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Paper

Losartan reduces sympathetic nerve outflow from the brain of rats with chronic renal failure

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Keywords:
losartan, sympathetic nerve activity, chronic renal failure, nitric oxide, nitric oxide synthase, interleukin-1β, posterior hypothalamus

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Abstract

Sympathetic nervous system (SNS) activity, measured by norepinephrine (NE) turnover rate, was greater in the posterior hypothalamic (PH) nuclei, the paraventricular nuclei (PVN), and the locus coeruleus (LC) of 5/6 nephrectomised (CRF) rats than of control rats. NE secretion from the PH was also greater in CRF than in control rats. These findings demonstrate that SNS activity plays an important role in the genesis of hypertension associated with CRF. The increase in central SNS activity was mitigated by increased local expression of nitric oxide synthase (NOS)-mRNA and nitric oxide (NOx) production. Because angiotensin II may stimulate the central SNS, we tested the hypothesis that losartan, a specific angiotensin II AT_1-receptor antagonist, may lower blood pressure (BP), at least in part, by central noradrenergic inhibition. To this end, we studied two groups of CRF rats. One group received losartan (10 mg/kg body weight) in drinking water between the 3rd and 4th week after nephrectomy, the second group received drinking water without losartan. SNS activity was measured by NE secretion from the PH using the microdialysis technique. NOS-mRNA gene expression was also measured by RT-PCR in the PH, PVN, and LC of CRF and control rats. Losartan reduced systolic BP from 184±3.7 to 152±3.1 mmHg and NE secretion from the PH from 340±9.7 to 247±4.8 pg/ml. CRF rats treated with losartan manifested a significant ($p<0.01$) increase in the expression of nNOS-mRNA in the PH (from 84±1.2 to 99±2.6), the PVN (from 44±1.5 to 63±2.1), and the LC (from 59±6.7 to 76±2.1). CRF rats also manifested a significant increase ($p<0.01$) in the expression of IL-1β in the PH (from 41.6±2.8 to 54.3±1.4), PVN (from 44±1.9 to 54±1.5), and LC (from 35.5±1.6 to 53.5±1.9).

In conclusion, these studies suggest that the antihypertensive action of losartan in CRF rats may be mediated, at least in part, by inhibition of central SNS outflow. The studies also suggest that the inhibitory action of losartan on the SNS may be mediated by activation of IL-1β, which, in turn, stimulates nNOS, an important modulator of central SNS activity.

Introduction

Hypertension remains a significant clinical problem in patients with chronic renal failure, and is an important factor in the pathogenesis of renal failure. Uncontrolled hypertension may hasten the progression to end stage renal disease, and may contribute to cardiovascular morbidity and mortality in these patients.

Several factors may play a role in the pathogenesis of renal hypertension, including sodium retention, volume expansion, and increased activity of the renin-angiotensin-aldosterone system (RAAS)[1,2] or the sympathetic nervous system (SNS).[3-7] We have shown a greater turnover rate of norepinephrine (NE) in the posterior hypothalamic nuclei (PH) and in the locus coeruleus of rats with chronic renal failure (CRF) than in control rats.[8] Also, the secretion of NE from the PH of CRF rats was greater than that of control rats.[9] Bilateral dorsal rhizotomy prevented the development of hypertension and the increase in NE turnover rate in the posterior hypothalamic nuclei and in the locus coeruleus of CRF rats.[10] In addition, the decrease in arterial pressure observed in uremic patients after bilateral nephrectomy was associated with lower sympathetic nerve firing and lower regional vascular resistance.[11] These findings suggest that afferent impulses from the kidney of rats and human subjects with renal disease may activate areas of the brain involved in the noradrenergic regulation of blood pressure and contribute to the development and maintenance of hypertension associated with CRF.

Angiotensin II increases blood pressure through a variety of mechanisms which include vasoconstriction, sodium retention, vascular remodelling, and increased SNS activity.[12-14] Angiotensin II AT_1-receptor antagonists (AIIAs) specifically inhibit angiotensin II binding to AT_1-receptors, resulting in vasodilatation, sodium excretion and lower blood pressure.[15] It is not known whether losartan has any antihypertensive effects mediated by the central nervous system.[16] However, experimental work has shown that losartan may bind to presynaptic AT_1-receptors of sympathetic neurons and decrease NE secretion.[17] Theoretically, these drugs should be able to inhibit SNS activity in situations in which the RAAS is activated. However, the evidence supporting this hypothesis is scanty, and limited to the observation of Brooks *et al.*[18] who has recently shown that eprosartan, a selective AIIA, inhibits the pressor response induced by activation of sympathetic outflow through spinal cord stimulation in pithed rats.

