Losartan Reduces Central and Peripheral Sympathetic Nerve Activity in a Rat Model of Neurogenic Hypertension

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Abstract—We have developed a new model of neurogenic hypertension in the rat, in which hypertension is caused by injecting 50 μL of 10% phenol in the lower pole of one kidney. Administration of phenol in the kidney causes an immediate and persistent rise in blood pressure (BP), norepinephrine (NE) secretion from the posterior hypothalamic nuclei (PH), and renal sympathetic nerve activity (RSNA). Because angiotensin II (Ang II) is known to stimulate central and peripheral sympathetic nervous system (SNS) activity, we have tested the hypothesis that losartan, a specific Ang II AT1 receptor antagonist, may lower BP, at least in part, by SNS inhibition. To this end, we studied the effects of losartan on BP and SNS activity following intrarenal phenol injection. Central SNS activity was measured by NE secretion from the PH using a microdialysis technique, and peripheral SNS activity was measured by direct recording of renal nerve activity. At the end of the experiments, brains were isolated and interleukin (IL)-1β and nitric oxide synthase (NOS) mRNA gene expression was measured by RT-PCR in the PH, paraventricular nuclei (PVN), and locus ceruleus (LC). The intrarenal injection of phenol raised BP, as well as central and renal SNS activity, but reduced the abundance of IL-1β and neuronal NOS (nNOS) mRNA in the PH, PVN, and LC. Whether injected intravenously or in the lateral ventricle, losartan caused a significant (P<0.01) and dose-dependent inhibition of the effects of phenol on BP, NE secretion from the PH, and RSNA. Losartan also caused a significant (P<0.01) and dose-dependent rise in IL-1β and nNOS-mRNA gene expression in the PH, PVN, and LC of phenol-injected rats. In conclusion, these studies have shown that the intrarenal injection of phenol causes a rise in central and renal SNS activity and a decrease in IL-1β and nNOS-mRNA in the PH, PVN, and LC. Losartan prevented the rise in BP and SNS activity, as well as the decrease in IL-1β and nNOS mRNA abundance caused by phenol. These studies have demonstrated that the antihypertensive action of losartan in the phenol renal injury model is largely mediated by inhibition of central and peripheral SNS activity and suggest that activation of IL-1β and nNOS, 2 important modulators of central SNS activity, mediates the inhibitory action of losartan on SNS activity. *(Hypertension. 2002;39:1101-1106.)*

Key Words: losartan ■ sympathetic nervous system ■ hypertension, renal ■ nitric oxide synthase ■ interleukins ■ hypothalamus

Hypertension remains a significant clinical problem in patients with renal diseases, and it is an important factor in the pathogenesis of renal failure. When uncontrolled, hypertension may hasten the progression to end-stage renal disease, and it may greatly contribute to cardiovascular morbidity and mortality.

Several factors may play a role in the pathogenesis of renal hypertension, including sodium retention, volume expansion, increased activity of the renin-angiotensin system, and increased sympathetic nervous system (SNS) activity. In the 5/6 nephrectomized (CRF) rat, we observed a greater turnover rate of norepinephrine (NE) and greater secretion of NE from the posterior hypothalamic nuclei (PH) compared with control rats. Bilateral dorsal rhizotomy prevented the development of hypertension and the increase in SNS activity in these rats. In dialysis patients, Converse et al found that the rate of SNS discharge recorded from postganglionic sympathetic fibers in the peroneal nerves was greater in patients with their native kidneys than in those with bilateral nephrectomy. Collectively, these findings support the notion that increased afferent nervous impulses from injured kidneys to the central nervous system may activate the SNS and raise BP.

To further support this notion, we observed that a renal injury caused by the injection of 50 μL of phenol in the lower pole of one kidney results in an immediate and persistent elevation of blood pressure (BP), which is preceded by a rise in norepinephrine (NE) secretion from the PH and renal SNS activity (RSNA) without any measurable alteration in kidney function. Renal denervation prevents the rise in BP and NE secretion from the PH, and nephrectomy of the injured kidney 3 to 4 weeks after the injection of phenol results in normalization of BP.

