 8 weeks and then were killed for additional functional and histological studies. Seven of the 13 rats who received a high salt diet developed severe hypertension and were killed after only 4 to 6 weeks of the high salt diet. As shown in Table 1, PE-treated rats that were placed on a high salt diet had greater urinary protein excretion than either PE-treated rats on a low salt diet or control (vehicle-treated) rats on a high salt diet. The final GFR also tended to be lower in both PE-treated groups, being 50% reduced in the low salt group and 30% reduced in the high salt group (Table 1). In contrast, the RBF was significantly lower in both PE-treated groups, being 50% reduced in the low salt group and 30% reduced in the high salt group (Table 1).

Histological studies obtained at the time of death continued to show evidence of tubulointerstitial damage (Table 2). Although focal areas of tubulointerstitial damage persisted, they appeared less when compared with tissue obtained at the end of the PE infusion. The decrease in injury noted by routine light microscopy was also associated with less osteopontin, α-smooth muscle actin, and TGF-β expression (Table 2 and data not shown). Glomeruli also remained normal, although occasional glomeruli in the high salt group showed glomerular collapse or focal synechiae and the early development of segmental sclerosis.

**Correlations**

An analysis was performed to identify factors that could predict the subsequent BP response to the high salt diet in the rats treated with PE. Both the mean systolic BP and the severity of labile hypertension (defined as the mean BP +1 SD) during the PE infusion strongly correlated with the subsequent BP response to a high salt diet ($r^2=0.987$ and $r^2=0.986$, respectively; $P<0.0001$). The degree of tubulointerstitial damage in the initial biopsy also could predict the subsequent development of hypertension with a high salt diet in PE-treated rats. Although the correlation was relatively weak by standard periodic acid–Schiff scores of tubulointerstitial damage ($r^2=0.471; P<0.003$), it was improved when the correlation was made with the osteopontin scores ($r^2=0.744; P<0.001$) (Figure 4). Interestingly, RBF at the time of death did not correlate with the final BP in PE-treated rats on a high salt diet, although the final GFR did show a weak correlation ($r^2=0.582; P<0.003$).

**Discussion**

We report a new model of salt-sensitive hypertension induced by the chronic exposure (8 weeks) of rats to catecholamines (ie, PE). Infusion of PE resulted in severe labile hypertension and the development of focal microvascular and tubulointerstitial damage in the kidney. When the PE infusion was stopped, both mean BP and BP variability returned to normal, but the subsequent exposure to a high salt diet led to the redevelopment of hypertension. The degree of BP elevation correlated with the BP during the PE infusion and also with the degree of tubulointerstitial damage induced.

The first observation was that the dose of PE used resulted in severe labile hypertension. The overall mean systolic BP during the PE infusion was only 140 to 145 mm Hg. However, there was a dramatic increase in BP variability, an observation that has been noted previously, resulting in frequent BP elevations of ≥170 mm Hg (Figure 1). The finding of mildly elevated mean systolic BP with episodic fluctuations is often used to define the syndrome of borderline hypertension, which is also linked with an elevated SNS, and is also reminiscent of pheochromocytoma, another elevated catecholamine state.

The second observation was that a focal tubulointerstitial lesion was induced with the PE infusion. The tubulointerstitial damage often appeared in stripes radiating out from the outer medulla, suggestive of a vascular pattern of injury (Figure 2). Glomeruli were generally spared, although some glomeruli displayed capillary collapse consistent with renal ischemia. However, the major finding was of focal tubular injury with dilation and cast formation and with focal rarefaction. There was also some evidence of capillary repair, as documented by the presence of proliferating capillary endothelial cells in or near the sites of damage (Figure 3). These histological findings are similar to those observed with other types of tubulointerstitial injury, such as glomerulone-
Figure 3. Microvascular injury induced with PE infusion. Whereas control kidneys (A) (magnification ×200) showed a normal lacy pattern of peritubular capillary staining with the RECA-1 antibody, kidneys from PE-infused rats (B) (magnification ×200) showed shrinkage, rounding, and focal rarefaction of the peritubular capillaries. In addition, some peritubular capillary endothelial cell proliferation (C) (magnification ×630; arrows) could be identified in the distorted capillaries, as noted by double immunostaining for PCNA (nuclear stain) and RECA-1 (cell membrane stain).
phritis, urinary tract obstruction, and cyclosporine toxicity, with aging, and in various hypertensive models. The observation that PE could induce chronic tubulointerstitial disease is of interest. The dose of PE used resulted in mean systolic BP in a range (142 to 145 mm Hg) not generally considered to induce renal damage, at least in humans. We have also reported that rats with 2-kidney, 1 clip hypertension do not develop glomerular and tubulointerstitial injury in the unclipped, hypertensive kidney if the mean systolic BP is <160 mm Hg. However, PE infusion markedly stimulated BP variability, leading to labile hypertension (Figure 1), and it is possible that the renal damage was due to the episodic elevations in pressure. Although the glomerulus and distal capillary beds are thought to be protected from elevations in BP as a result of renal autoregulation in which the afferent arteriole and interlobular artery vasoconstrict, this autoregulatory response is instantaneous, and some transmission of pressure may occur. Indeed, micropuncture studies have documented that norepinephrine infusion will cause significant increases in glomerular and peritubular capillary hydrostatic pressure. Sudden elevations in BP might be expected to induce some peritubular capillary damage, especially because these capillaries are without surrounding pericytes.