Recent studies have provided evidence that nitric oxide synthase (NOS) is present in specific areas of the brain involved in the neurogenic control of blood pressure.[19,20] The neuronal isoform of NO synthase (nNOS) is an important component

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of the transduction pathways that tonically inhibit the sympathetic outflow from the brain stem.[21] The hypertensive response to L-NAME, an inhibitor of NOS, in normal rats is greatly attenuated by sympathectomy[22] or by renal denervation.[23] We have shown that, in normal rats, the basal activity of the central SNS is inhibited by local NO production.[24] In 5/6 nephrectomised rats, nNOS-mRNA gene expression and NO_2/NO_3 content were greater than in control rats. L-NAME increased blood pressure and NE turnover rate in the brain of CRF rats, suggesting that NO partially mitigates the increase in SNS activity in these rats. We have also shown that IL-1β modulates the activity of the central SNS and that this is mediated by increased local expression of nNOS-mRNA.[25]

In these studies, we examined the effects of losartan, an AT_1-receptor antagonist, on blood pressure and NE secretion from the PH of CRF rats. Moreover, to determine the mechanisms of the potential SNS inhibitory action of losartan, we measured the quantity of IL-1β and nNOS mRNA in several brain nuclei of Sprague-Dawley rats subjected to 5/6 nephrectomy (CRF) with or without administration of losartan.

Materials and methods

Animal preparation

Male Sprague-Dawley rats weighing 250–300 g were used for these studies. Rats received normal rat chow (ICN Nutritional Biochemical, Cleveland, OH) and tap water. Rats were anesthetised with an intraperitoneal injection of sodium pentobarbital (35 mg/kg) and subjected to 5/6 nephrectomy (CRF) as previously described.[26] They were followed for four weeks, and during the last week they received losartan (10 mg/kg body weight in the drinking water), or water without losartan. Blood pressure was measured before surgery and weekly thereafter by the tail-cuff method using an electrosphygmomanometer and physiograph recorder MK-III (Narco Bio-Systems, Houston, TX). Each blood pressure recorded was the average of six to eight readings over 30–40 minutes. Blood samples were drawn from the tail of the animals before and four weeks after the 5/6 nephrectomy for measurement of serum creatinine by autoanalyser.

Norepinephrine (NE) secretion from the posterior hypothalamic (PH) nuclei

Four weeks after nephrectomy, rats were anesthetised with an intraperitoneal injection of sodium pentobarbital (35 mg/kg). Subsequently, the heads of rats were accurately placed in a stereotaxic apparatus and a 2-mm long Teflon 22-gauge guide cannula (IV Catheter Placement Unit; Critikon, Inc., Tampa, FL) was implanted in the PH using coordinates anteroposterior -4.0 mm, lateral ± 0.4 mm, and vertical 8 mm. The guide was 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 posterior hypothalamus, namely -8.5 mm from the skull surface.

Microdialysis probes were constructed from 25-gauge stainless tubing (Critikon) and 1-mm lengths of cuproammonium rayon dialysis tubes, which have a molecular weight cutoff of 10,000 and 206 μm o.d., taken from a hollow fiber dialyzer (Asahi Medical Co., Ltd., Japan). 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 x inside diameter = 150 x 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 microinfusion pump (Razel Model A-99), and an infusion of artificial cerebrospinal fluid (aCSF) prepared by us (in mM: Na^+ 150, K^+ 3.0, Ca^{2+} 1.4, Mg^{2+} 0.8, P 1.0 mM, Cl^- 155, pH 7.2), was initiated at the rate of 1.7 μl per minute. PE-10 tubings were 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 120 minutes of dialysis equilibration, dialysate samples were collected for five minutes each. All samples were immediately frozen and stored at -70°C until the time of assay.

Norepinephrine microassay

All brain samples were sonicated in 0.03 N perchloric acid and then centrifuged (10,000 x g for 30 seconds). The supernatants were assayed for NE by a highly sensitive micro-radioenzymatic assay.[20] Ten μl dialysate were added to 5 μl reaction mixture containing 1 μl of 3.7 mol/l Tris base (with 0.37 mol/l EGTA and 1.8 mol/l $MgCl_2$, pH 8.2), 0.06 μl of 36 mmol/l benzoxylamine, 1.5 μl of S-[methyl-^{3}H]adenosyl-L-methionine and 2.4 μl of partially purified catechol-O-methyltransferase and incubated for 60 minutes at 37°C. The sensitivity of this method is 0.5 pg.

