Cardiopulmonary Baroreflex Function in Nephrotic Rats[1,2]

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(J. Am. Soc. Nephrol. 1995; 5:2082–2086)

ABSTRACT

Efferent renal sympathetic nerve activity is increased in experimental nephrotic syndrome and exhibits attenuated cardiopulmonary baroreflex inhibition during volume expansion in anesthetized rats. Additional studies were performed in conscious rats to avoid the potentially confounding influences of anesthesia; these studies used another more specific standardized stimulus for cardiopulmonary baroreflex activation. Sprague Dawley rats were studied 3 to 4 wk after adriamycin injection (3.5 mg/kg iv); all rats developed proteinuria. In sinoaortic denervated rats (anesthetized), graded frequency stimulation of the central end of the cut right vagus nerve produced frequency-dependent decreases in mean arterial pressure, heart rate, and efferent renal sympathetic nerve activity. The decreases in mean arterial pressure and heart rate were similar in control and nephrotic rats, but efferent renal sympathetic nerve activity decreased significantly less in nephrotic than control rats over the entire frequency range ($P < 0.02$). In sinoaortic denervated rats (conscious), 10% body weight isotonic saline volume expansion decreased mean arterial pressure, heart rate, and efferent renal sympathetic nerve activity. The decreases in mean arterial pressure and heart rate were similar in control and nephrotic rats, but efferent renal sympathetic nerve activity decreased significantly less in nephrotic than control rats over the entire period of volume expansion ($P < 0.04$). In nephrotic syndrome, the cardiopulmonary baroreflex inhibition of efferent renal sympathetic nerve activity is decreased; the defect lies in the central portion of the reflex. This may contribute to the observed increase in efferent renal sympathetic nerve activity in nephrotic syndrome.

Key Words: *Kidney, cardiac volume receptor reflex, renal sympathetic nerves, edema*

[1] Received December 5, 1994. Accepted January 31, 1995.

[2] The manuscript was sent to Franklyn Knox, MD, for review by expert referees, for editorial decision, and for final disposition.

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1046-6673/0512-2082$03.00/0
Journal of the American Society of Nephrology

The nephrotic syndrome (NS) is a disease state characterized by renal sodium and water retention, edema formation, and increased efferent renal sympathetic nerve activity (ERSNA; (1–5). Increased ERSNA can directly increase renal tubular sodium and water reabsorption throughout the nephron without altering renal hemodynamics (5). By use of the rat model of adriamycin-induced NS, reflex regulation of ERSNA has been shown to be abnormal. In conscious rats with intact arterial and cardiopulmonary baroreflexes, intravenous volume expansion resulted in a lesser inhibition of ERSNA in the nephrotic than the control rats. In anesthetized rats, arterial baroreflex regulation of ERSNA is normal but there is decreased cardiopulmonary baroreflex inhibition of ERSNA during intravenous volume expansion (3). This defect was shown to be in the central portion of the cardiopulmonary baroreflex arc and was unmasked by sinoaortic denervation (SAD). This failure to normally suppress ERSNA may contribute to the increased level of ERSNA in NS and, therefore, to the renal sodium and water retention that results in edema formation. These studies were undertaken to further characterize the cardiopulmonary baroreflex dysfunction in conscious rats to avoid the potentially confounding influences of anesthesia, to use another more specific standardized stimulus for cardiopulmonary baroreflex activation, central vagal nerve stimulation, and to avoid possibly confounding contributions from intact sinoaortic baroreceptors wherein slight alterations in arterial pressure would produce alterations in ERSNA independent of the cardiopulmonary baroreflex.

METHODS

Animal Preparation

All animal experimentation was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats (350 ± 5 g body wt) were anesthetized with methohexital sodium (50 mg/kg ip) and were injected through the tail vein with adriamycin (3.5 mg/kg) to induce NS (1–4). They were subjected to SAD, either simultaneously for the conscious protocol or at the time of study for the anesthetized protocol. The rats were returned to individual metabolism cages and allowed free access to standard laboratory rat chow and tap water. Control rats were not given adriamycin and were subjected to SAD either 1 wk before the conscious volume expansion protocol or at the time of the anesthetized vagal stimulation protocol. At the time of study, proteinuria was confirmed in NS rats by urine precipitation with trichloroacetic acid (6).

Procedures

Catheterization. The rats were instrumented with polyethylene catheters in the left femoral vein for the infusion of isotonic saline at 0.05 mL/min and the abdominal aorta via the left femoral artery for the measurement of mean arterial pressure (MAP) and heart rate (HR).

