A Limited Renal Injury May Cause a Permanent Form of Neurogenic Hypertension

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Previously, we have shown that an acute injury to the kidney produced by an intrarenal injection of phenol causes an immediate increase in blood pressure and in norepinephrine (NE) secretion from the posterior hypothalamus. The studies suggest that in this model afferent impulses from the kidney to central integrative structures in the brain may be responsible for the increase in blood pressure.

To further evaluate whether a renal injury caused by the intrarenal injection of phenol leads to a permanent elevation of blood pressure and whether this is mediated by increased sympathetic nervous system activity, we examined the chronic effects (4 weeks) of an intrarenal injection of 50 µL of 10% phenol on blood pressure and NE secretion from the posterior hypothalamus.

Systolic blood pressure increased from 128 ± 2.1 to 176 ± 1.5 mm Hg (P < .01) 4 weeks after receiving the intrarenal injection of phenol, but it did not change in rats that received the vehicle (128 ± 2.4 and 135 ± 1.7 mm Hg) and in rats that were subjected to renal denervation (127 ± 3.4 and 124 ± 1.0 mm Hg). The secretion of NE from the posterior hypothalamic nuclei was greater (P < .01) in rats that received phenol (253 ± 9.6 pg/mL) than in controls (158 ± 8.6 pg/mL) and denervated rats (170 ± 2.1 pg/mL).

These studies have shown that a limited injury to one kidney may cause a permanent elevation of blood pressure and this is associated with increased sympathetic nervous system activity. Am J Hypertens 1998;11:723–728 © 1998 American Journal of Hypertension, Ltd.

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Hypertension remains a significant clinical issue in the patient with renal disease. Several factors may play a role in the pathogenesis of renal hypertension, including sodium retention, volume expansion, and increased activity of the renin-angiotensin system1,2 and of the sympathetic nervous system.3–7 We have shown greater turnover rates of norepinephrine (NE) in the posterior hypothalamic nuclei and in the locus ceruleus of rats with chronic renal failure (CRF) than control rats.8 Bilateral dorsal rhizotomy (T-10 to L-2) prevented the development of hypertension and the increase in NE turnover rates in the posterior hypothalamic nuclei and in the locus ceruleus of CRF rats.9 The secretion of NE, measured by the microdialysis technique, was greater from the posterior hypothalamus of CRF than control rats.10 The decrease in arterial pressure observed in uremic patients after bilateral nephrectomy is associated with lower sympathetic activity of the renin-angiotensin system1,2 and of the sympathetic nervous system.3–7 We have shown greater turnover rates of norepinephrine (NE) in the posterior hypothalamic nuclei and in the locus ceruleus of rats with chronic renal failure (CRF) than control rats.8 Bilateral dorsal rhizotomy (T-10 to L-2) prevented the development of hypertension and the increase in NE turnover rates in the posterior hypothalamic nuclei and in the locus ceruleus of CRF rats.9 The secretion of NE, measured by the microdialysis technique, was greater from the posterior hypothalamus of CRF than control rats.10 The decrease in arterial pressure observed in uremic patients after bilateral nephrectomy is associated with lower sympathetic activity of the renin-angiotensin system1,2 and of the sympathetic nervous system.3–7 We have shown greater turnover rates of norepinephrine (NE) in the posterior hypothalamic nuclei and in the locus ceruleus of rats with chronic renal failure (CRF) than control rats.8 Bilateral dorsal rhizotomy (T-10 to L-2) prevented the development of hypertension and the increase in NE turnover rates in the posterior hypothalamic nuclei and in the locus ceruleus of CRF rats.9 The secretion of NE, measured by the microdialysis technique, was greater from the posterior hypothalamus of CRF than control rats.10 The decrease in arterial pressure observed in uremic patients after bilateral nephrectomy is associated with lower sympathetic
nerve firing and lower regional vascular resistance. These findings suggest that afferent impulses from the kidney of rats and human subjects with chronic renal failure may activate areas of the brain involved in the noradrenergic regulation of blood pressure and contribute to the development of hypertension.

We have also shown that an acute renal injury caused by an intrarenal injection of phenol raises blood pressure and increases the secretion of NE from the posterior hypothalamic nuclei. Renal denervation prevents the increase in blood pressure and NE secretion from the hypothalamus caused by the intrarenal injection of phenol. These studies suggest that afferent impulses from an acutely injured kidney may increase blood pressure and NE secretion from the posterior hypothalamus.