Measurements of IL-1β and nNOS mRNA abundance in the brain

Four weeks after 5/6 nephrectomy, rats were sacrificed by decapitation, and the brains were immediately removed, frozen in dry ice, and stored at -70°C until assay, but for no longer than three weeks. Later, the brains were cut into consecutive 200 μm sections in a cryostat at -20°C and bilateral micropunches 0.5 mm in diameter from several brain nuclei were obtained according to the Paxinos and Watson rat atlas.[27-29] The coordinates for the PH were A-P from -3.5 to -4.1 mm; Lat. ±0.4 mm; V=8 mm; for the PVN were A-P from -1.4 to -2.0 mm; Lat. ± 0.3 mm; V= 7.9 mm; for the locus coeruleus (LC) were A-P from -9.8 mm to -10.2

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Table 1 Oligonucleotide primers for PCR used in the study

		Primer sequences	Size (bp)
nNOS	Upstream:	5′-AAGAGGGTCAAGGCGACCATTC-3′	397
	Bases:	(2599-2620)	
	Downstream:	5′-CGAACACTGAGAACCTCACATTGG-3′	
	Bases:	(2995-2972)	
IL-1β	Upstream:	5'-TGATGTTCCCATTAGACAGCGA-3'	378
	Bases:		
	Downstream:	5'GGTGCTGATGTACCAGTT-3'	
	Bases:		
β-actin	Upstream:	5′-TTCTACAATGAGCTGCGTGTGG-3′	539
	Bases:	(268-289)	
	Downstream:	5′-ATACCCAGGAAGGAAGGCTGGAAG	
	Bases:	(806-783)	

mm; Lat. ±1.4 mm; V=7.2 mm. The nuclei so isolated were used to measure IL-1β and nNOS m-RNA gene expression.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from the tissues with the TRIzol Reagent (Life Technologies,) which is modification of the single-step RNA isolation method described by Chomczyski and Sacchi.[30] The quantity and purity of total RNA for each sample was measured by O.D. at 260 nm and at 280 nm (Du-64 Sprectrophotometer. Beckman Instruments, Inc., Fullerton, CA 92634). Total RNA measurement of all samples ranged between 0.2 and 0.8 µg. All samples were stored at -70°C for the next part of the experiments.

For reverse transcription (RT), total RNA (0.2-0.8 µg) was mixed with 3 µl of random hexamers primers (0.5 ng/µl), incubated at 70°C for 10 minutes and then transferred on ice for five minutes. Nine µl of RT reaction mixture (containing 4 µl of 5 x reaction buffer, 2 µl of 25 mM MgCl2, 1 µl of 10 mM dNTP and 2 µl of 0.1 DTT) were added to each sample tube. The mixture was incubated at 25°C for five minutes. Thereafter, 1 µl (200 units) of Super Scrip™ II RT was added, and samples were incubated at 25°C for 10 minutes and at 42°C for 50 minutes. Subsequently, the reaction mixture was heated to 70°C for 15 minutes to inactivate the reverse transcriptase, then chilled on ice for five minutes. A total of four µl of cDNA template were used for each PCR reaction.

PCR was performed on the resulting RT product using specific oligonucleotide primers for either neural-NOS (n-NOS) or interleukin-1β derived from cDNAs cloned from rat brain[31] (Genbank, Accession X59949) or rat liver[32] (Table 1). A master mix of PCR reagents was made for duplex reactions containing primers for the 'house-keeping' gene β-actin (Genbank, Accession JOO691) and primers for either neuronal NOS (Genbank, Accession X59949), or interleukin-1β (accession number M98820). The PCR reaction mixture contained 10 µl of 10 x PCR buffer, 5 units Taq DNA polymerase, 4 µl cDNA, $MgCl_2$ 2 mM, dNTP 0.2 mM, and 0.1 µM each Primer set. The final volume of each PCR was 100 µl. Each reaction mixture tube was overlaid with 50 µl mineral oil. The PCR was performed with DNA Thermal Cycler 480 (Perkin Elmer, Branchburg, New Jersey). The cycling programs were as follows: denaturation for one minute at 94°C, annealing for 1.5 minutes at 58°C, extension for 1.5 minutes at 72°C. After completion of PCR (25 cycles for beta-actin, 28 cycles for IL-1β, and 28 cycles for NOS), the thermal cycler was stopped in the course of an extension and 80 µl of the reaction volume removed through the mineral oil from each vial to be used for quantification of RT-PCR. To make sure that the PCR reaction was appropriate, the remaining 20 µl of the PCR mixture were subjected to an additional 15 cycles of amplification. Later, PCR products were separated on 1.5% agarose gel electrophoresis stained with ethidium bromide (EtBr). Only PCR products with a distinct target band corresponding to the appropriate product on the electrophoresis gel were used for further analysis.

