The renin-angiotensin-aldosterone system excites hypothalamic paraventricular nucleus neurons in heart failure

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Zhang, Zhi-Hua, Joseph Francis, Robert M. Weiss, and Robert B. Felder. The renin-angiotensin-aldosterone system excites hypothalamic paraventricular nucleus neurons in heart failure. Am J Physiol Heart Circ Physiol 283: H423–H433, 2002; 10.1152/ajpheart.00685.2001.—The paraventricular nucleus (PVN) of the hypothalamus has critical homeostatic functions, including the regulation of fluid balance and sympathetic drive. It has been suggested that altered activity of this nucleus contributes to the progression of congestive heart failure (HF). We hypothesized that forebrain influences of the renin-angiotensin-aldosterone system augment the activity of PVN neurons in HF. The rate of PVN neurons (n = 68) from rats with ischemia-induced HF was higher than that of PVN neurons (n = 42) from sham-operated controls (8.7 ± 0.8 vs. 2.7 ± 0.3 spikes/s, P < 0.001, HF vs. SHAM). Forebrain-directed intracarotid artery injections of the angiotensin type 1 receptor antagonist losartan, the angiotensin-converting enzyme inhibitor captopril, and the mineralocorticoid receptor antagonist spironolactone all significantly (P < 0.05) reduced PVN neuronal activity in HF rats. These findings demonstrate that the renin-angiotensin-aldosterone system drives PVN neuronal activity in HF, likely resulting in increased sympathetic drive and volume accumulation. This mechanism of neurohumoral excitation in HF is accessible to manipulation by blood-borne therapeutic agents.

angiotensin type 1 receptors; angiotensin-converting enzyme; mineralocorticoid receptors; spironolactone; baroreceptors

METHODS

All experiments were performed on adult male Sprague-Dawley rats (350–450 g; Harlan Sprague). The animals were housed in the University of Iowa Animal Care Facility and exposed to a normal 12:12-h light-dark cycle. All experimental procedures were approved by the institution’s Animal Care and Use Committee and are in accordance with the guidelines of the American Veterinary Association.

Induction of HF or SHAM. HF was induced using the coronary artery ligation technique, which is widely described in the literature. The details of this procedure are described elsewhere (6). In brief, the left anterior descending coronary artery was ligated under sterile conditions via a left thoracotomy in anesthetized (90 mg/kg ketamine/10 mg/kg xylazine ip), mechanically ventilated rats. SHAM rats underwent the same surgery, but did not undergo coronary artery ligation.

Echocardiographic assessment of left ventricular function. Echocardiography was performed under ketamine (25 mg/kg ip) sedation to confirm impairment or preservation of left ventricular (LV) function, usually within 24 h after coronary

In rats with ischemia-induced congestive heart failure (HF), metabolic activity within the PVN is increased (22). Blockade of forebrain angiotensin type 1 (AT1) receptors (3, 35) or of mineralocorticoid receptors (5) can reduce sympathetic drive and improve baroreflex regulation of renal sympathetic nerve activity in rats with ischemia-induced HF. Thus we hypothesized that activation of forebrain components of the renin-angiotensin-aldosterone system (RAAS) increases the discharge rate of PVN neurons in HF, potentially contributing to the volume accumulation and augmented sympathetic drive characteristic of the HF syndrome. To test this hypothesis, we obtained extracellular recordings from single PVN neurons in rats with ischemia-induced HF and examined their responses to the selective blockade of several specific components of the RAAS at the forebrain level. We observed a generalized increase in PVN neuronal discharge in HF versus sham-operated rats that could be attributed largely to activation of forebrain elements of the RAAS.
ligation. Details of the technique have been published elsewhere (6). In brief, two-dimensional images were acquired at a frame rate of 100/s with an Acuson (Mountainview, CA) Sequoia model 256 clinical echocardiograph fitted with an 8-MHz sector-scanning probe. Short- and long-axis images of the left ventricle were analyzed. LV volumes and LV mass (LVM) were calculated using the area-length method, which has been validated in rodents (11) and humans (27). Infarct size was roughly estimated by planimetric measurement of the percentage of the left ventricle that demonstrated systolic akinesis or dyskinesis. From these measurements, percent infarct size, LV ejection fraction (LVEF), LV end-diastolic volume (LVEDV), and LVEDV-to-LVM ratio, all indexes of severity of HF, were reported. After completion of two-dimensional imaging, pulse-wave Doppler interrogation of mitral inflow was performed to determine heart rate. Cardiac output was calculated as the product of heart rate and stroke volume. The age-matched control rats that did not undergo an operative procedure (NORM) were not examined with echocardiography.