We have previously shown that losartan, a selective angiotensin II type-1 receptor (AT1) antagonist, reduces blood
pressure in CRF rats largely through inhibition of central SNS activity. Moreover, the inhibitory action of losartan on central SNS activity appeared to be mediated by local activation of interleukin (IL)-1β and nNOS in the PH.9

In the present studies, we have used the phenol renal injury model of neurogenic hypertension to further evaluate whether losartan lowers BP by inhibiting central and peripheral SNS activity and whether this inhibition is mediated by increased abundance of IL-1β and neuronal nitric oxide synthase (nNOS) mRNA in the brain.

Methods

Animal Preparation
Male Sprague-Dawley rats (Harlan, Indianapolis, Ind) weighing 280 to 300 g were used for these studies. Rats received normal rat chow (ICN Nutritional Biochemical) and tap water. After anesthesia with sodium pentobarbital (50 mg/kg, IP), we implanted catheters (PE-10) in a femoral artery and vein for subsequent measurements of arterial pressure and administration of drugs. NE secretion from the PH was measured according to a method previously described by us.9 NE was measured by a highly sensitive microradioenzymatic assay.10

Effect of Intrarenal Phenol on BP and NE Secretion From the PH
After a dorsal incision, we exposed the left kidney and injected 50 μL of 10% phenol or normal saline within the cortex of the lower pole. We continuously recorded arterial BP (Physiograph, Grass Instrument) and collected the dialysate from the PH immediately before the infusion and every 5 minutes thereafter for the entire duration of the experiment.

Effect of Intrarenal Phenol on Renal Sympathetic Nerve Activity
Rats were prepared for renal nerve recording according to the method of Lundin and Thoren,11 as modified by DiBona et al.12 The left kidney, left renal artery, and abdominal aorta were exposed retroperitoneally via a flank incision. A renal nerve branch usually found in the angle between the aorta and the renal artery was dissected free from fat and connective tissue for a length of approximately 10 mm. The nerves were placed on thin bipolar platinum electrodes (Cooner Wire Company) connected to a Grass high-impedance probe (HP 511, Grass Instrument Co). Renal sympathetic nerve activity (RSNA) was amplified (10,000 to 300,000) and filtered with a Grass 511 bandpass amplifier. The amplified and filtered signal was channeled to a Tektronix 5113 oscilloscope (Tektronix, Inc) for visual evaluation, to an audio-amplifier/loudspeaker (Grass AM8 audio monitor) for auditory evaluation, and to a rectifying voltage integrator (Grass 7P10). The voltage-integrated frequency discharge was then displayed on a Grass polygraph. The quality of RSNA was assessed during the operation by examining the magnitude of changes in recorded RSNA during sinoaortic baroreceptor loading and unloading with injections of NE (5 μg, IV) or acetylcholine (1 μg, IV), respectively. When an optimal recording was achieved, the nerve on the electrode was isolated with silicone rubber (Wacker Sil-Gel 604, Wacker, Inc). During this time the animals were kept warm under heated lamps and received an infusion of 30 μL/min of saline. Arterial pressure, heart rate, and RSNA were continuously monitored.

Effect of Losartan IV or ICV on BP, NE Secretion From the PH, and RSNA
Rats received losartan (0.05, 0.1, or 0.3 mg/kg, intravenously) 15 minutes before the intrarenal injection of phenol. Control rats received saline before the phenol injection. BP, NE secretion from the PH, and RSNA were monitored before and for additional 60 minutes after the injection of phenol.

In 2 additional groups of rats, losartan (0.01 mg/kg body weight dissolved in 10 μL of artificial cerebrospinal fluid [aCSF]) or aCSF were infused in the right lateral ventricle over a 5-minute period. Fifteen minutes later, phenol was injected into the lower pole of the kidney. BP, heart rate, NE secretion from the PH, and RSNA were continuously recorded before and after the infusion of losartan.

