Role of angiotensin in renal sympathetic activation in nephrotic syndrome

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Sanchez-Palacios, Manuel, Susan Y. Jones, and Gerald F. DiBona. Role of angiotensin in renal sympathetic activation in nephrotic syndrome. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R808–R813, 1998.—The effect of type 1 angiotensin II receptor antagonist treatment (losartan) on cardiac baroreflex regulation of renal sympathetic nerve activity (RSNA) and renal sodium handling in rats with nephrotic syndrome was examined. After intravenous losartan administration, with arterial pressure normalized by intravenous methoxamine, basal RSNA was decreased 14 ± 3% in arterial baroreceptor-intact rats and by 21 ± 5% in arterial baroreceptor-denervated rats. Intracerebroventricular losartan, which did not affect arterial pressure, decreased basal RSNA activity by 15 ± 1%. Both intravenous and intracerebroventricular losartan augmented the renal sympathetic inhibitory response to acute volume loading, and this was associated with an enhanced natriuretic response to the acute volume load. In nephrotic syndrome, acute losartan administration improved cardiac baroreflex regulation of RSNA, which was associated with improved ability to excrete acute sodium loads.

losartan; renal sympathetic nerve activity; cardiac baroreflex; sodium

The nephrotic syndrome (NS) is a clinical disorder characterized by proteinuria (albuminuria), hypoalbuminemia, increased renal sodium and water retention, and edema formation. One of the mechanisms that contributes to the increased renal sodium retention is increased renal sympathetic nerve activity (RSNA) (15). The role of increased RSNA in the renal sodium retention of the rat model of NS has been well characterized (4, 11, 13).

The mechanisms responsible for this heightened level of RSNA in NS have been studied (12, 20). Normally, RSNA is substantially regulated by both high-pressure arterial (sinoaortic, 17) and low-pressure cardiac (cardiopulmonary, 9) baroreceptors. Arterial baroreflex regulation of RSNA is normal in NS (12). However, cardiac baroreflex regulation of RSNA is abnormal in NS, with the defect being observed in the central component of the reflex (12). That is, in response to increases in cardiac filling pressure produced by volume loading, the increases in afferent vagal nerve activity are similar in control and NS rats. However, for a given degree of increase in afferent vagal nerve activity, the inhibition of RSNA is less in NS than in control rats.

The central nervous system (CNS) alterations underlying this cardiac baroreflex defect have not been defined. Several lines of evidence suggest that angiotensin II (ANG II) is involved in the regulation of sympathetic nerve activity (2, 23, 27), including RSNA. ANG II receptors are located in CNS areas that are capable of influencing RSNA, either directly via connections to the intermediolateral column of the spinal cord or indirectly through modulation of arterial and cardiac baroreflex function (2, 26).

The activity of the renin-angiotensin system, as reflected by circulating concentrations of renin and ANG II, is increased in NS (29). This raises the possibility that CNS effects of ANG II may contribute to both the increased basal level of RSNA and the abnormal cardiac baroreflex regulation of RSNA in NS.

We sought to determine the role of ANG II AT1 receptors in both the increased basal level of RSNA in NS and the abnormal cardiac baroreflex regulation of RSNA in rats with NS.

METHODS

Animals

Adult male Sprague-Dawley rats, 300–325 g, were allowed free access to normal-sodium rat pellet diet (Teklad, Na + 172 meq/kg), and tap water drinking fluid was used for all experiments. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the University of Iowa Animal Care and Use Committee.

Anesthesia

Rats were anesthetized intraperitoneally with methohexitol (short duration) or pentobarbital sodium (long duration) at 50 mg/kg.

Model Preparation

Using a technique previously described and validated for this laboratory (4, 11–13, 20), we administered adriamycin (3.5 mg/kg iv) to produce NS. After recovery from anesthesia, rats were returned to individual metabolism cages with free access to normal-sodium rat pellet diet and tap water drinking fluid. All subsequent studies were performed between 3 and 4 wk thereafter, when ongoing renal sodium retention and edema formation have been shown to be present (11).

Procedures

Catheterization. Catheters were inserted in a jugular vein for drug and solution infusion and in a carotid artery for measurement of mean arterial pressure (MAP) and heart rate (HR).

Intracerebroventricular cannulation. Three to four days before the acute experiment, the rats were anesthetized and placed in a cranial stereotaxic apparatus. A stainless steel cannula was inserted into the lateral cerebral ventricle by methods previously described (14). Coordinates were 0.3 mm posterior to the bregma, 1.4 mm lateral to the midline, and 4.0 mm below the cortical surface (21). The cannula was held...
in place with stainless steel jeweler's screws and cranioplastic cement. At the end of the acute experiment, 2 µl of isotonic saline colored with methylene blue were injected intracerebroventricularly and placement of the cannula was confirmed at autopsy.

