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 ($r^2=0.99$) and the degree of BP lability ($r^2=0.74$) during the phenylephrine infusion, the amount of osteopontin expressed in the initial biopsy/nephrectomy ($r^2=0.58$), and the final glomerular filtration rate ($r^2=0.58$). 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. 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. Adrenergic stimulation can be associated with left ventricular hypertrophy as well as hypertrophy 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.

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). Infusions of norepinephrine can also induce microalbuminuria in both normal and diabetic individuals. A combination of intrarenal and intravenous norepinephrine in dogs resulted in mild tubulointerstitial disease involving 7% of the renal cortex. Acute renal failure thought to be secondary to ischemia can also be induced with larger intrarenal doses of norepinephrine.

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, which is at a level that is usually not associated with renal damage. However, continuous infusions of catecholamines markedly increase BP lability (defined as the amplitude of BP fluctuations).

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. BP was obtained over a 7- to 14-day period after the

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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; α-smooth muscle actin (a marker of renal interstitial myofibroblasts) (Sigma); OP199, a goat anti-rat osteopontin antibody (gift of C. Giachelli); goat anti-human and anti-bovine type IV collagen (Southern Biotech); monoclonal antibody F37.2D12, an antibody to human renin (gift of M. Laprade, Sanofi Recherche, Montpellier, France); and RECA-1, a monoclonal anti-rat endothelial antibody. Detection of antigens was followed by incubation with a biotinylated secondary antibody directed against the primary antibody, followed by avidin-biotin-peroxidase, diaminobenzidine, and colorimetric reaction, as detailed previously. Controls included omission of the primary antibody or substitution with an irrelevant antibody of the same species and isotype.

Capillary endothelial cell proliferation was detected by double immunostaining tissue sections with the RECA-1 antibody and an antibody to the proliferating nuclear cell antigen (PCNA) (19A2, a monoclonal IgM; Coulter) as described elsewhere, again with the use of controls in which either primary antibody was substituted with an irrelevant monoclonal antibody of the same isotype.

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 alpha-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).

Salt-Sensitive Hypertension Develops in Rats After PE Exposure

At the end of 8 weeks, the PE infusion was stopped, and the pumps were removed. Systolic BP and BP variability quickly returned to the normal range (Table 1, Figure 1). Rats were

<table>
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<th>TABLE 1. Hemodynamic and Renal Functional Changes With PE Infusion</th>
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<tr>
<td>Measurement</td>
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<tr>
<td>Mean systolic BP, mm Hg</td>
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<tr>
<td>PE, then high NaCl (n=13)</td>
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<tr>
<td>PE, then low NaCl (7)</td>
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<tr>
<td>Vehicle, high NaCl (4)</td>
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<tr>
<td>BP variability, mm Hg</td>
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<tr>
<td>PE, then high NaCl</td>
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<tr>
<td>PE, then low NaCl</td>
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<tr>
<td>Vehicle, high NaCl</td>
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<tr>
<td>Serum creatinine, mg/dL</td>
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<tr>
<td>PE, then high NaCl</td>
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<tr>
<td>PE, then low NaCl</td>
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<tr>
<td>Vehicle, high NaCl</td>
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<tr>
<td>Urinary protein, mg/d</td>
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<tr>
<td>PE, then high NaCl</td>
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<td>PE, then low NaCl</td>
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<td>Vehicle, high NaCl</td>
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<tr>
<td>GFR, mL/min</td>
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<tr>
<td>PE, then high NaCl</td>
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<td>PE, then low NaCl</td>
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<tr>
<td>Vehicle, high NaCl</td>
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<tr>
<td>RBF, mL/min</td>
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<tr>
<td>PE, then high NaCl</td>
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<tr>
<td>PE, then low NaCl</td>
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<tr>
<td>Vehicle, high NaCl</td>
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</tbody>
</table>

Values are mean±SD. ND indicates not determined.
*Basal BP was defined as average systolic BP during the final 2 days before initiation of PE infusion.
†P<0.05 compared with vehicle.
‡P<0.05 compared with PE, then low NaCl.

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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).
TABLE 2. Histological Changes Associated With PE Infusion

<table>
<thead>
<tr>
<th>Histological Changes</th>
<th>At End of PE (8 wk)</th>
<th>At End of Diet (Death)</th>
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</thead>
<tbody>
<tr>
<td>Tubulointerstitial score (0–5+)</td>
<td></td>
<td></td>
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<tr>
<td>PE, then high NaCl</td>
<td>3.1±0.3†</td>
<td>3.0±0.4†</td>
</tr>
<tr>
<td>PE, then low NaCl</td>
<td>3.4±0.5†</td>
<td>2.6±0.5†</td>
</tr>
<tr>
<td>Vehicle, high NaCl</td>
<td>0.1±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Osteopontin, % area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE, then high NaCl</td>
<td>3.4±0.8†</td>
<td>2.2±0.9</td>
</tr>
<tr>
<td>PE, then low NaCl</td>
<td>3.5±0.9†</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Vehicle, high NaCl</td>
<td>0.2±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Macrophages (0–4+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE, then high NaCl</td>
<td>2.5±0.3</td>
<td>2.8±0.3†</td>
</tr>
<tr>
<td>PE, then low NaCl</td>
<td>2.1±0.3†</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>Vehicle, high NaCl</td>
<td>1.1±0.3</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Renin, % glomeruli with adjacent renal staining*</td>
<td>PE, then high NaCl</td>
<td>31±2</td>
</tr>
<tr>
<td></td>
<td>PE, then low NaCl</td>
<td>31±5</td>
</tr>
<tr>
<td></td>
<td>Vehicle, high NaCl</td>
<td>25±1</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*Has been shown to correlate with tissue renin content.
†P<0.05 vs vehicle.

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, although it was significant only in the low 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 (r²=0.987 and r²=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 (r²=0.471; P<0.003), it was improved when the correlation was made with the osteopontin scores (r²=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 (r²=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,3,10 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,1 and is also reminiscent of pheochromocytoma, another elevated catecholamine state.21,22

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 accumulations of macrophages, α-actin–positive fibroblasts, and TGF-β expression and collagen deposition. In addition, there was an alteration in the peritubular capillary morphology at the sites of injury, with rounding, shrinkage, and 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-

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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).
Phenylephrine and PE can also cause marked renal vasoconstriction, and 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 lability 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 a-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 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.
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