Effects of Intrarenal Phenol on nNOS and IL-1β–mRNA Abundance in the Brain of Rats
Sixty minutes after the intrarenal injection of phenol or solvent, rats were decapitated, and brains were isolated and immediately frozen and stored at −80°C until assay. Later, brains were cut into consecutive 200-μm sections in a cryostat at −20°C, and bilateral micropunches, 0.5 mm in diameter, were obtained from the PH, paraventricular nuclei (PVN), and locus ceruleus (LC) according to the Paxinos and Watson rat atlas.13-14 Total RNA was extracted from brain nuclei with the TRizol Reagent (Life Technologies). Polymerase chain reaction (PCR) was performed on the resulting reverse transcription (RT) product using a method previously described by us.15

Statistical Analyses
Data were analyzed by one-way analysis of variance (ANOVA) by the Fisher test for comparisons among groups using the computer programs Statview and Graphics 4.01 (Labacus Concepts, Inc). When indicated, repeated-measure ANOVA was performed. Results are expressed as mean±SEM. The accepted level of significance was P<0.05.

Results

Effect of Losartan Infused in the Lateral Ventricle on Blood Pressure, Norepinephrine Secretion From the PH, and RSNA
Infusion of losartan (10 μg in 10 μL of aCSF) in the right lateral ventricle caused a marked inhibition (P<0.01) of the effects of intrarenal phenol injection on blood pressure (Figure 1A), NE secretion from the PH (Figure 1B), and RSNA (Figure 2).

Effect of Intravenous Losartan on Blood Pressure and Norepinephrine Secretion From the PH
To address a potentially more pertinent clinical issue, we evaluated the effects of losartan given intravenously (rather than intracerebroventricularly) before the intrarenal injection of phenol on BP and SNS activity. Pretreatment with losartan in increasing doses of 0.05 to 0.3 mg/kg body weight given intravenously reduced dose-dependently the effects of phenol on BP and NE secretion from the PH (Figures 3A and 3B). Pretreatment with losartan (0.3 mg/kg body weight given IV 15 minutes before injection of phenol) also reduced (P<0.001) the increase in RSNA caused by the administration of phenol (Figure 4).

Effects of Intravenous Losartan on nNOS and IL-1β–mRNA Abundance in the Brain of Rats
Losartan given IV caused a dose-dependent increase in the abundance of nNOS (Figure 5) and IL-1β (Figure 6) in the PH, PVN, and LC of rats injected with intrarenal phenol or vehicle.

Effects of an Intrarenal Injection of Phenol on nNOS and IL-1β–mRNA Abundance in the Brain of Rats
An intrarenal injection of phenol caused a significant (P<0.0001) decrease in IL-1β and nNOS-mRNA in the PH, PVN, and LC (Figures 7A and 7B).

In 2 additional groups of rats, losartan (0.01 mg/kg body weight dissolved in 10 μL of artificial cerebrospinal fluid [aCSF]) or aCSF were infused in the right lateral ventricle over a 5-minute period. Fifteen minutes later, phenol was injected into the lower pole of the kidney. BP, heart rate, NE secretion from the PH, and RSNA were continuously recorded before and after the infusion of losartan.
We have shown that intrarenal injection of phenol results in increased SNS activity and sustained elevation of BP. We postulated that renal afferent stimuli from the injured kidney integrate with areas of the brain involved in the noradrenergic control of BP resulting in SNS activation and elevation of BP. The current studies suggest that central activation of the SNS caused by the renal injury may be the result of inhibition of IL-1β and nNOS mRNA in brain nuclei involved in the noradrenergic control of BP.

Our studies have also demonstrated that losartan inhibits central and peripheral sympathetic nerve activity while increasing the abundance of IL-1β and nNOS mRNA in brain nuclei involved in the noradrenergic control of BP.

Our studies have also demonstrated that losartan inhibits central and peripheral sympathetic nerve activity while increasing the abundance of IL-1β and nNOS mRNA in brain nuclei involved in the noradrenergic control of BP. The classic explanation for the inhibitory action of losartan on SNS activity is that this drug binds to selective AT1 receptors and prevents the effects of Ang II on SNS activation. Ohlstein et al.28 however, have suggested that the antihypertensive effects of Ang II receptor antagonists may not be due solely to Ang II receptor antagonism. This possibility is supported by our studies. In the phenol renal injury model, the rise in BP and in NE secretion from the PH was completely prevented by renal denervation. This suggests that in this model central activation of the SNS is not likely due to effects of circulating Ang II released by the kidney.