Surgical preparation for recording. Terminal studies for PVN neuronal recording were performed in HF and SHAM rats 4–6 wk after coronary ligation or sham coronary ligation, and in age-matched NORM rats that had not undergone a prior surgical procedure. Rats were anesthetized with urethane (1.5 g/kg ip), and supplemental doses of urethane (0.2–0.4 g/kg ip or iv) were given as needed. The level of anesthesia was periodically reassessed during the surgical procedures and experimental recording by examining pupillary size and nociceptive reflex responses and by continuously monitoring blood pressure and heart rate. The left femoral artery was cannulated with polyethylene (PE)-50 tubing filled with heparinized saline (10 U/ml) for the recording of arterial pressure. The left femoral vein and left carotid artery were cannulated with PE-20 tubing for the administration of drugs. The animals' core temperature was maintained at 37 ± 0.2°C with a rectal thermometer and a temperature controller (model K-100, Baxter Healthcare; Valencia, CA). The animals were not ventilated. The rats were fixed in a stereotoxic frame (David Kopf Instrument; Tujunga, CA), and a small midline craniotomy was made above the PVN region.

Electrophysiological recording. PVN neuronal recordings were obtained from HF, SHAM, and unoperated NORM rats. The recording session began at least an hour after completion of the surgical preparation. Single-unit extracellular recordings were obtained using a glass micropipette (resistance 6–10 MΩ) filled with 0.5 M sodium acetate dissolved in 2% Pontamine sky blue (pH 7.6). The micropipette was advanced into the left PVN (stereotoxic coordinates: anterior-posterior, –1.6 to –2.3 mm from bregma; medial-lateral 0.2–0.5 mm; dorsal ventral 7.0–8.0 mm from brain surface) (23) in a precise stepwise fashion (5- to 10-μm steps) using a micropositioner (Kopf model 660, David Kopf Instrument). Action potentials were amplified with a Dagan 2400A extracellular preamplifier (Minneapolis, MN) and monitored visually on a Tektronix digital oscilloscope (TDS 3014, Tektronix; Wilsonville, OR) and auditorily with a Grass AM8 audio monitor (Grass Instruments; Quincy, MA).

Baroreceptor activation. To determine whether a neuron was sensitive to baroreceptor afferents input, arterial pressure was raised and lowered with bolus intravenous injections of phenylephrine hydrochloride (PE, 10 μg/kg) and sodium nitroprusside (SNP, 5 μg/kg), respectively, administered in 50 μl of saline.

Baroreceptor denervation. In theory, close injection of vasoactive drugs in the vicinity of the carotid sinus might alter baroreceptor or chemoreceptor afferent activity, either through a direct effect on the receptors themselves or indirectly by altering vascular compliance. To control for this possibility, some experiments were performed in animals with baroreceptor denervation. In these rats, the superior laryngeal nerves, cervical sympathetic nerves, and aortic depressor nerves were sectioned bilaterally. Each carotid bifurcation was then stripped of connective tissue and painted with 10% phenol in ethanol. The completeness of baroreceptor denervation was confirmed by demonstrating that the reflex inhibition of heart rate during an increase in arterial pressure induced by infusion of PE (5–10 μg/kg iv) was eliminated. Rats were allowed to recover for at least 1 h before the PVN recording session.

Intracarotid cannulation. Previous work (9) has demonstrated that centrally directed intracarotid artery (ICA) injections in the rat predominantly target the ipsilateral forebrain, and therefore provide a means of delivering drugs to that region by the natural blood-borne route. This study took advantage of that observation. The left common carotid artery was cannulated at a cephalad direction, ipsilateral to the PVN recording site, for the administration of drugs to the forebrain. The catheter was tied in place with its tip below the carotid sinus.

Drugs administered. PE, SNP, angiotensin I (ANG I), angiotensin II (ANG II), captopril, and spironolactone were obtained from Sigma (St. Louis, MO). Losartan was obtained from DuPont Pharmaceuticals (Wilmington, DE). Drugs were dissolved in saline (captopril, losartan, ANG I, ANG II, PE, and SNP) or 0.5% ethanol in saline (spironolactone). ICA injections were given in a volume of 10–20 μl flushed by 25 μl of artificial cerebrospinal fluid (aCSF, pH 7.5). ICA injections of 0.5% ethanol in saline and aCSF were administered in the same volume to control for effects of vessel distension.

