Brain angiotensin-converting enzyme activity and autonomic regulation in heart failure

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Francis, Joseph, Shun-Guang Wei, Robert M. Weiss, and Robert B. Felder. Brain angiotensin-converting enzyme activity and autonomic dysregulation characteristic of rats with congestive heart failure. Rats with heart failure (HF) induced by coronary artery ligation and sham-operated control (SHAM) rats were treated with chronic (28 days) third cerebral ventricle (intracerebroventricular (ICV)) or intraperitoneal (IP) infusion of a low dose of the ACE inhibitor enalaprilat (ENL) or vehicle (VEH). VEH-treated HF rats had increased sodium consumption, reduced urine sodium and urine volume, and increased sympathetic nerve activity with impaired baroreflex regulation. These responses were minimized or prevented by ICV ENL started 24 h after coronary ligation. IP ENL at the low dose used in these studies had no beneficial effects on HF rats. Neither IP nor ICV ENL had any substantial effect on the SHAM rats. The findings confirm a critically important contribution of the brain renin-angiotensin system to the pathophysiology of congestive heart failure.

METHODS

Animals

These studies were performed in accordance with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” (1). The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Adult male Sprague-Dawley rats (3–4 mo old, 300–325 g body wt; Harlan Sprague Dawley, Indianapolis, IN) were housed in temperature-controlled (23 ± 2°C) and light-controlled (lights on between 0700 and 1900) animal quarters; rat chow was provided ad libitum.

All rats were subjected to three or four survival surgeries and one echocardiography session. After each procedure, they recovered from anesthesia under observation in the laboratory before they were returned to their cages. Surgery was performed using sterile technique, and animals received buprenorphine for treatment of postoperative pain as indicated for each specific procedure.

General Experimental Protocol

Some rats underwent initial placement of a cannula into the third cerebral ventricle for central drug infusion. All rats then became acclimated over a 2-wk interval to metabolic cages in which they had access to water and a 1.8% NaCl solution. Baseline body weight,

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consumption of tap water and 1.8% NaCl, and urine volume and urine sodium excretion were measured near the end of this interval. All rats then underwent coronary artery ligation to induce congestive HF (HF rats) or a sham procedure (SHAM rats). At 24 h after coronary ligation, echocardiography was performed to confirm the condition of the LV, and animals were assigned to treatment groups. Immediately thereafter, osmotic minipumps were implanted for continuous intra-cerebroventricular (ICV) or intraperitoneal (IP) infusion of the ACE inhibitor enalaprilat (ENL). Repeated measurements of body weight, consumption of tap water and the NaCl solution, and urine volume and sodium content were made over the course of the 28-day protocol. The rats then underwent surgery for placement of renal nerve recording electrodes and arterial and venous cannulas, with subsequent conscious renal sympathetic nerve recording and baroreflex testing. At the conclusion of the study, animals were euthanized with an overdose of anesthesia.

**Specific Methods**

**ICV cannula implantation.** Rats were anesthetized with an Equithesin-like anesthetic cocktail (0.97 g pentobarbital sodium and 4.25 g of chloral hydrate per 100 ml of distilled water, 0.33% NaCl/100 ml body wt), and a cannula for chronic drug infusion was implanted in the third cerebral ventricle, and fixed to the cranium with use of dental acrylic and small screws, as described previously (15, 16). Stereotaxic coordinates used for cannula placement were 1.0 mm caudal to bregma, 1.5 mm lateral to midline, and 8.7 mm ventral to dura, with the cannula angled 10° toward the midline. Metal tubing (30 gauge) was used as an obturator to keep the cannula patent. Buprenorphine (0.1 mg/kg sc) was administered immediately after surgery for management of postoperative pain.

**Induction of HF or SHAM.** HF was induced by ligation of the left anterior descending coronary artery, as previously described (14–17). Briefly, animals under ketamine + xylazine (90 and 10 mg/kg ip, respectively) anesthesia were endotracheally intubated and mechanically ventilated, and the left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium. SHAM rats underwent the same surgery but did not undergo coronary ligation. The heart was returned to the chest cavity, the lungs were reinfated, and the chest incision was closed. Posturgically, animals were given benzathine penicillin (30,000 U im) and buprenorphine (0.1 mg/kg sc) immediately after surgery and 12 h later.

**Echocardiographic assessment of LV function.** At 24 h after coronary artery ligation, echocardiography was performed under ketamine (25 mg/kg ip) sedation, as previously described (14–17), using an Acuson (Mountainview, CA) Sequoia model 256 clinical imager fitted with an 8-MHz sector-array probe, which generates two-dimensional images at a rate of ~100/s. Short- and long-axis images of the LV were analyzed. Ischemic zone was estimated by planimetry of the region of the LV endocardial silhouette that demonstrated akinesis or dyskinesis, expressed as a percentage of the whole (percent ischemic zone). The percent ischemic zone, LV ejection fraction (LVEF), LV mass, and LV end-diastolic volume (LVEDV) were reported. After completion of two-dimensional imaging, pulse-wave Doppler interrogation of mitral inflow was performed to determine heart rate (HR). Only animals with large infarctions (range 50.1–56.4% ischemic zone) were used in the study.