The data suggest that afferent impulses triggered by a renal injury may activate Ang II formation in areas of the brain involved in the noradrenergic control of BP and stimulate SNS activity. We also propose that the effects of Ang II on SNS activity are mediated by inhibition of IL-1β and nNOS in PH, PVN, and LC. In fact, losartan attenuates the effect of phenol on SNS activity through a rise in the abundance of IL-1β and nNOS mRNA in these brain nuclei.

Discussion
We have shown that intrarenal injection of phenol results in increased SNS activity and sustained elevation of BP. We postulated that renal afferent stimuli from the injured kidney integrate with areas of the brain involved in the noradrenergic control of BP resulting in SNS activation and elevation of BP. The current studies suggest that central activation of the SNS caused by the renal injury may be the result of inhibition of IL-1β and nNOS mRNA in brain nuclei involved in the noradrenergic control of BP.

Our studies have also demonstrated that losartan inhibits central and peripheral sympathetic nerve activity while increasing the abundance of IL-1β and nNOS mRNA in the PH, PVN, and LC in this rat model of neurogenic hypertension.

Although the intravenous dose of losartan required to inhibit SNS activity was far greater than that necessary to achieve the same results when given intracerebroventricularly, qualitatively the results were the same. This suggests that, even when given intravenously, losartan may penetrate into the brain in sufficient amounts to inhibit central SNS activity.
This, however, does not rule out the possibility that, in response to renal afferent stimuli, locally produced angiotensin II facilitates the activation of central SNS activity. In this case, losartan would reduce SNS activity by blocking the binding of Ang II to selective AT1-receptors in brain noradrenergic neurons.

Our studies indicate that postreceptor effects of Ang II may mediate central SNS activation. Intracerebroventricular infusion of Ang II raised BP, RSNA, and NE secretion from the PH. Ang II also reduced the abundance of IL-1β and nNOS mRNA in the PH, PVN, and LC. Pretreatment with losartan (0.05, 0.1, or 0.3 mg/kg body weight, IV) 15 minutes before the intrarenal administration of 50 μL of 10% phenol. Each group comprised 5 rats. Values are expressed as mean±SEM. All doses of losartan significantly (P<0.01) reduced NE concentration in the dialysate compared with phenol injection only. Each group comprised 5 rats. By contrast, the decrease in BP caused by phentolamine was associated with an increase, rather than a decrease, in NE secretion from the PH and by a decrease in IL-1β and nNOS-mRNA abundance in the PH. Moreover, intracerebroventricular administration of Ang II resulted in inhibition of IL-1β and nNOS and an increase in inhibitory action on SNS activity, the decrease in NO expression caused by Ang II is probably responsible for the increase in SNS activity.

One could speculate that the effects of losartan on SNS activity are secondary to effects on baroreflex mechanisms. This possibility, however, does not seem plausible because the reflex effects of changes in BP on IL-1β and nNOS-mRNA abundance are different from those observed with losartan. In fact, the rise in BP caused by intravenous (not ICV) administration of Ang II results in a significant decrease in NE secretion from the PH and a rise in IL-1β and nNOS-mRNA abundance. By contrast, the decrease in BP caused by phentolamine was associated with an increase, rather than a decrease, in NE secretion from the PH and by a decrease in IL-1β and nNOS-mRNA abundance in the PH. Moreover, intracerebroventricular administration of Ang II resulted in inhibition of IL-1β and nNOS and an increase in inhibitory action on SNS activity, the decrease in NO expression caused by Ang II is probably responsible for the increase in SNS activity.

![Figure 3](image_url) A, Losartan and NE secretion from the PH. The line graphs show NE concentrations in the dialysate obtained from the PH of Sprague-Dawley rats that received losartan (0, 0.05, 0.1, or 0.3 mg/kg body weight, IV) 15 minutes before the intrarenal administration of 50 μL of 10% phenol. Values are expressed as mean±SEM. All doses of losartan significantly (P<0.01) reduced NE concentration in the dialysate compared with phenol injection only. Each group comprised 5 rats. B, Losartan, IV, and mean arterial pressure. The line graphs show levels of mean arterial pressure in Sprague-Dawley rats that received losartan (0.05, 0.1, or 0.3 mg/kg body weight, IV) 15 minutes before the intrarenal administration of 50 μL of 10% phenol. Each group comprised 5 rats. Values are expressed as mean±SEM. All doses of losartan significantly (P<0.01) reduced mean arterial pressure compared with phenol injection only.