SAD. All rats underwent SAD by the method of Krieger (7). Efficacy was assessed by noting the absence of a bradycardia in response to a 50-mm Hg increase in MAP produced by an intravenous bolus injection of norepinephrine (3 μg).

Renal Sympathetic Nerve Activity Recording Electrode. The left kidney was exposed through a left flank incision via a retroperitoneal approach. With a dissecting microscope (×25), a renal nerve branch from the aorticorenal ganglion was isolated, carefully dissected free, and placed on a bipolar platinum wire (Cooner Wire Company, Chatsworth, CA) electrode. Renal sympathetic nerve activity was amplified (10,000 to 50,000 times) and filtered (low, 30; high, 3,000 Hz) with a bandpass amplifier (Model P511; Grass Instrument Co., Quincy, MA). The amplified and filtered signal was channeled to an oscilloscope (Model 5113; Tektronix, Inc., Beaverton, OR) and a Grass Model 7DA or Beckman Model R611 polygraph (Beckman Instruments, Fullerton, CA) for visual evaluation, to an audio amplifier/loudspeaker (Grass Model AM 8 audio monitor) for auditory evaluation, and to a rectifying voltage integrator (Grass Model 7P10 or Beckman Model 9873B) with a reset time of 1 s. When an optimal recording was obtained, the nerve was sectioned distally and the recording electrode was fixed to the proximal portion of the renal nerve branch with a silicone cement (Wacker Sil-Gel 604; Wacker-Chemie, Munich, Germany). For conscious rat studies, the electrode cable was secured in position by suturing it to the abdominal trunk muscles and the flank incision was closed in layers. At the completion of all experiments, rats were euthanized with an overdose of pentobarbital or methohexital and the postmortem ERSNA signal was recorded. This postmortem background signal was subtracted from all experimental values of ERSNA.

Central Vagal Stimulation. Via a midline cervical incision, the right cervical vagus was isolated and sectioned distally and the central end was placed on a bipolar platinum wire (Cooner Wire Company) electrode. The nerve was secured to the electrode with a silicone cement (Wacker Sil-Gel 604; Wacker-Chemie). The electrode was connected to a Grass Model S9 stimulator via a Grass Model SIU5A stimulation isolation unit.

Experimental Protocol

Central Vagal Stimulation. Three to four weeks after adriamycin injection, rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg ip followed by an infusion of 0.13 mg/kg per minute iv; Abbott Laboratories, North Chicago, IL), intubated, mechanically ventilated with room air, catheterized, subjected to SAD, and instrumented with a renal sympathetic nerve activity recording electrode and a right vagal nerve stimulating electrode. A 60-min postsurgical equilibration period followed. Thereafter, continuous measurements of MAP, HR, and ERSNA were made during consecutive 5-min periods in which electrical stimulation of the central portion of the sectioned right vagus nerve was alternated with control observations. The stimulation parameters were 4 V, 2.0 ms, and 0 (control), 0.5, 1, 2, 4, 8 and 16 Hz (8,9).

Volume Expansion. Three to four weeks after adriamycin injection and SAD, rats were anesthetized with methohexital sodium (50 mg/kg ip, supplemented with 3 to 5 mg iv as needed), catheterized, and instrumented with a renal sympathetic nerve activity recording electrode. Rats were allowed to recover from anesthesia in individual restraining cages during a 120-min postsurgical equilibration period. Thereafter, control measurements of MAP, ERSNA, and HR were made for 5 min. Then, isotonic saline was infused intravenously in a volume equal to 10% body weight at 2.0 mL/min with continuous measurement of MAP, ERSNA, and HR. Rats were allowed to recover for 30 min, and a 5-min recovery period was recorded.

Analysis

MAP, HR, and integrated ERSNA were continuously recorded on videotape via a pulse code modulation recording adapter (Vetter Model 4000A; Vetter, Rebersburg, PA) connected to a standard videocassette recorder (Fisher Model FVH-6200; Chatsworth, CA). Analog signals were then played back via an analog-to-digital converter (Model DT2801; Data Translation Inc., Marlborough, MA) with Labtech Notebook Version 4.2 (Laboratory Technologies, Wilmington, MA) and a personal computer. The sampling rate was 5 Hz. In the central vagal stimulation protocol, data from the last 2 min of each 5-min stimulation period were averaged to give a single value for that stimulation frequency. In the volume expansion protocol, data from the 0.5 min before and the 0.5 min after 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 fibers and the nature of fiber-electrode contact, absolute values of integrated voltage from multifiber sympathetic nerve recordings cannot be compared between rats or groups of rats. Therefore, the data were analyzed as percent change from baseline control period in each rat. Integrated ERSNA, MAP, and HR were analyzed by the use of percent change from baseline control period and were plotted against stimulation frequency in the vagal stimulation protocol or against percent volume load administered in the volume expansion protocol. Statistical analysis was performed by the use of repeated-measures analysis of variance for main effects and interactions, with subsequent use of Scheffe's test for simultaneous multiple comparisons within groups (10). Differences were considered statistically significant when $P < 0.05$.