Data acquisition. Amplified action potentials were passed through a nerve traffic analyzer (University of Iowa Bioengineering 706C) that produced a transistor-transistor logic pulse for each action potential falling within a voltage window set above the background noise level. Arterial pressure was monitored with a Hewlett-Packard 775A chart recorder (HP Medical Products Group; Andover, MA). These signals (spike activity and arterial pressure) were fed into an on-line data acquisition system consisting of a Cambridge Electronics Design 1401 Plus (CED, Cambridge; UK) computer interface coupled with a Gateway Pentium personal computer. Digitized data were stored for subsequent off-line analysis with Spike2 (CED) software.

Statistical analysis. The baseline discharge rate and arterial pressure were averaged over 60-s intervals immediately preceding an experimental intervention. Peak responses were averaged over 30-s intervals. Changes in PVN neuronal discharge were calculated as a percent change from the baseline activity. A net change in discharge rate of ±30% or more that could be repeated without a noticeable change in the amplitude of the action potential was considered a significant effect of an experimental intervention. Statistical analysis of paired or unpaired data employed Student's t-test.

Echocardiographic data regarding percentage of ischemic injury, LVEF, LVEDV, LVEDV-to-LVM ratio, cardiac output, and heart rate were analyzed using a one-way ANOVA followed by a Student's t-test.

Values are expressed as means ± SE. P < 0.05 was considered to indicate statistical significance.

Anatomy/histology. The last unit recorded in each experiment was marked with iontophoresis of Pontamine sky blue. At the conclusion of each experiment, an overdose of ure-
thanne was injected, and the heart, lungs, and brain were removed for examination. The brain was fixed in a 10% formalin solution for at least 3 days and then sectioned (40 μm) on a cryostatic microtome (OM2563, Triangle Biomedical Sciences; Durham, NC). The recording sites marked with Pontamine sky blue were identified with a light microscope, and the locations of other recording sites were identified with respect to this reference point. Recording sites were plotted on representative schematic tracings of the PVN based on the standard rat atlas of Paxinos and Watson (23).

For the HF rats, the presence or absence of ischemic injury, as indicated by LV scarring, was confirmed by visual inspection. The heart-to-body weight and lung-to-body weight ratio were determined.

RESULTS

Characteristics of study groups. Infarct size in the HF rats, estimated by echocardiography, was 38.6 ± 1.4% of the LV wall. Compared with SHAM rats (n = 15), HF rats (n = 24) had increased LVEDV and LVEDV-to-LVM ratio and reduced LVEF (Fig. 1). In these sedated animals, there was no difference in heart rate between HF (365 ± 12 beats/min) and SHAM (361 ± 13 beats/min) rats. Cardiac output was lower in the HF rats (78 ± 8 vs. 98 ± 7 ml/min, P < 0.05).

At the conclusion of the study, there was no difference in body weight between the HF versus SHAM rats (425 ± 13 vs. 423 ± 16 g). Heart weight-to-body weight ratio was higher (P < 0.05) in the HF than the SHAM rats (0.65 ± 0.04% vs. 0.50 ± 0.03%), as was lung weight-to-body weight ratio (0.80 ± 0.05% vs. 0.49 ± 0.02%).

Spontaneous discharge of PVN neurons in HF, SHAM, and NORM rats. Figure 2 shows that the baseline discharge rate of PVN neurons in HF rats was significantly (P < 0.001) higher (8.7 ± 0.8 spikes/s, range 0.4–33.2 spikes/s, n = 68, 25 rats) than that of PVN neurons from SHAM (2.7 ± 0.3 spikes/s, range 0.2–10.1 spikes/s, n = 42, 16 rats) and NORM (2.9 ± 0.4 spikes/s, range 0.1–13.9 spikes/s, n = 84, 21 rats) rats. Figure 3 shows recordings from a PVN neuron of a HF (left) and a SHAM (right) rat, illustrating the more rapid discharge rate in the HF rat. There were no significant differences in baseline mean arterial pressure among the three groups: NORM, 95.5 ± 2.2 mmHg (n = 21); HF, 91.2 ± 2.3 mmHg (n = 25); SHAM, 93.7 ± 2.4 mmHg (n = 16).