Norepinephrine and PE can also cause marked renal vasoconstriction, and micropuncture studies have documented a 30% to 40% decrease in peritubular capillary blood flow. Whether this reduction in blood flow is sufficient to cause local ischemia to the surrounding tubules and interstitium is unclear, but it is of interest that similar reductions in RBF have been observed with other agents that also cause tubulointerstitial damage, including angiotensin II, cyclosporine, and nitric oxide synthase inhibitors. It is also possible that PE could be inducing some of its effects through nonhemodynamic mechanisms, because it is known, for example, that catecholamines can induce smooth muscle cell hypertrophy and proliferation in vitro. The major new finding was that despite the transient return of both mean systolic BP and BP variability to normal on cessation of the PE infusion, the subsequent placement of the rats on a high salt diet resulted in the redevelopment of hypertension. Interestingly, the hypertension persisted despite histological evidence that the renal injury was lessening (Table 2). This suggests that alterations in the ability of the kidney to excrete salt were induced by the PE infusion. Several mechanisms could be operative, including renal injury, which is known to activate renal afferents and stimulate central SNS activity (reviewed in Reference 27); local osteopontin expression, which may inhibit intrarenal nitric oxide generation; and peritubular capillary damage and interstitial fibrosis, both of which could shift the pressure natriuresis curve to the right. The lower GFR observed might also contribute to a relative impairment in salt excretion, although it is known that glomerulotubular balance will maintain sodium excretion despite moderate changes in GFR. This study may be relevant to the natural history of pheochromocytomas, in which it is known that surgical removal is more effective at curing hypertension in the early phases when the hypertension is episodic and there is less evidence of target organ damage.

These studies may also provide a potential pathogenic pathway to explain the linkage between SNS overactivity and the subsequent development of persistent hypertension. However, the model would have to be considered an accelerated version of what occurs in humans, because the BP variability in this model is more severe than that typically observed in human labile hypertension, and the development of persistent hypertension is also more rapid. Nevertheless, this model may be relevant to the hypertension that occurs in blacks, in which evidence for involvement of the SNS includes a greater pressor response to exercise and to norepinephrine, which may relate to increased \( \alpha \)-adrenergic responsiveness. The salt-sensitive hypertension that characterizes this population is associated with substantial renal injury, characterized by arteriolosclerosis, glomerulosclerosis, tubulointerstitial fibrosis, and a reduction in the GFR, findings similar to those observed in our experimental study. Another example is obesity-associated hypertension, which is associated with SNS activation, salt-sensitive hypertension, and renal damage with glomerulosclerosis and tubulointerstitial fibrosis. Similarly, one might speculate that the routine but sometimes marked fluctuations in BP that occur daily in “normotensive” patients and that are known to be triggered by the SNS may be relevant to the age-related development of glomerulosclerosis and tubulointerstitial fibrosis, the age-related decline in GFR, and the development of salt-sensitive hypertension in this population. Finally, one wonders whether a similar mechanism may be involved in the transition from labile to persistent hypertension that occurs in “white-coat” hypertension, with sleep apnea, or with exercise-induced hypertension, all of which are SNS-driven responses. Although certainly speculative, the studies presented here are consistent with recently proposed mechanisms for the development of persistent hypertension in humans.
Acknowledgments
This study was supported by US Public Health Service grants DK-13422, DK-47659, DK-52121, and DK-40426. Dr Suga was supported by a Banyu fellowship and the Kanae Foundation for Life and Socio-Medical Science.

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Hypertension. 1999;34:151-159
doi: 10.1161/01.HYP.34.1.151

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