RESULTS

Central Vagal Stimulation (Anesthesia)

Baseline values in control ($N = 8$) and nephrotic ($N = 8$) rats, respectively, were 353 ± 9 *versus* 352 ± 5 g for body weight, 122 ± 3 *versus* 126 ± 7 mm Hg for MAP, and 370 ± 15 *versus* 387 ± 13 beats/min for HR. There were no significant differences between control and nephrotic rats in body weight, MAP, or HR. As seen in Figure 1, in both control and nephrotic rats, there were frequency-dependent decreases in MAP (top panel) that were statistically significant at stimulation frequencies of 1 Hz or more, with the maximum decrease being achieved at 4 Hz. However, there were no statistically significant differences between the responses of control and nephrotic rats. HR (middle panel) decreased minimally during vagal stimulation; the changes were not statistically significant in either control or nephrotic rats. In both control and nephrotic rats, the decrease in ERSNA (bottom panel) was statistically significant at all stimulation frequencies, with the maximum decrease being achieved at 2 Hz for control rats and at 4 Hz for nephrotic rats. The inhibition of ERSNA in nephrotic rats was signifi-

cantly less than that in control rats at all stimulation frequencies ($P < 0.02$).

Volume Expansion (Conscious)

Baseline values in control ($N = 8$) and nephrotic ($N = 11$) rats, respectively, were 325 ± 12 *versus* 364 ± 10 g for body weight, 141 ± 7 *versus* 135 ± 4 mm Hg for MAP, and 424 ± 13 *versus* 426 ± 14 beats/min for HR. Recovery values in control and nephrotic rats were 142 ± 8 *versus* 134 ± 5 mm Hg for MAP and 395 ± 19 *versus* 415 ± 14 beats/min for HR. The body weight in nephrotic rats was significantly higher than that in control rats ($P < 0.05$), which may be accounted for by the fact that the nephrotic rats were approximately 2 wk older than the control rats. There were no significant differences between control and nephrotic rats in MAP or HR. In both control and nephrotic rats, MAP and HR are somewhat increased over values usually observed in conscious chronically instrumented or anesthetized rats in our laboratory. However, these rats had undergone SAD and were being monitored under mild restraint in preparation for volume expansion. It is known that SAD rats exhibit increased lability of MAP and HR in response to alerting stimuli (3). Of importance, there were no differences in MAP or HR between control and nephrotic rats in either the baseline control or the recovery period. As seen in Figure 2, in both control and nephrotic rats, MAP (top panel) decreased progressively beginning at 30% volume expansion but did not reach statistical significance until 70% volume expansion. The changes in MAP were not significantly different between control and nephrotic rats. HR (middle panel) decreased significantly in both control and nephrotic rats at 30% volume expansion and continued to decrease thereafter to a maximum decrease of −13 ± 6% in control rats and −13 ± 5% in nephrotic rats, with no significant difference between them. In both control and nephrotic rats, ERSNA (bottom panel) decreased progressively during volume expansion but the inhibition was significantly less throughout volume expansion in nephrotic than in control rats ($P < 0.04$). The decrease in ERSNA became statistically significant in control rats at 20% volume expansion, but this did not occur in nephrotic rats until 40% volume expansion. Maximum inhibition of ERSNA was significantly less in nephrotic rats (−39 ± 10%) than in control rats (−65 ± 5%; $P < 0.05$).