Effects of baroreceptor denervation. In a subpopulation of neurons from HF rats with the carotid sinus and aortic depressor nerves sectioned, the spontaneous discharge rate of PVN neurons did not differ from that of neurally intact HF rats (8.9 ± 1.0 spikes/s, n = 56 vs. 7.7 ± 1.5 spikes/s, n = 12; intact vs. denervated, P = not significant). The spontaneous discharge rate of PVN neurons (3.0 ± 0.7 spikes; n = 5) recorded from SHAM rats (n = 2) that had undergone carotid sinus and aortic depressor nerve section was also unaffected (P = not significant). The responses to ICA administration of captopril, losartan, and spironolactone, reported below, were similar in magnitude and direction in baroreceptor-denervated and intact rats. Accordingly, data obtained from the neurally intact and the denervated rats were pooled for statistical analysis.

Effect of ICA captopril on PVN neuronal activity in HF and SHAM rats. As illustrated by the tracings in Fig. 4 (top), ICA captopril reduced both PVN neuronal activity and arterial pressure in HF rats. In contrast, a falling arterial pressure induced by intravenous SNP increased the activity of this unit. As shown in Fig. 4 (bottom), ICA captopril (50 μg/kg) reduced the firing rate (−73.3 ± 4.2%, P < 0.01) of nine neurons from HF rats baseline discharge 7.9 ± 1.2 spikes/s, range 4.2–14.5 spikes/s, five from intact rats, (open circles) and four from baroreceptor-denervated (filled circles) rats. The responses of the baroreceptor-denervated rats to
ICA captopril did not differ from those of the baroreceptor intact rats, thus eliminating direct effects of the captopril on vascular compliance or carotid sinus baroreceptor activity as an explanation for the observed reductions in PVN neuronal activity. Among the five captopril-responsive neurons from baroreceptor intact rats, three increased their firing rate in response to a comparable decrease in mean arterial pressure induced by SNP and two were unaffected by SNP. Thus the responses of these PVN neurons to a reduction in arterial pressure were opposite in direction to the responses to ICA captopril. We cannot exclude the possibility that baroreceptor input might have minimized the reduction in activity of several of these neurons, but the distribution of responses of neurons from the intact and barodenervated rats (Fig. 4, bottom) does not support that suggestion. ICA captopril had a variable effect on neurons from the SHAM rats, with an overall reduction in mean discharge rate (44.4 ± 11.0%; n = 10; P = 0.048). Not surprisingly, considering the low baseline firing rate in the SHAM rats, the decrease in PVN neuronal activity was significantly (P = 0.03) greater in neurons from HF versus SHAM rats.

ICA captopril induced similar reductions in mean arterial pressure in HF (24.3 ± 3.4% from baseline of 93.2 ± 3.1 mmHg; n = 9) and SHAM (26.2 ± 2.6% from baseline of 94.6 ± 3.7 mmHg; n = 10) rats. The dose of ICA captopril used in these studies completely abolished the pressor response to ICA ANG I (HF: from 12.1 ± 1.8% to 1.3 ± 0.4%, n = 7; SHAM from 11.7 ± 1.4% to 1.6 ± 0.9%, n = 8) but had no affect on the pressor response to ICA ANG II (HF: from 12.1 ± 1.5% to 11.7 ± 1.6%, n = 7; SHAM from 12.8 ± 1.7% to 12.2 ± 1.6%, n = 6). ICA captopril also blocked neural activation induced by ICA ANG I (50 μg/kg) from 104.1 ± 20.4% to 7.2 ± 4.4% in 12 units tested (6 HF and 6 SHAM), but did not affect the neural responses to ICA ANG II (50 μg/kg) in eight units tested (5 HF and 3 SHAM) (96.8 ± 13.4% increase before vs. 88.2 ± 15.2% increase after). Captopril blockade of the ANG I effects lasted 50–60 min. ICA injection of aCSF had no effect on PVN neuronal activity or mean arterial pressure.