The RT-PCR products were quantified by a method based on that of Higuchi *et al*.[33] Fluorescence was measured in a fluorescence spectrofluorometer (F-2000, Hitachi Ltd., Tokyo, Japan). Excitation was at 280 nm and emitted light was selected at 590 nm. Results were expressed as a ratio of the resultant optical densities for the specific gene to β-actin.

Random hexamers, dithiothretol (DTT), Super Scrip™ Super reverse transcriptase with reaction buffer (5 x) (20 mM Tris-HCl, 10 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP 40, and 50% glycerol), Taq DNA polymerase with reaction buffer (10 x) (50 mM Tris-HCl, 10 mM NaCl, 0.1 mM EDTA, 5 mM DTT, and 50% glycerol, and 1.0% Triton X-100), deoxynucleotide mixture (dNTP) and $MgCl_2$ were purchased from GIBC/BRL (Gaithersburg, Maryland, USA).

Location of probes

At the end of the experiments, while rats were still anesthetised, the dialysis probes were removed and rats were sacrificed by decapitation. The brains were immediately removed, frozen in dry ice, and stored at -70°C. Later, brains were sliced in 200 µm sections and the proper location of the

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Figure 1 The line graphs show levels of systolic blood pressure in 5/6 nephrectomised Sprague-Dawley rats who received losartan (10 mg/kg body weight) in drinking water during the last week of the study (CRF + losartan, continuous line), or CRF rats who received no medication in drinking water (CRF, dotted line). Both groups comprised six rats each. Values are expressed as means ± SEM. * = p<0.001

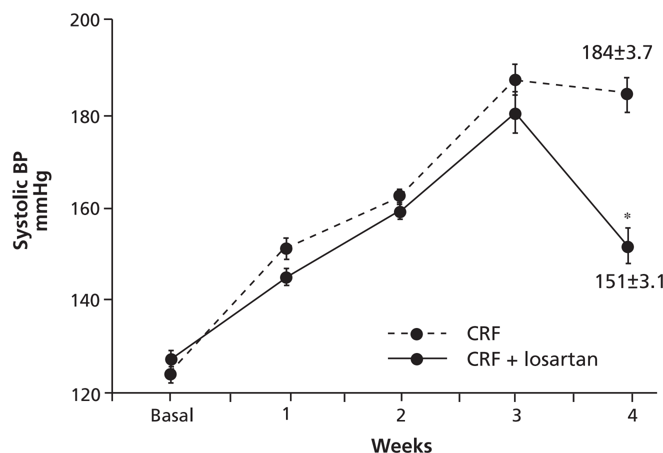
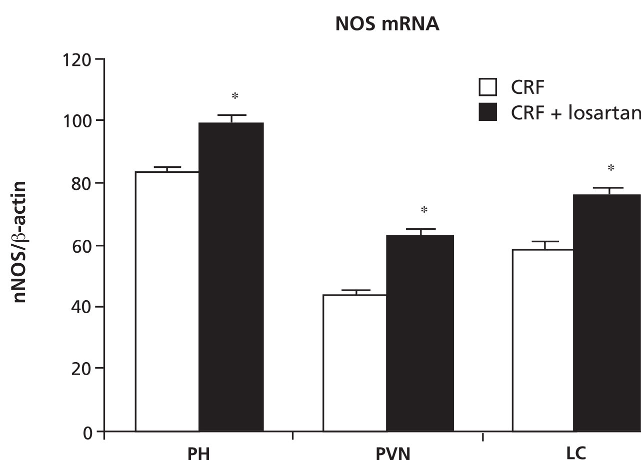


Figure 2 Bar graphs show the relative amounts of nNOS-mRNA compared with β-actin mRNA in the posterior hypothalamic nuclei (PH), locus coeruleus (LC) and paraventricular nuclei (PVN) of 5/6 nephrectomised Sprague-Dawley rats who received losartan (10 mg/kg body weight) in drinking water during the last week of the study (CRF + losartan), or CRF rats who received no medication in drinking water (CRF). Each group comprised six rats. Data represent means ± SEM. * p<0.001 *vs* control.



lesion in the posterior hypothalamic nuclei identified. Only rats with probes properly implanted in the posterior hypothalamic nuclei were considered for further analysis.