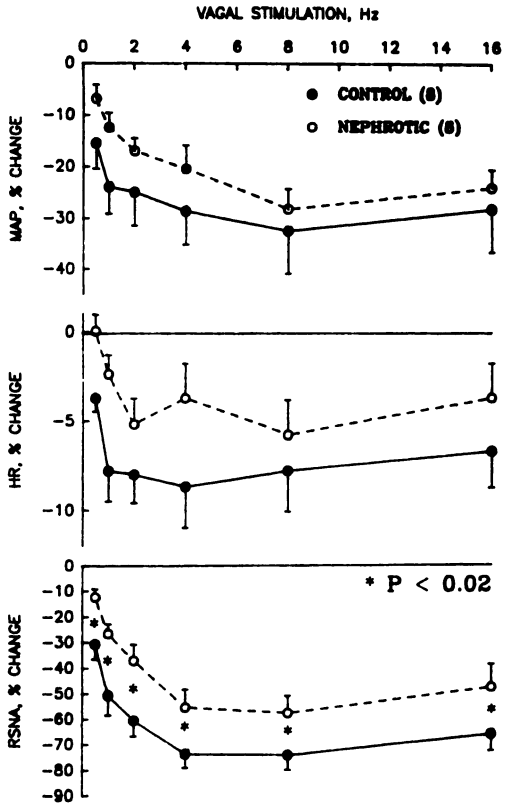

Figure 1. Percent change in MAP, HR, and ERSNA in control (filled circles) and nephrotic (open circles) rats during graded frequency stimulation of the central vagus nerve (vagal stimulation, Hertz). The inhibition of efferent RSNA in nephrotic rats was significantly less than that in control rats at all stimulation frequencies (*$P < 0.02$).

DISCUSSION

The experimental rat model of adriamycin-induced NS exhibits renal sodium and water retention with edema formation, as is seen clinically in nephrotic humans. In response to intravenous isotonic saline volume expansion, conscious nephrotic rats exhibit an attenuated suppression of ERSNA in association with an impaired diuretic and natriuretic response that is markedly improved after renal denervation (1). This suggests that the impaired renal excretory responses are at least partially secondary to failure to adequately suppress ERSNA in the face of volume expansion. Subsequent studies demonstrated that, after SAD in anesthetized rats, intravenous isotonic saline volume expansion elicited normal increases in afferent vagal nerve activity but attenuated the inhibition of ERSNA (3). Together, the conscious and anesthetized studies identified a defect in cardiopulmonary baroreflex inhibition of ERSNA that was localized to the central portion of the reflex arc. However, in those experiments, intravenous volume expansion was used as the stimulus for the activation of the cardiopulmonary baroreflex. In addition to increases in cardiac filling pressure with the stimulation of cardiopulmonary baroreceptors, there are multiple other dilutional and hormonal consequences (*e.g.*, renin angiotensin, atrial natriuretic peptide) of intravenous volume expansion that could have influenced the results. Also, the results could have been influenced by the potentially confounding effects of anesthesia, well known to influence reflex responses. Thus, these studies were performed in order to circumvent these potentially confounding factors.

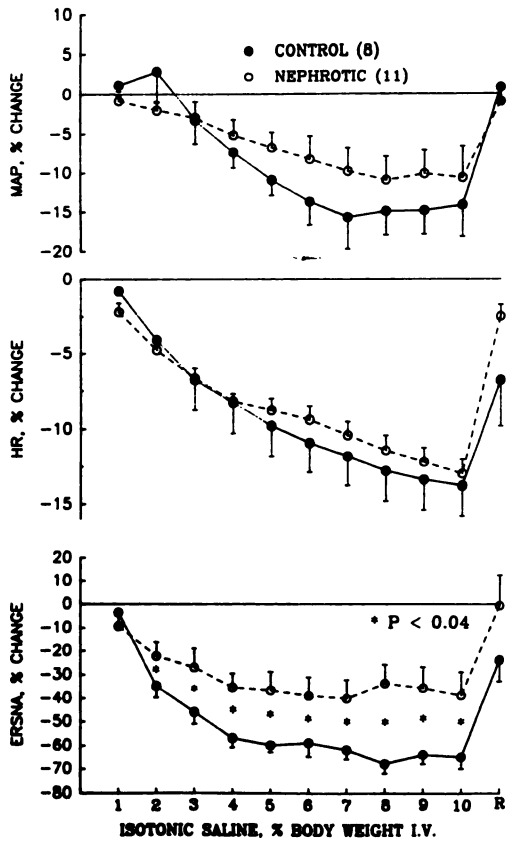

Figure 2. Percent change in MAP, HR, and ERSNA in control (filled circles) and nephrotic (open circles) rats during 10% body weight intravenous isotonic saline volume expansion. In both control and nephrotic rats, ERSNA (bottom panel) decreased progressively during volume expansion but the inhibition was significantly less throughout volume expansion in nephrotic than in control rats (*$P < 0.04$).