![Figure 4](image_url) Losartan IV and renal sympathetic nerve activity. The line graphs show the integrated renal sympathetic nerve activity expressed in percentage changes from baseline in Sprague-Dawley rats that received an intrarenal injection of 50 μL of 10% phenol (closed circles), rats pretreated with losartan (0.3 mg/kg body weight, IV) 15 minutes before the intrarenal administration of phenol (open triangles), and control rats (open circles). Values are expressed as mean±SEM. ∗P<0.01 versus the other 2 groups by ANOVA. Each group comprised 5 rats.

![Figure 5](image_url) Losartan and IL-1β mRNA. Bar graphs show the relative amounts of IL-1β compared with β-actin mRNA in the PH, locus caeruleus (LC), and paraventricular nuclei (PVN) of Sprague-Dawley rats that received losartan (0.05, 0.1, or 0.3 mg/kg body weight, IV) or vehicle 15 minutes before the intrarenal administration of 50 μL of 10% phenol. Each group comprised 5 rats. Values are expressed as mean±SEM. All values postlosartan are significantly (P<0.01) greater than values in rats receiving phenol without losartan.
Losartan and nNOS-mRNA. Bar graphs show the relative amounts of nNOS-mRNA compared with β-actin mRNA in the PH, LC, and PVN of Sprague-Dawley rats that received losartan (0, 0.05, 0.1, or 0.3 mg/kg body weight, IV) or vehicle 15 minutes before the intrarenal administration of 50 μL of 10% phenol. Each group comprised 5 rats. Values are expressed as mean±SEM. All values postlosartan are significantly (P<0.01) greater than values in rats receiving phenol without losartan.

SNS activity. Other investigators have also shown that NE turnover in the PH increases when arterial pressure falls and decreases when arterial pressure rises.29,30

Recent studies have provided evidence that neuronal nitric oxide synthase (nNOS) is present in specific areas of the brain involved in the neurogenic control of BP31,32 and is an important component of transduction pathways that tonically inhibit the sympathetic outflow from the brain stem.33–35

Complex relationships exist between cytokines, SNS activity, and nitric oxide.36–38 IL-1β activates NOS expression in several organs.39,40 Administration of IL-1β into the lateral ventricle of control and CRF rats causes a dose-dependent increase in nNOS-mRNA abundance in several brain nuclei and a decrease in BP and NE secretion from the PH. Infusion of a specific anti-rat IL-1β antibody in the lateral ventricle raised BP and NE secretion from the PH of control and CRF rats. Administration of an anti-rat IL-1β antibody decreased NOS-mRNA expression in the PH, PVN, and LC of both control and CRF rats. In all, these studies suggest that IL-1β modulates the activity of the SNS in the central nervous system and that this modulation is mediated by local expression of nNOS-mRNA abundance.

Other studies, however, have shown that IL-1β may stimulate SNS activity.41 The difference in results between these studies may depend on the way IL-1β is administered and the experimental model used. Intravenous administration of IL-1β may cause hypotension due to effects on the peripheral circulation, which may result in increased SNS activity. On the other hand, infusion IL-1β directly into the lateral ventricle may result in inhibition of SNS activity and BP.

In conclusion, these studies have shown that intrarenal injection of phenol causes a rise in central and renal SNS activity. The antihypertensive action of losartan in the phenol renal injury model is mediated, at least in part, by inhibition of central and peripheral SNS activity. Stimulation of central SNS activity triggered by renal afferent impulses may be mediated by local activation of Ang II. Ang II may stimulate SNS activity via inhibition of IL-1β and NOS-mRNA abundance in brain nuclei involved in the noradrenergic control of BP. The inhibitory action of losartan on central and peripheral SNS may be mediated by blockage of the local effects of Ang II on the brain, resulting in upregulation of IL-1β and nNOS-mRNA, 2 important modulators of central SNS activity.

References


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