**Effect of ICA losartan on PVN neuronal activity in HF and SHAM rats.** As illustrated by the record in Fig. 5 (top), losartan reduced both the PVN neuronal activity and the arterial pressure in a HF rat. The discharge
of this neuron was not altered by a comparable decrease in mean arterial pressure induced by SNP. As shown in Fig. 5 (bottom), ICA losartan (200 μg/kg) substantially reduced the baseline firing rate (−75.9 ± 2.9%; P < 0.01) in 12 neurons from HF rats (baseline discharge 8.6 ± 1.4 spikes/s, range 3.8 ± 15.3 spikes/s), 6 from baroreceptor intact (open circles), and 6 from baroreceptor-denervated (filled circles) rats. The responses of the baroreceptor-denervated rats to ICA losartan did not differ from those of the baroreceptor intact rats, eliminating local effects of losartan at the site of carotid injection as an explanation for the observed reductions in PVN neuronal activity. Among the neurons from the baroreceptor intact rats, 4 losartan-responsive neurons increased their firing rate after a similar decrease in arterial pressure produced by SNP; whereas 2 were unaffected. Again, the responses of these PVN neurons to a drop in arterial pressure were opposite in direction to the responses to ICA losartan. Although it is possible that the PVN response to ICA losartan might have been modulated by baroreceptors in the intact rats, a perusal of the data points in Fig. 5 does not support such an argument; i.e., the distribution of the responses of neurons from the intact and barodenervated rats overlap. In the SHAM rats responses to ICA losartan were minimal and highly variable (−28.5 ± 15.6%; n = 8), but the baseline activity of these neurons was low.

The dose of losartan used for these studies blocked the pressor effects and neural responses to both ANG I (n = 11) and ANG II (n = 13). ICA losartan induced similar decreases in mean arterial pressure in HF (−20.3 ± 2.8% from baseline of 90.8 ± 3.4 mmHg; n = 12, 6 intact, 6 denervated) and SHAM (−17.8 ± 1.2% from baseline of 91.3 ± 3.6 mmHg; n = 8) rats.

Effect of ICA spironolactone on PVN neuronal activity in HF and SHAM rats. Figure 6 (top) illustrates the effect of ICA spironolactone on a PVN neuron from a HF rat. Spironolactone also reduced the discharge rate of PVN neurons in HF rats, but this effect was slower in onset (onset latency ranging from ~5 to 10 min) and longer in duration (~15–25 min) than the effects of captopril and losartan and was not associated with a reduction in arterial pressure. As shown in Fig. 6 (bottom), ICA spironolactone (100 μg/kg) significantly
reduced PVN neuronal discharge rate = 74.0 ± 5.1%; 
\(n = 21, P < 0.01\) in the HF rats (baseline discharge 7.6 ± 1.6 spikes/s, range 3.3–15.8 spikes/s). Two neurons (filled circles) from baroreceptor-denervated rats are included in this data set, and their responses were similar in magnitude to those from the baroreceptor intact animals. The same dose of spironolactone induced no significant change in PVN neural activity (from 2.3 ± 0.5 to 2.2 ± 0.4 spike/s, 4.3 ± 6.3%, \(n = 9, P > 0.05\)) of SHAM rats. Spironolactone had no effects on baseline blood pressure (90.4 ± 3.8 and 91.8 ± 3.8 mmHg, respectively, \(P > 0.05\)) in HF or SHAM rats. ICA injection of vehicle also had no effect on PVN activity or mean arterial pressure.

Convergent inputs to PVN neurons from forebrain and hindbrain. Figure 7 illustrates the convergence of descending signals from ANG activation of the forebrain and ascending signals from baroreceptor inputs to hindbrain on a single PVN neuron from a HF rat. Responses to ICA ANG I, ANG II, and baroreceptor activation were tested in 52 (31 HF; 21 SHAM) units. Thirty (17 HF; 13 SHAM) of 52 (58%) neurons were excited by ANG I and 24 (13 HF; 11 SHAM) of 52 (46%) were excited by ANG II. Most PVN neurons responsive to ANG I also responded to ANG II.

Of the ANG-responsive units, 23 (12 HF; 11 SHAM) of 33 (70%) tested also responded to baroreceptor activation: 9 (4 HF; 5 SHAM) were excited and 14 (8 HF; 6 SHAM) were inhibited by increases in arterial pressure induced by intravenous PE. The PE-induced rise in mean arterial pressure required to excite PVN neurons was substantially larger than the mean arterial pressure increase associated with ICA ANG. For example, an ICA ANG-induced increase in PVN neuronal activity of 123.7 ± 18.4% was associated with a 12.1 ± 1.7% increase in mean arterial pressure, but a 131.5 ± 24.4% PE-induced increase in PVN neuronal activity required a 41.3 ± 3.4% increase in mean arterial pressure. Thus, among those neurons (a minority) that could be excited both by ANG I or ANG II and by baroreceptor stimulation, the small rise in pressure associated with ICA ANG was not sufficient to account for an increase in PVN neuronal activity of the magnitude observed.