Graded frequency stimulation of the central end of the right vagus nerve was used to provide a standardized stimulus for the activation of afferent vagal fibers subserving the cardiopulmonary baroreflex (8,9). Intravenous isotonic saline volume expansion was performed in conscious rats. In both protocols, SAD was performed to remove potential influences of alterations in the sinoaortic baroreflex regulation of ERSNA.

In the central vagal stimulation protocol, the voltage and duration parameters of the electrical stimulation used have been shown to selectively activate the predominantly nonmyelinated (at low frequencies) cardiac vagal afferents in the rat (8,9). This provided a standardized afferent input to the central nervous system neuronal pools involved in the cardiopulmonary baroreflex in the absence of the associated dilutional and hormonal alterations associated with intravenous volume expansion. The results from the central vagal stimulation protocol demonstrate that, for a similar degree of afferent input, there is decreased cardiopulmonary baroreflex inhibition of ERSNA in nephrotic compared with control rats. This defect is specific in that the magnitude of the decreases in MAP and HR were not different between nephrotic and control rats. These data confirm our previous studies (3) using intravenous volume expansion and localize the site of this defect to the central portion of the cardiopulmonary baroreflex arc, affecting those central neuron pools that regulate ERSNA but not those involved in MAP and HR regulation.

Given the technical difficulties of performing central vagal stimulation in conscious rats, the effect of intravenous volume expansion was examined in conscious rats, *i.e.*, in the absence of the potentially confounding effects of anesthesia. The results from the volume expansion protocol demonstrate that, for a similar magnitude of volume expansion, there is decreased cardiopulmonary baroreflex inhibition of ERSNA in nephrotic compared with control rats. This defect is specific in that the magnitude of the decreases in MAP and HR were not different between nephrotic and control rats. These data confirm our previous studies with intravenous volume expansion in both conscious rats with intact arterial baroreflexes (*i.e.*, no SAD; 1) as well as in anesthetized rats (3) and extend them by demonstrating that this abnormality is specific for the cardiopulmonary baroreflex regulation of ERSNA, with cardiopulmonary baroreflex regulation of MAP and HR being unaffected. The reductions in MAP and HR are due to the cardiopulmonary baroreflex–mediated inhibition of sympathetic nerve activity to the peripheral arterial resistance vessels and heart, respectively.

These studies confirm the failure of ERSNA to suppress normally in NS in response to an acute intravenous volume load (1). This is known to contribute to the associated attenuated diuretic and natriuretic response to the acute intravenous volume load (1) and to the chronic excess renal sodium and water retention and edema formation during the development of the NS (2). As suggested by the previous studies (3) and confirmed herein by the results of the vagal stimulation protocol, the defect can be localized to a decreased gain of the central portion of the cardiac volume receptor reflex arc.

As to candidate mechanisms for the defect in the central portion of the cardiopulmonary baroreflex control of ERSNA in NS, consideration should be given to the associated alterations in hormonal systems. NS is characterized by increased activity of the renin-angiotensin-aldosterone, atrial natriuretic peptide, vasopressin, and circulating catecholamine systems. Although it is thought that this neurohormonal activation reflects an underfilled circulation, this has been difficult to substantiate with measurements of plasma or blood volume. In our previous studies (3), nephrotic rats had a decreased right atrial pressure and normal MAP in the face of a reduced cardiac index, which was maintained by an increase in total peripheral resistance index. Although not conclusive,

these findings offer supportive evidence for the presence of an underfilled circulation. Of these hormonal systems, there is evidence in normal animals that angiotensin II affects the arterial baroreflex control of HR and ERSNA in a pressure-independent fashion to result in chronically elevated levels of HR and ERSNA (reviewed in Refs. 11 and 12). It is known that angiotensin II is necessary for normal arterial baroreflex control of HR during sodium and water deprivation. It has been speculated that the increased activity of the renin-angiotensin system in cardiac failure contributes to the chronically increased levels of HR and ERSNA observed via this pressure-independent effect of angiotensin II on arterial baroreflex function. Although arterial baroreflex regulation of ERSNA is normal in NS (3), it is a testable hypothesis that increased activity of the renin-angiotensin system in NS may contribute to the observed central defect in the cardiopulmonary baroreflex regulation of ERSNA, possibly via an effect on the area postrema or nucleus tractus solitarius. This could occur via access of circulating angiotensin II to these central nervous system sites or by local increases in central nervous system renin-angiotensin system activity.

ACKNOWLEDGMENTS

This study was supported by Grants DK 15843 and HL 44546 from the NIH and grants from the Department of Veterans Affairs.

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