Statistical analyses

Data were analysed by one-way analysis of variance, by the Fisher's test for comparisons among groups using the computer program Statview and Graphics 4.01 (Labacus Concepts, Inc.). When indicated, repeated measure ANOVA was performed. Simple regression analyses were performed to determine correlations between different parameters. Results are expressed as mean±SEM. The accepted level of significance was p<0.05.

Results

Blood pressure and norepinephrine secretion from the posterior hypothalamic nuclei (PH)

In CRF rats, blood pressure increased progressively during the first three weeks following 5/6 nephrectomy. However, during the last week of observation, rats that received losartan had significantly lower systolic blood pressure than that of rats receiving no medication (p<0.001) (184±3.7 and 152±3.1 mmHg, respectively) (Figure 1). Rats that received losartan also demonstrated significantly lower NE secretion rate from the PH than control rats (247±4.8 and 340±9.7 pg/ml, respectively; p<0.001) (Figure 2).

Effects of losartan on nNOS and IL-1β-mRNA abundance in the brain of CRF rats

CRF rats treated with losartan manifested a significantly greater (p<0.01) abundance of NOS-mRNA compared with untreated rats in the PH (99±2.6 *vs* 84±1.2), the PVN (63±2.1 *vs* 44±1.5), and the locus coeruleus (76±2.1 *vs* 59±6.7) (Figure 2). CRF rats also manifested a significant greater (p<0.01) abundance of IL-1β compared with

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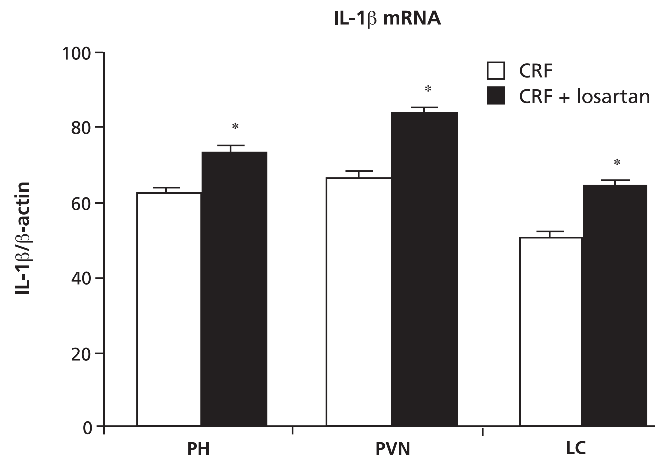
Figure 3 Relative amounts of IL-1β-mRNA compared with β-actin mRNA in the posterior hypothalamic nuclei (PH) locus coeruleus (LC) and paraventricular nuclei (PVN) of 5/6 nephrectomised Sprague-Dawley rats who received losartan (10 mg/kg body weight) in the drinking water during the last week of the study (CRF + losartan), or CRF rats who received no medication in the drinking water (CRF). Each group comprised six rats. Data represent means ± SEM. * $p<0.001$ *vs* control.



untreated rats in the PH (54.3±1.4 *vs* 41.6±2.8), the PVN (54±1.5 *vs* 44±1.9), and the locus coeruleus (53.5±1.9 *vs* 35.5±1.6) (Figure 3).

Discussion

These studies have shown that the antihypertensive action of losartan in CRF rats may be due, at least in part, to inhibition of sympathetic nervous system activity. The studies have also shown that the administration of losartan is associated with an increase in the abundance of IL-1β and nNOS in several brain nuclei involved in the noradrenergic control of blood pressure. We speculate that the increased abundance of IL-1β and nNOS mRNA may mediate the inhibition of central SNS activity.

We have previously shown that hypertension in CRF rats is largely dependent upon increased activity of the SNS; afferent stimuli from the injured kidney connect with brain nuclei involved in the noradrenergic control of blood pressure and result in activation of the SNS and an increase in blood pressure.[8-10]