Dose-dependent responses of PVN neurons to intravenous captopril in HF and SHAM rats. Figure 8 and Fig. 9 illustrate a dose-dependent differential effect of intravenous captopril on PVN neuronal activity. Intravenous captopril acts peripherally on the vasculature to reduce blood pressure by decreasing production of the vasoconstrictor ANG II and preventing the breakdown of the vasodilator bradykinin. As illustrated in the tracing in Fig. 8 (top), the lower dose (50 \(\mu g/kg\)) caused a reduction in arterial pressure and an increase

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**Fig. 7.** Example of a PVN neuron activated by both angiotensin (ANG) and baroreceptor stimulation in HF rat. This neuron is excited by both ICA ANG I and ANG II and by a drop in pressure induced by sodium nitroprusside (SNP). PVN firing is displayed as ratemeter recordings. Arrows indicate time of injections.
in PVN activity in a SHAM rat (the anticipated response of a PVN neuron to hypotension in an animal with intact baroreflexes). Figure 8 (bottom) shows the group data from SHAM and HF rats, showing a larger ($P < 0.05$) increase in PVN activity in the SHAM (153.3 ± 44.1%, $n = 11$) than in the HF (38.8 ± 8.8%, $n = 7$, 4 from intact, 3 from denervated) rats, consistent with the diminished (or absent) baroreflex regulation in these animals.

As shown in Fig. 9 (left), the higher dose of captopril (3 mg/kg) reduced arterial pressure and inhibited PVN neuronal activity in a HF rat. Figure 9 (bottom) shows the group data for HF and SHAM rats, demonstrating that the higher dose of captopril produced a significant inhibition of the spontaneous PVN neural activity in both groups of rats: HF −72.9 ± 6.9%, $n = 7$; 4 from intact, 3 from denervated; SHAM −70.9 ± 7.1%; $n = 6$. The magnitude of the change was not different ($P > 0.05$) between HF and SHAM rats, although the fall in blood pressure produced by captopril was slightly less ($P = 0.09$) in SHAM rats. This parallel reduction in PVN neuronal activity and mean arterial pressure suggests that the high dose of captopril has a central influence to depress PVN neuronal activity that overcomes or prevents the anticipated baroreceptor-mediated increase in PVN activity in response to its peripheral influence on arterial pressure.

**Recording sites in PVN.** Figure 10 shows the recovered recording sites for the groups of PVN neurons tested for effects of ICA captopril, losartan, and spironolactone, as defined by extrapolation from a Pontamine blue mark placed at the conclusion of the recording session. Recordings were made from sites throughout the PVN, with a good representation of neurons from parvocellular PVN, in regions that are strongly associated with sympathetic outflow. However, neurons mediating other homeostatic functions, including many that project to median eminence to regulate the hypothalamic-pituitary-adrenal axis, are also found in this medial region of PVN. By anatomic assessment, magnocellular neurons were also included among the recorded neurons. Thus the findings reported are not representative of neuronal population mediating a specific function but likely reflect a sampling across multiple cell types subserving a variety of homeostatic functions. The implication is that HF in-
creases the activity of PVN neurons in a nonspecific manner, consistent with the results of a previous study of metabolic activity (22).

DISCUSSION

This study provides the first direct evidence that neuronal activity in the PVN is increased in rats with ischemia-induced HF. This observation confirms a hypothesis arising from an earlier metabolic study (22). Novel to the present study are the observations that intrinsic brain RAAS contributes to the increased PVN neuronal activity in rats with ischemia-induced HF and that the increased activity of PVN neurons in rats with ischemia-induced HF can be attenuated by blood-borne manipulations of the RAAS. The preserved sensitivity of these neurons to changes in the humoral environment has important implications for the clinical management of heart failure.