RSNA recording electrode. The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope, a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was placed on a recording electrode. RSNA was amplified (×20,000–30,000) and filtered (low, 30 Hz; high, 3,000 Hz) via a Grass HIP511 high-impedance probe, leading to a Grass P511 bandpass amplifier. The amplified and filtered neurogram was then channeled to a Tektronix 5113 oscilloscope and Grass model 7D polygraph for visual evaluation, to an audio amplifier/loudspeaker (Grass model AM8) for auditory evaluation, and to a rectifying resistance-capacitance voltage integrator (Grass model 7P3). The quality of the RSNA signal was assessed by its pulse-synchronous rhythmicity and by examining the magnitude of decrease in recorded RSNA during sinoaortic nerve recordings cannot be compared between rats or groups due to the use of different numbers of nerve fibers and the degree of nerve fiber-electrode contact, absolute values of integrated voltage from multifiber sympathetic nerve recordings cannot be compared between rats or groups of rats. Therefore, data were analyzed as percent change from baseline control period (C2, after iv or icv vehicle or losartan), and the magnitude of the overall response was calculated as the area above the curve in each rat. Urine was collected during the 10-min control period (C1) and C2 periods and during and after the volume expansion period for a total of 120 min.

We have previously demonstrated that losartan (10 µmol/kg [4.6 µg/kg] iv) (less than one-half of the dose used herein) completely prevented the pressor response (45 mmHg) to intravenous administration of 100 pmol ANG II, and intracerebroventricular administration of 10 nmol losartan (dose used herein) completely prevented the pressor response (20 mmHg) to intracerebroventricular administration of 1 nmol ANG II (22).

At autopsy, both pleural spaces and peritoneal cavity were inspected for evidence of fluid collection. Bladder urine was taken for quantitative measurement of proteinuria with the use of trichloroacetic acid precipitation (0 = lack of turbidity, 4+ = dense precipitate). The kidneys were removed, blotted, and weighed.

Analysis

MAP, HR, and RSNA data were acquired digitally at a 1-Hz sampling rate using a Data Translation DT-2801 analog-to-digital board, Labtech notebook v. 4.2 software (Laboratory Technology, Wilmington, MA), and a PC. During the volume expansion period, data from the 1-min time interval centered on each 1% increment of the 10% body weight volume expansion were averaged to give a single value for that increment. Because of potential differences in the numbers of nerve fibers and the degree of nerve fiber-electrode contact, absolute values of integrated voltage from multifiber sympathetic nerve recordings cannot be compared between rats or groups of rats. Therefore, data were analyzed as percent change from the baseline control period (C2, after iv or icv vehicle or losartan), and the magnitude of the overall response was measured as the area above the curve in each rat. Urine volume was determined gravimetrically, and urinary sodium concentration (U\text{Na}, meq/l) was measured by flame photometry. Urinary sodium excretion was calculated as U\text{Na,V} = U\text{Na} \times V, where V = urinary flow rate; U\text{Na,V} is presented per gram kidney weight.

Statistical analysis was performed with two-factor (group, response) analysis of variance with repeated measures on one factor and Duncan’s post hoc test (30).

RESULTS

Baseline data (C1) on body weight, MAP, HR, and RSNA are shown for all groups in Table 1. At autopsy,
all rats had bilateral hydrothorax and/or ascites. Bladder urine in each rat showed 4+ reaction for protein.

**Intravenous Losartan**

In IV Los-Intact rats, after the nadir of MAP after intravenous losartan, the intravenous administration of methoxamine restored MAP to a level (C2) not significantly different from the baseline control value (C1) (Table 1). At this unchanged MAP, HR and RSNA were significantly decreased by 9 ± 3 and 14 ± 3%, respectively (P < 0.05 for both). In contrast, no effects were seen in IV Veh-Los rats. Although the methoxamine maneuver was successful in restoring MAP to the baseline control level in IV Los-Intact rats, this protocol was repeated in rats with SAD to more rigorously eliminate the possibility of confounding influences derived from input to peripheral arterial baroreceptors. In IV Los-SAD rats, after the nadir of MAP after intravenous losartan, the intravenous administration of methoxamine restored MAP to a level (C2) not significantly different from the baseline control value (C1). At this unchanged MAP, HR and RSNA were significantly decreased by 12 ± 4 and 21 ± 5%, respectively (P < 0.05 for both).