The evidence from this study that losartan reduces central SNS activity and blood pressure in CRF rats, lends further support (albeit indirect) to the notion that central activation of the SNS may play a role in the pathogenesis of hypertension in this model. One could speculate that the effects of losartan on the CNS could be mediated indirectly through reflex or compensatory mechanisms, or could represent epi-phenomena rather than being causal. This possibility, however, does not seem very plausible since the reflex effects resulting from a reduction in blood pressure are different from those observed with losartan. Indeed, we have previously shown that administration of angiotensin II in doses that elevate blood pressure up to 180 mmHg, cause a significant decrease in NE secretion from the posterior hypothalamic nuclei and an increase in IL-1β and nNOS mRNA abundance. By contrast, the decrease in blood pressure caused by phentolamine was associated with an increase, not a decrease, in NE secretion and by a decrease in IL-1β and nNOS mRNA abundance in the PH.[25] Other investigators have also shown that NE turnover in this region increases when arterial pressure falls and decreases when arterial pressure rises.[34,35]

We selected NE secretion from the PH as a marker of SNS activity because some evidence has shown that an increase in noradrenergic activity in the PH is associated with an increase in peripheral SNS activity and in blood pressure. Electrical stimulation of the PH increases blood pressure[36,37] while destruction of these areas decreases blood pressure in rats.[38] Local perfusion of hypertonic saline or phenylephrine in the PH caused an increase in blood pressure, heart rate and NE release.[39] All these studies provide supportive evidence, albeit indirect, that the PH is an important component of the central noradrenergic regulation of blood pressure.

Complex relationships exist between cytokines, the sympathetic nervous system and nitric oxide.[40-45] IL-1β activates NOS expression in several organs[46,47] including smooth muscle and endothelial cells.[48,49] Post-inflammatory cytokines increase the expression of an inducible form of NO synthase in airway epithelial cells,[50] and in rat microvascular brain endothelial cells.[51] Some evidence also suggests that NO is involved in the IL-1β-induced central activation of sympathetic outflow in rats.[52] We have shown that administration of IL-1β in the lateral ventricle of control and CRF rats caused a dose-dependent decrease in blood pressure and NE secretion from the PH, and an increase in nNOS-mRNA abundance in several brain nuclei. Moreover, infusion of a specific anti-rat IL-1β antibody in the lateral ventricle led to an increase in BP and NE secretion from the PH of control rats, and to a further increase in blood pressure and NE secretion from the PH of CRF

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rats. Administration of an anti-rat IL-1β antibody decreased NOS-mRNA expression in the PH, PVN and locus coeruleus of both control and CRF rats.[25]

In all, these findings suggest that IL-1β mediates a negative feed-back loop on the activity of the sympathetic nervous system via activation of nNOS. With these studies we cannot rule out the possibility that an increase in angiotensin II levels secondary to angiotensin II receptor inhibition may play a role in upregulating IL-1β and nNOS mRNA. Further studies using AT_2-receptor blockers are necessary to address this possibility.

The activation of IL-1β could also be secondary to the uremic state. However, we have shown that the abundance of IL-1β increases when blood pressure increases independently of alterations in kidney function, such as after administration of angiotensin II,[25] or in the phenol renal injury model of hypertension (data not presented and unpublished), which is a neurogenic model of hypertension developed in our laboratory.[53]

In conclusion, these studies suggest that the antihypertensive action of losartan in CRF rats may be mediated, at least in part, by central inhibition of the sympathetic nervous system outflow. The studies also suggest that the inhibitory action of losartan on the SNS may be mediated by activation of IL-1β, which, in turn, stimulates nNOS, an important modulator of central SNS activity.

Activation of the renin-angiotensin-aldosterone system is another important pathogenetic factor for hypertension in this model.[1-2] Angiotensin II can increase blood pressure through direct vasoconstriction, aldosterone secretion, sodium retention, and activation of the sympathetic nervous system both, centrally and peripherally.[12-14]

References

1 . Schalekamp MADH, Schalekamp-Kuyken MPA, deMoor-Fruytier M, Meininger T, Vaandrager-Kranenburg DJ, Birkenhager WH. Interrelationships between blood pressure, renin, renin substrate and blood volume in terminal renal failure. *Clin Sci Mol Med* 1973; **45**:417-28.

2 . Lazarus JM, Hampers CL, Merrill JP. Hypertension in chronic renal failure. Treatment with hemodialysis and nephrectomy. *Arch Intern Med* 1974; **133**:1059-66.

3 . Atuk NO, Bailey CJ, Turner S, Peach MJ, Westervelt FB, Jr. Red blood cell catechol-o-methyl transferase, plasma catecholamines and renin in renal failure. *Trans Am Soc Artif Intern Organs* 1976; **22**:195-200.

4 . Lake CR, Ziegler MG, Coleman MD, Kopin IJ. Plasma levels of norepinephrine and dopamine-beta-hydroxylase in CRF patients treated with dialysis. *Cardiovasc Med* 1979; **1**:1099-1111.