In normal rats, circulating and intrinsic components of RAAS interact at forebrain level to regulate sympathetic drive and extracellular fluid volume. Blood-borne ANG II and ANG I are too large to cross the blood-brain barrier, but the AT1 receptors that mediate the effects of ANG II in the forebrain and the angiotensin-converting enzyme (ACE) are abundant in forebrain circumventricular organs, such as the subfornical organ (18, 25), where they are accessible to circulating peptides. Blocking forebrain ACE inhibits the sympathetic drive in normal rats, suggesting a tonic excitatory influence of intrinsic forebrain RAAS (31). Neurons in the forebrain circumventricular organs project to PVN (16, 20), another hypothalamic nucleus where intrinsic RAAS is active (2, 18, 21, 28). Chronic infusions of ANG II appear to upregulate AT1 receptors in the hypothalamus (33), although aldosterone, another product of RAAS activation, is also increased under those conditions and can increase the binding of ANG II to AT1 receptors in subfornical organ and PVN (2). Interestingly, aldosterone may access the brain from the circulation (7, 10) or, like ANG II, may be produced locally within the brain tissue (8).

Recent work from this laboratory and others suggests a role for forebrain RAAS mechanisms in the pathogenesis of HF. At least in rats with high-output HF, the expression of AT1 receptors in subfornical organ and PVN is increased (34). In rats with ischemia-induced HF, sympathetic drive can be reduced by blockade central of AT1 receptors (3, 35) or of central mineralocorticoid receptors (5); the latter intervention also reduces volume accumulation in these animals. The results of present study strongly support the hypothesis that forebrain mechanisms contribute to the progression of HF, demonstrating for the first time with direct electrophysiological recordings that the RAAS drives PVN neuronal activity in rats with ischemia-induced HF.

The data also clearly demonstrate that the activity of PVN neurons, and thus perhaps resulting adverse downstream effects of PVN activity in heart failure, can be reduced by blood-borne agents targeting the central neural effects of the RAAS. The fact that PVN neurons remain responsive to moment-to-moment changes in the circulating humoral milieu suggests that blood-borne mediators provide a continuous source of stimulation to PVN neurons in HF. The concept of modulating these blood-borne inputs to the central sites of neurohumoral activation in HF has not received much attention in the clinical literature, which focuses primarily on treating peripheral mani-
festations of HF (4). In animal studies, however, strong evidence suggests that modifying the effects of RAAS at the central nervous system level has beneficial effects in heart failure. As mentioned above, direct interventions in RAAS activity at forebrain level can ameliorate the sympathetic drive in the rat model of ischemia-induced HF (3, 5, 35), and systemically administered losartan (3), captopril (31), and spironolactone (5) have been shown to reduce sympathetic drive in HF.

Data from the present study therefore strengthen the case for the importance of central mechanisms in HF and further suggest that failure to recognize the central contribution may exacerbate the HF syndrome. For example, first-line treatment for decompensated heart failure in humans is the coadministration of a powerful diuretic and an ACE inhibitor. In normal rats, this combination increases circulating ANG I, which can be converted in the forebrain to ANG II to induce thirst and sodium appetite (30) and to increase sympathetic drive (31). In the present study, low-dose captopril lowered arterial pressure (a desired peripheral effect) but actually increased PVN neuronal activity, a result that might be explained either by increased ANG I production or by baroreceptor withdrawal. In contrast, high doses of captopril decreased both arterial pressure and PVN neuronal activity, indicating a blockade of central as well as peripheral ACE activity. This finding is consistent with the observation that tissue concentrations of ACE are substantially higher in certain regions of the forebrain than in the periphery (26). In a functional anatomy study addressing this point (17), low-dose captopril (0.5 mg/kg sc) dramatically increased the expression of immediate early gene c-fos in the subfornical organ and organum vasculosum of lamina terminalis (i.e., stimulated neuronal activity), whereas high-dose captopril (50 mg/kg subcutaneously) had no effect (i.e., blocked neuronal activity).