The responses of RSNA during the volume loading are shown in Fig. 1. For additional comparison, the response of NS rats with SAD that received intravenous vehicle, IV Veh-SAD, is shown. These rats were prepared in an identical fashion and studied with a similar experimental protocol in the same laboratory by the same investigators (20). Statistical comparisons of the magnitudes of the overall responses (calculated as area above the curve) showed significant differences (all at P < 0.05 or better) for the following: IV Veh-Intact vs. IV Los-Intact, IV Veh-SAD vs. IV Los-SAD, IV Veh-Intact vs. IV Veh-SAD, and IV Los-Intact vs. IV Los-SAD.

During volume loading, both MAP and HR decreased as part of generalized sympathetic withdrawal. At 10% body weight volume loading, the peak decreases in MAP and HR were −8 ± 2 mmHg and −68 ± 6 beats/min in IV Veh-Intact, −24 ± 5 mmHg and −72 ± 8 beats/min in IV Veh-SAD, −12 ± 2 mmHg and −79 ± 5 beats/min in IV Los-Intact, and −25 ± 3 mmHg and −67 ± 4 beats/min in IV Los-SAD, respectively (all P < 0.05). The decreases in MAP were significantly greater in IV Veh-SAD than in IV Veh-Intact and were greater in IV Los-SAD than in IV Los-Intact (P < 0.05), whereas the decreases in HR were not significantly different, respectively.

**Intracerebroventricular Losartan**

To avoid the confounding variables of changing MAP (i.e., changing input to peripheral arterial baroreceptors) and compensatory arterial baroreflex adjustments, intracerebroventricular losartan was used because its use in previous studies in both normal and pathophysiological conditions (5, 6) has not been associated with significant changes in MAP. Neither MAP nor

### Table 1. Baseline data for body weight, mean arterial pressure, heart rate, and renal sympathetic nerve activity

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<tr>
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Data are means ± SE; n = no. of rats. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; IV, intravenous; ICV, intracerebroventricular; Veh, vehicle; Los, losartan; SAD, sinoaortic denervated; ND, not determined. *P < 0.05, second control period (C2) vs. first control period (C1); ICV Veh-Intact and ICV Los-Intact groups used for measurement of natriuretic response to volume loading only.

Fig. 1. Effect of 10% body weight volume loading with isotonic saline on renal sympathetic nerve activity (RSNA). All rats have nephrotic syndrome (NS). Statistical comparisons of magnitudes of overall responses (calculated as area above the curve) showed significant differences (all at P < 0.05 or better) for the following: intravenous (IV) vehicle (Veh)-sinoaortic baroreceptor innervation intact (Intact) (○) vs. IV losartan (Los)-Intact (○), IV Veh-sinoaortic baroreceptor denervation (SAD) (■) vs. IV Los-SAD (□), IV Veh-Intact vs. IV Veh-SAD, and IV Los-Intact vs. IV Los-SAD.

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HR were significantly affected by intracerebroventricular administration of vehicle in the (ICV Veh-Intact) or losartan (ICV Los-Intact) group (Table 1, C2 vs. C1). In the ICV Veh-Intact group, intracerebroventricular vehicle did not affect RSNA, whereas in the ICV Los-Intact group, intracerebroventricular losartan decreased basal RSNA by 15% (P < 0.05). As seen in Fig. 2, the decrease in RSNA produced by progressive volume loading in ICV Los-Intact was sustained for the entire period of volume loading, whereas the reduction in RSNA in ICV Veh-Intact was short lived. When analyzed as area over the curve, there was 36% less renal sympathoinhibition in ICV Veh-Intact than in ICV Los-Intact. During the volume loading, significant changes in MAP and HR did not occur in either ICV Veh-Intact or ICV Los-Intact groups.

**DISCUSSION**

The results demonstrate that ANG II AT1 receptor antagonist administration, either intravenous or intracerebroventricular, decreased the basal level of RSNA and improved cardiac baroreflex regulation of RSNA in NS rats. This resulted in an enhanced renal sympathoinhibitory response to progressive volume loading, which was associated with an enhanced natriuretic response.

In normal rats, we have previously demonstrated that cardiac baroreflex gain of RSNA (percent ΔRSNA/mmHg right atrial pressure during iv volume loading) was lower in high-sodium-diet rats than in normal or low-sodium-diet rats (7). These results suggested that endogenous ANG II activity, influenced by alterations in dietary sodium intake, might affect cardiac baroreflex regulation of RSNA. In a subsequent study in normal rats, it was shown that intracerebroventricular losartan did not significantly affect MAP but decreased RSNA in low and normal but not in high-sodium-diet rats (5). These results indicate that physiological alterations in endogenous ANG II activity tonically influence basal levels of RSNA.