To further evaluate whether a renal injury caused by the intrarenal injection of phenol leads to a permanent elevation of blood pressure and whether this is mediated by increased sympathetic nervous system activity, we have examined the chronic effects of an intrarenal injection of phenol on blood pressure and NE secretion from the posterior hypothalamic nuclei.

METHODS

Animals and Surgical Methods Male Sprague-Dawley rats weighing 200 to 250 g were used for these studies. Rats received normal rat chow (ICN Nutritional Biochemical, Cleveland, OH) and tap water. After anesthesia with an intraperitoneal injection of sodium pentobarbital (35 mg/kg) and placed in a stereotaxic apparatus. A 2-mm long Teflon 22-gauge guide cannula (IV Catheter Placement Unit; Critikon, Inc., Tampa, FL) was implanted using coordinates anteroposterior -4.0 mm, lateral ±0.4 mm, and vertical 8 mm, and secured in place with dental cement. A 28-gauge stainless steel stylus was lowered through the guide cannula to a depth 1.5 mm dorsal to the dorsoventral coordinate for the posterior hypothalamus, namely -8.5 mm from the skull surface.

Microdialysis Probe Probes were constructed from 25-gauge stainless tubing (Critikon) and 1-mm long dialysis membrane (Cuprophor, 0.2 mm diameter blood cutoff; Cobe, Lakewood, CO). One end of the dialysis tube was sealed with epoxy resin (Rapid Araldite; Ciba-Geigy, Summit, NJ). Two lengths of fused silica capillary tubing (outside diameter × inside diameter = 150 × 75 μm) were inserted into the 25-gauge tubing and the long one, which formed the inlet, was inserted into the dialysis tube with the tip 200 μm from the sealed end. The short capillary formed the outlet of the probe. The inlet and outlet fused silica tubes were covered with 10 mm of 27-gauge stainless steel tubing for connection of polyethylene tubing.

The stylus was removed from the guide cannula and replaced with the dialysis probe, which was secured to the guide with sticky wax. The inlet tubing of the dialysis probe was connected by polyethylene 20 tubing to a 1-mL disposable syringe driven by a micropump. The perfusate (pH 7.2) contained Na+ 150 mmol/L, K+ 3.0 mmol/L, Ca2+ 1.4 mmol/L, Mg2+ 0.8 mmol/L, phosphorus 1.0 mmol/L, and Cl− 155 mmol/L. The polyethylene 10 tubing was attached to the outlet side of the probe and the free end led to a 0.5-mL vial set in a small box of ice. The vial contained 2 μL of 0.1 N HCl for preservation of NE. After 90 min of dialysis equilibration, dialysate samples were collected for 5 min each. All samples were immediately frozen and stored at −80°C until the time of assay.

Location of Probe At the end of the experiments, we deeply anesthetized the rats by intravenous sodium pentobarbital (60 mg/kg) and we perfused transcardially a 10% formaldehyde solution. We removed the brains and stored them in formalin for at least 3 days at which time we cut 50-μm serial slices and stained with cresyl violet. Only rats with probes properly implanted in the posterior hypothalamic nuclei were considered for further analysis.

Norepinephrine Microassay We used a highly sensitive microradioenzymatic assay. We add 10 μL of dialysate to 5 μL of reaction mixture containing 1 μL of 3.7 mol/L Tris base (with 0.37 mol/L EGTA and 1.8 mol/L MgCl2, pH 8.2), 0.06 μL of 36 mmol/L benzoxylamine, 1.5 μL of S-[methyl-3H]adenosyl-L-methionine and 2.4 μL of partially purified catechol-O-methyltransferase and incubate for 60 min at 37°C. The sensitivity of this method is 0.5 pg.

Histologic Evaluation Kidneys injected with phenol or vehicle were collected 2 h or 4 weeks after the phenol injection. Kidneys were immediately sectioned...
and post-fixed in Bouin's fixative and imbedded in plastic for histologic examination, which consisted of hematoxylin and eosin-stained sections cut at 3 μm.

**Statistical Analysis**  Data were analyzed by one-way analysis of variance and by the Fisher's test for comparisons among the three groups. Each group included at least five rats and the results are expressed as mean ± SEM.

**RESULTS**

Effects of Intrarenal Injection of Phenol on Blood Pressure and NE Secretion From the Posterior Hypothalamic Nuclei  Intrarenal injection of 10% phenol (50 μL) caused a significant increase in blood pressure. Systolic blood pressure increased from 128 ± 2.1 to 176 ± 1.5 mm Hg (P < .01) 4 weeks after receiving the intrarenal injection of phenol, but it did not change in rats that received the vehicle (128 ± 2.4 and 135 ± 1.7 mm Hg) (Figure 1). The increase in blood pressure was already significant 1 week after the intrarenal injection of phenol. In rats that were subjected to renal denervation blood pressure did not change (127 ± 3.4 and 124 ± 1.0 mm Hg).