5. Henrich WL, Katz FH, Molinoff PB, Schrier RW. Competitive effects of hypokalemia and volume depletion on plasma renin activity, aldosterone and catecholamine concentrations in hemodialysis patients. *Kidney Int* 1977;**12**:279-84.

6 . Izzo JL, Izzo MS, Sterns RH, Freeman RB. Sympathetic nervous system hyperactivity in maintenance hemodialysis patients. Trans. *Am Soc Artif Organs*1982; **28**: 604-7.

7 . Cuche JL, Prinseau J, Selz F, Ruget G, Baglin A. Plasma free, sulfo- and glucuro-conjugated catecholamines in uremic patients. *Kidney Int* 1986;**30**:566-72.

8 . Bigazzi R, Kogosov E, Campese VM. Altered norepinephrine turnover in the brain of rats with chronic renal failure. *J Am Soc Nephrol* 1994; **4**:1901-7.

9. Ye S, Ozgur B, Campese VM. Renal afferent impulses, the posterior hypothalamus, and hypertension in rats with chronic renal failure. *Kidney Int* 1997;**51**:722-7.

10. Campese VM, Kogosov E. Renal afferent denervation prevents hypertension in rats with chronic renal failure. *Hypertension* 1995; **25**[part 2]:878-82.

11. Converse RL Jr., Jacobsen TN, Toto RD *et al*. Sympathetic overactivity in patients with CRF. *New Engl J Med* 1992;**327**:1912-8.

12. Ferrario CM, Gildenberg PL, McCubbin JW. Cardiovascular effects of angiotensin mediated by the central nervous system. *Circ Res* 1972:**30**:257-62.

13. Zimmerman BG, Sybertz EJ, Wong PC. Interaction between sympathetic and renin-angiotensin system. *J Hypertens* 1984;**2**:581-8.

14. McGiff JC, Fasy TM. The relationship of the renal vascular activity of angiotensin II to the autonomic nervous system. *J Clin Invest* 1965;**4**:1911-23.

15. Chiu AT, McCall DE, Price WA *et al*. Nonpeptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Ther* 1990;**252**:711-8.

16. Wong PC, Price WA, Chiu AT *et al*. Nonpeptide angiotensin II receptor antagonists. XI. Pharmacology of EXP3174, an active metabolite of DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Ther* 1990;**255**:211-7.

17. Ohlstein EH, Gellai M, Brooks DP *et al*. The antihypertensive effects of the angiotensin II receptor antagonist DuP 753 may not be due solely to angiotensin II receptor antagonism. *J Pharmacol Exp Ther* 1992;**262**:595-601.

18. Brooks DP, Ohlstein EH, Ruffolo RR, Jr. Pharmacology of eprosartan, an angiotensin II receptor antagonist: Exploring hypotheses from clinical data. *AM Heart J* 1999;**138**:246-51.

19. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. *Nature* 1990; **347**:768-70.

20. Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* 1992;**46**:755-84.

21. Tseng CJ, Liu HY, Lin HC, Ger LP, Tung CS, Yen MH. Cardiovascular effects of nitric oxide in the brain stem nuclei of rats. *Hypertension* 1996;**27**:36-42.

22. Sander M, Hansen PG, Victor RG. Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. *Hypertension* 1995;**26**:691-5.

23. Matsuoka H, Nishida H, Nomura G, van Vliet BN, Toshima H. Hypertension induced by nitric oxide synthesis inhibition is renal nerve dependent. *Hypertension* 1994;**23**[part 2]:971-5.

24. Yee S, Nosrati S, Campese VM. Nitric oxide (NO) modulates the neurogenic control of blood pressure in rats with chronic renal failure. *J Clin Invest* 1997;**99**:540-8.

25. Ye S, Mozayeni P, Gamburd M, Zhong H, Campese VM. Interleukin 1-ß and the neurogenic control of blood pressure in normal rats and rats with chronic renal failure. *Am J Physiol* (In press).

26. Bigazzi R, Kogosov E, Campese VM. Altered norepinephrine turnover in the brain of rats with chronic renal failure. *J Am Soc Nephrol* 1994;**4**:1901-7.

27. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic 1986.

28. Glowinski J, Iversen L. Regional studies of catecholamines in the rat brain. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J Neurochem* 1966;**13**: 665-9.

29. Palkovits M, Zaborszky L. Neuroanatomy of central cardiovascular control. In: W. de Jong, A.P. Provoost and A.P. Shapiro (Eds), Hypertension and Brain Mechanisms, Progress in Brain Research, Vol 47, Elsevier, Amsterdam, 1977: 9-34.

30. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;**162**:156-9.

31. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991; **351**:714-8.

32. Scotte M, Hiron M, Masson S. Differential expression of cytokine genes in monocytes, peritoneal macrophages and liver fllowing endotoxin- or turpentine-induced inflammation in rat. *Cytokine* 1996;**8**:115-20.

33. Higuchi R, Dollinger G. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* 1992; **10**:413-7.

34. Patel KP, RL Kline. Influence of renal nerves on noradrenergic responses to changes in arterial pressure. *Am J Physiol* 1984;**247**:R615-20.

35. Chalmers JP. Brain amines and models of experimental hypertension. *Circ Res* 1975;**36**:469-80.

36. Patel KP, Kline RL. Influence of renal nerves on noradren-

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ergic responses to changes in arterial pressure. *Am J Physiol* 1984;**247**:R615-20.
37. Chalmers JP. Brain amines and models of experimental hypertension. *Circ Res* 1975;**36**:469-80.
38. Bunag RD, Eferakeya AE. Immediate hypotensive aftereffects of posterior hypothalamic lesions in awake rats with spontaneous, renal, or DOCA hypertension. *Cardiovasc Res* 1976;**10**:663-71.
39. Nakata T, Berard W, Kogosov E, Alexander N. Microdialysis in the posterior hypothalamus: sodium chloride affects norepinephrine release, mean arterial pressure, heart rate and behavior in awake rats. *Brain Res Bull* 1990;**25**:593-8.
40. Ruhl A, Hurst S, Collins SM. Synergism between interleukins 1β and 6 on noradrenergic nerves in rat myenteric plexus. *Gastroenterology* 1994;**107**:993-1001.
41. Ruhl A, Berezin I, Collins SM. Involvement of eicosanoids and macrophage-like cells in cytokine-mediated changes in rat myenteric nerves. *Gastroenterology* 1995;**109**:1852-62.
42. Ichijo T, Katafuchi T, Hori T. Central interleukin-1β enhances splenic sympathetic nerve activity in rats. *Brain Res Bull* 1994;**34**:547-53.
43. Terao A, Oikawa M, Saito M. Tissue-specific increase in norepinephrine turnover by central interleukin-1, but not by interleukin-6, in rats. *Am J Physiol* 1994;**266**:R400-4.
44. Niijima A, Hori T, Aou S, Oomura Y. The effects of interleukin-1β on the activity of adrenal splenic and renal sympathetic nerves in the rat. *J Autonom Nerv Syst* 1991;**36**:183-92.
45. Murakami Y, Yokotani K, Okuma Y, Osumi Y. Nitric oxide mediates central activation of sympathetic outflow induced by interleukin-1ß in rats. *Europ J Pharmacol* 1996;**317**:61-6.
46. Liu S, Adcock IM, Ols RW, Barnes PJ, Evans TW. Lipopolysaccharide treatment in vivo induces widespread tissue expression of inducible nitric oxide synthase mRNA. *Biochem Biophys Res Commun* 1993;**196**:1208-13.
47. Robbins RA, Springall DR, Warren JB *et al*. Inducible nitric oxide synthase is increased in murine lung epithelial cells by cytokine stimulation. *Biochem Byophys Res Commun* 1994;**198**:835-43.
48. Tanazawa T, Suzuki Y, Anzai M, Tsugane S, Takayasu M, Shibuya M. Vasodilation by intrathecal lipopolysaccharide of the cerebral arteries after subarachnoid haemorrhage in dogs. *Acta Neurochir* 1996;**138**:330-7.
49. Shibata M, Parfenova H, Zuckerman SL, Seyer JM, Krueger JM, Leffler CW. Interleukin-1β peptides induce cerebral pial arteriolar dilation in anesthetised newborn pigs. *Am J Physiol* 1996;**270**:R1044-50.
50. Barnes PJ. NO or no NO in asthma? *Thorax* 1996;**51**: 218-20.
51. Bonmann E, Suschek C, Spranger M, Kolb-Bachofen V. The dominant role of exogenous interleukin-1 beta on expression and activity of inducible nitric oxide synthase in rat microvascular brain endothelial cells. *Neurosci Lett* 1997;**230**:109-12.
52. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. *Nature* 1990;**347**:768-70.
53. Ye S, Ozgur B, Campese VM. Renal afferent impulses, the posterior hypothalamus, and hypertension in rats with chronic renal failure. *Kidney Int* 1997;**51**:722-7.

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