In addition to a central defect in the cardiac baroreflex regulation of RSNA (12), NS rats have activation of the renin-angiotensin system (29). Acute intravenous

**Natriuretic Response to Isotonic Saline Volume Load**

This protocol evaluated the possibility that the beneficial effect of intracerebroventricular losartan on the renal sympathoinhibitory response to volume loading in NS was associated with an improved natriuretic response to volume loading. Intracerebroventricular vehicle and losartan did not affect basal MAP or HR (Table 1, C2 vs. C1) or UNaV (Fig. 3, C2 vs. C1). Volume loading significantly increased UNaV in both groups (P < 0.01), but both the absolute magnitude of UNaV (P < 0.01) and the increment in UNaV from C2 to volume-loading period (P < 0.01) were significantly greater in ICV Los-Intact than in ICV Veh-Intact.
and intracerebroventricular administration of losartan improved the cardiac baroreflex regulation of RSNA in NS, resulting in enhanced renal sympathoinhibition during volume loading. Whereas there was a clear beneficial effect of intravenous losartan in the presence of intact sinoaortic baroreceptors (IV Los-Intact vs. IV Veh-Intact), it is apparent that intact sinoaortic baroreceptors attenuate the extent of renal sympathoinhibition during volume loading. Thus the extent of renal sympathoinhibition was greater in IV Veh-SAD than in IV Veh-Intact groups and greater in IV Los-SAD than in IV Los-Intact groups. This pattern was similar to that of our previous studies in which the central defect in cardiac baroreflex regulation of RSNA was more prominently observed after SAD (12). These findings suggest that, despite attempts to normalize MAP after intravenous losartan with intravenous methoxamine, small decreases in MAP may have, via unloading of peripheral arterial baroreceptors, reflexly stimulated RSNA and attenuated the degree of renal sympathoinhibition observed during the subsequent volume loading. This interpretation is further supported by the results of intracerebroventricular losartan administration, in which MAP was not significantly affected and intravenous methoxamine was not required. Under these circumstances, enhanced renal sympathoinhibition during the subsequent volume loading was also observed.

The greater decrease in RSNA during volume loading after intracerebroventricular losartan administration was associated with a greater natriuretic response to the volume loading. Despite the fact that losartan can, with time, traverse the blood-brain barrier (18), the time course of this effect and the small dose of intracerebroventricular losartan used (−0.1% of the iv dose) make it unlikely that the greater natriuretic response resulted from a peripheral (direct renal) effect of losartan that had leaked into the systemic circulation. An additional argument is that whatever amount of losartan that appeared in the systemic circulation after intracerebroventricular losartan administration was insufficient to affect MAP.

As discussed previously (5, 6, 8, 31, 32), there are several possible CNS sites of action of intracerebroventricular losartan. As intracerebroventricular losartan decreases basal RSNA and influences both arterial (6) and cardiac baroreflex regulation of RSNA, this focuses attention on the rostral ventrolateral medulla (RVLM), a region that receives inputs from many peripheral receptors, including arterial and cardiac baroreceptors (8). These inputs are generally relayed by medullary nuclei, such as the nucleus of the solitary tract or the caudal ventrolateral medulla (3). Importantly, the RVLM of rats (25), rabbits (1), and humans (19) contains ANG II receptors predominantly of the AT1 subtypes. Microinjection of a non-subtype-selective peptide ANG II receptor antagonist into the RVLM decreases RSNA (23).

During the volume loading, the decreases in RSNA in the intracerebroventricular losartan groups were not accompanied by decreases in HR as were observed in the intravenous losartan groups. It is possible that some differences in experimental protocol can account for this discrepancy: 1) presence or absence of intracerebroventricular cannula and surgery related thereto and 2) adaptive or compensatory events occurring during the ≈3-day recovery period after intracerebroventricular cannula implantation with or without interaction with the same-day RSNA electrode implantation and acute experimental protocol.

In summary, acute administration of losartan to rats with NS improves cardiac baroreflex regulation of RSNA. This results in enhanced suppression of RSNA during volume loading, which is associated with improved ability of the kidney to excrete acute sodium loads.

**Perspectives**

In NS, in which ANG II concentrations are increased, blockade of ANG II’s effects on intrarenal AT1 receptors can contribute to the increased ability of the kidney to excrete sodium in several ways. These include the renal vasculature, with increased renal blood flow and glomerular filtration rate, the renal tubule, with decreased renal tubular sodium reabsorption; and the renal sympathetic nerve terminal, with diminished norepinephrine release. However, an additional factor contributing to the increased ability of the kidney to excrete sodium is blockade of ANG II’s effects on AT1 receptors, located in discrete CNS areas, which improves cardiac baroreflex regulation of RSNA, facilitates renal sympathoinhibition during volume loading, and enhances the ability of the kidney to excrete acute sodium loads.

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