In considering the present results, it is important to recognize that the same neurons that respond to manipulations of forebrain RAAS may also respond to signals from medullary sites processing cardiovascular afferent signals. Approximately two-thirds of the PVN neurons tested were affected by both baroreceptor stimuli and forebrain RAAS receptor blockade. In HF, it is well known that the balance of inhibitory and excitatory effects of cardiovascular receptor systems is shifted in favor of mechanisms increasing sympathetic excitatory drive; arterial and cardiac vagal baroreflexes are blunted (36), and chemoreceptor (29) and cardiocardiac sympathetic reflexes (15) are exaggerated. Thus an alternative mechanism for activation of PVN neurons in HF is altered ascending input from hindbrain regions mediating sensory inputs from the cardiovascular system. Notably, however, the spontaneous discharge rate of PVN neurons was higher in the HF rats even after eliminating inputs from the aortic and carotid sinus nerves. This finding argues against altered afferent signaling in these two nerves as a continuing source of excitatory drive to PVN neurons in chronic HF, but it does not exclude a role for ascending neural pathways activated by other mechanisms. Furthermore, the effects of ICA captopril, losartan, and spironolactone were present in the denervated rats, demonstrating that neither local effects on vascular compliance or on the afferent endings themselves nor baroreceptor-mediated responses to changes in arterial pressure mediated the salutary influence of these agents on PVN neuronal discharge in HF.

Several ancillary findings of this study deserve brief comment. First, captopril inhibited the activity of PVN neurons in SHAM as well as HF rats. Because bloodborne ANG I is not normally present in high levels, this finding suggests that the production of ANG II by the intrinsic forebrain RAAS provides a tonic excitatory drive to PVN neurons in normal rats, at least in anesthetized animals. Interestingly, losartan did not have a similar effect. Second, mineralocorticoid receptor blockade with spironolactone reduced PVN neuronal activity in HF rats without affecting arterial pressure. This finding contrasts with the effects of ACE inhibition and AT1 receptor blockade and suggests that spironolactone may have affected a population of PVN neurons more involved in volume regulation than in sympathetic drive. Alternatively, a higher dose or bilateral ICA infusion of spironolactone might have affected a larger population of neurons and, by so doing, may have altered sympathetic drive. These possibilities were not tested. Finally, spironolactone had no effect on the activity of PVN neurons in the SHAM rats, indicating that mineralocorticoid receptor activation may contribute to the increased activity of PVN neurons in HF, but not under normal conditions.

Our methods do not permit a functional analysis of the specific components of the RAAS that are altered in HF or a localization of drug effect within the forebrain. For example, the finding of a reduction in PVN neuronal activity with intracarotid captopril clearly implicates the involvement of forebrain ACE but does not differentiate between increased conversion of ANG I to ANG II versus increased number or affinity of AT1 receptors as the mechanism for increased PVN neuronal discharge in the untreated HF rat. The delayed time course of the spironolactone influence on PVN neuronal discharge argues strongly against a direct action to block aldosterone effects on PVN neurons. However, the more rapid effects of losartan and captopril might well be attributed to a local reduction in AT1 receptor-mediated excitation. In a previous study of PVN neuronal responses to subfornical organ stimulation (14), intravenously administered losartan had an immediate effect that was attributed to passage of the drug across the blood-brain barrier. Although captopril also crosses blood-brain barrier during chronic systemic administration (32), its effects on PVN firing in an acute bolus injection study like this one might reasonably be attributed to inhibition of ACE in the more readily accessible circumventricular organs (see Refs. 12 and 24).

Finally, it is important to note that the projection sites of the PVN neurons reported here were not deter-
mined in this initial study so that a link between the observed RAAS effects and physiological functions of the recorded neurons cannot be established. The diffuse distribution of recovered recording sites within PNVT suggests that the recorded neurons subserved a variety of functions, likely including regulation of volume, sympathetic drive, and the hypothalamic-pituitary-adrenal axis. The present results suggest the need for further studies to define the contribution of RAAS to the specific pathophysiological abnormalities in HF.

In summary, the present study confirms with direct electrophysiological recordings a previous suggestion (22) that neurons in the PVN of rats with ischemia-induced heart failure are driven to a higher level of excitability. Our results carry this observation further by demonstrating that forebrain RAAS is primarily responsible for the exaggerated PVN neuronal activity in HF and that blood-borne manipulations of forebrain RAAS have the potential to normalize PVN neuronal activity in HF. Because the activation of PVN neurons is generally associated with the activation of homeostatic mechanisms, including thirst, sodium appetite, sympathetic drive, and the hypothalamic-pituitary-adrenal axis, it can be surmised that RAAS-dependent sympathetic drive, and the hypothalamic-pituitary-adrenal axis, can be surmised that RAAS-dependent regulations in HF, including thirst, sodium appetite, RAAS to the specific pathophysiological abnormalities in HF.

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