The secretion of NE from the posterior hypothalamic nuclei was greater (P < .01) in rats that received phenol (253 ± 9.6 pg/mL) than in controls (158 ± 8.6 pg/mL) and denervated rats (170 ± 2.1 pg/mL) (Figure 2).

Histologic Examination of the Injured Kidney  Kidneys removed 2 h after the acute administration of phenol showed a 1-mm wide area that extended from the cortex to the medulla in a roughly rectangular configuration. In the center of this area there was a parenchymal defect, likely corresponding to the needle track, filled with hemorrhage (Figure 3). Glomeruli in the cortex of the affected area showed intracapillary congestion or thrombosis (Figure 4). The interstitium showed multifocal hemorrhages and edema, and the tubules showed extensive damage, ranging from vacuolar cytoplasmic changes within the more superficial cortex to complete necrosis of both deeper cortical tubular and medullary tubular epithelium, replete with sloughing of the epithelium in the latter area of the pole of the epithelium in the latter (Figures 3 and 4). Peritubular capillaries were markedly congested and dilated in the area of the medulla affected by this damage. Arteries showed no evidence of thrombosis. Areas of the phenol-treated kidneys distant from the area of phenol-induced damage appeared normal.

The kidney removed 4 weeks after the injection of phenol showed a rather small area of linear scar at one pole of the kidney extending from the cortex into the superficial medulla. This area showed tubular atrophy and scarring with small tubules, a few of them containing hyaline casts. There was a moderate interstitial chronic inflammation including plasma cells and pigmented-laden (hemosiderin-laden) macrophages, as well as scant interstitial fibrosis. Glomeruli, interstitium, and arteries in the regions adjacent to this area and throughout the rest of the kidney appeared normal (Figure 5).

The kidney of animals injected with vehicle showed no abnormalities.

**DISCUSSION**

These studies have shown that a limited injury to one kidney may cause a permanent elevation of blood pressure.
pressure and this is mediated by increased sympathetic nervous system activity.

The kidney is a sensory organ richly innervated with baroreceptors and chemoreceptors.\textsuperscript{20–22} Renal afferent nerves project directly or indirectly to a number of areas in the central nervous system that contribute to blood pressure regulation.\textsuperscript{23,24} Stimulation of renal receptors by adenosine, urea, or electrical impulses, evoke reflex increases in sympathetic nerve activity and blood pressure.\textsuperscript{25–27} Renal afferent impulses play an important role in the genesis of hypertension in one-kidney one-clip and two-kidney one-clip Goldblatt hypertension in rats,\textsuperscript{28} but not in the deoxycorticosterone acetate-salt (DOCA-salt) hypertension in

\begin{figure}[h]
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\caption{Kidney, 2 h after injection. A lucent area in the deep cortex/medulla, corresponding to the injection site, lies adjacent to a focal area of acute tubular necrosis characterized by dilated tubules whose epithelium is lost. Normal tubular epithelium is seen below. (H & E stain, ×150.)}
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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Kidney, 2 h after injection. The renal cortex immediately adjacent to the injection site shows glomerular congestion and acute tubular necrosis. (H & E stain, ×300.)}
\end{figure}
rats, in the one-kidney one-wrap Grollman hypertension in the rat, or in the spontaneously hypertensive rat (SHR).\textsuperscript{29–32}

In a previous study we have shown that stimulation of renal sensory receptors produced by an intrarenal injection of phenol caused an immediate increase in blood pressure and a concomitant increase in NE secretion from the posterior hypothalamus. Renal denervation prevented both the increase in blood pressure and NE secretion from the posterior hypothalamus elicited by intrarenal injection of phenol. The increase in blood pressure and in NE secretion could not be attributed to a nonspecific pain stimulus, as intraperitoneal injection of phenol caused only a modest and transitory rise in blood pressure and in NE secretion. On the other hand, injection of phenol in the spleen caused a modest and transitory decrease in NE secretion and blood pressure. These studies showed that afferent signals from the kidneys cause a reflex increase in sympathetic outflow from the posterior hypothalamic nuclei.

The present study has extended these observations to prove that an injury to a limited portion of one kidney may cause a permanent elevation of blood pressure and this is associated with increased noradrenergic activity in the posterior hypothalamic nuclei, which are involved in the neurogenic control of blood pressure.

REFERENCES