**Implantation of osmotic minipumps.** The procedure for preparation and implantation of the minipumps has been described previously (15, 16). For ICV infusion, the pumps were implanted subcutaneously at the back of the neck. The obturator was removed from the ICV cannula, and the free end of the silicone rubber tubing was connected to the cannula and secured using dental acrylic. For IP infusion, the pumps were implanted in the peritoneal cavity. The minor surgery required was performed under ketamine (100 mg/kg) anesthesia. Buprenorphine (0.1 mg/kg sc) was administered immediately after surgery for management of postoperative pain.

**Drug infusion.** ENL (Baxter) was diluted in sterile water (Sigma) to a final concentration of 0.5 mg/ml. Alzet osmotic minipumps (model ML4, Alza, Palo Alto, CA) were filled with ENL or vehicle (VEH, sterile water) and attached to a flow moderator to obtain a continuous infusion of the drug. The pumps had an average flow rate of 2.5 μl/h, and the final dose of drug infused was 1.25 μg/h for 28 days. The infusion rates and final drug dose were the same regardless of the route (ICV or IP) of administration. In these studies, the rationale for IP infusion was to provide a control for effects of potential leakage of ENL from the cerebrospinal fluid into the systemic circulation during the 28-day protocol.

**Conscious sympathetic nerve recording and baroreflex testing.** Under pentobarbital sodium (50 mg/kg ip) anesthesia, animals were instrumented as previously described (14, 16, 17) for recording of integrated RSNA, arterial pressure, and HR and baroreflex testing in the conscious, freely mobile state. The rats were under anesthesia for ~1 h for the surgical procedure and recovered within the subsequent 1 h. Buprenorphine (0.06–0.08 mg/kg sc) was administered immediately after the surgery for management of postoperative pain. At 4–6 h after recovery from the anesthesia, at a time when the rats were alert and active in their cages, baseline measurements were obtained and baroreflex testing was performed using bolus intravenous injections of phenylephrine (2–10 μg/kg) and sodium nitroprusside (5–20 μg/kg). The data were analyzed using standard methods described previously (14, 16, 17).

Because of long-standing concerns regarding the effect that variability in multiphase nerve recordings may have on comparisons between groups of animals (6, 18, 21), we employed a pulse-triggered average of the integrated RSNA as an additional means of comparing baseline RSNA across treatment groups. The waveform average, triggered by the peak of the arterial pressure pulse, was derived from a 2-min recording interval while the animal was at rest. This method closely resembles in principle the “cycle activity” method described by Lundin et al. (26) to validate the increased sympathetic drive in the spontaneously hypertensive rat compared with the Wistar-Kyoto rat and subsequently employed by those investigators to demonstrate increased RSNA in HF after myocardial infarction vs. sham-operated controls (12). It provides a quantitative means of describing the nearly continuous nature of sympathetic discharge during all cardiac cycles in HF; i.e., those cardiac cycles with little or no sympathetic activity that create the typical waxing and waning of the renal nerve signal in the normal rat, characterized by Lundin et al. as “silent cycles,” are rare or absent in the untreated rat with heart failure. We previously used this method, which is independent of differences in HR, to represent the typical pattern of unrestrained RSNA in HF rats (14, 17).

**Metabolic cage assessment of fluid balance.** Ingestion of water and 1.8% NaCl and urine volume and urinary sodium content were measured at baseline, daily after coronary artery ligation for 14 days, and then on days 21 and 28. Urine sodium was measured using a sodium and potassium analyzer (NOVA Biomedical, Waltham, MA). The rats were weighed at the end of weeks 1, 2, and 4.

**Verification of cannula placement and minipump function.** At the end of the study, brains were removed to check the site of cannula implantation. The osmotic minipumps were also removed to check residual volume of the drug, if any, to ensure that the drugs were infused into the animals.

**Statistical Analysis**

Values are means ± SE. Differences between groups in body weight, consumption of water and 1.8% NaCl, urine volume, and urinary sodium were analyzed by two-way repeated-measures ANOVA followed by Fisher’s post hoc least significant difference test. As a nonlinear regression program (SigmaPlot, Jandel Scientific) was used to analyze the components defining the individual sigmoidal curve fits of the baroreflex data, and these values were averaged to construct representative baroreflex curves for each group relating...
Treatment with ICV ENL normalized compared with SHAM rats (Fig. 1), as previously reported in assigned to treatment with VEH vs. ENL. HF than in SHAM rats but did not differ between those animals either (IP or ICV) ENL treatment protocol. HR was higher in LVEF, and LVEDV did not differ between HF rats assigned to with the SHAM rats (Table 1). However, percent infarct zone, Characteristics of Study Groups study, ENL treatment had no apparent in the 2nd wk after ligation. Thus, over the time course of this groups, one ENL-treated and one VEH-treated HF rat died by assigned time point. Among the rats assigned to IP treatment VEH-treated HF rat and one SHAM rat died before the desig- assigned to ICV treatment groups, one ENL-treated and one coronary ligation survived the procedure. Among the rats died within 24 h of surgery. All 24 rats undergoing sham Survival

Ten of 40 rats (25%) undergoing coronary artery ligation survived the procedure. Among the rats assigned to ICV treatment groups, one ENL-treated and one VEH-treated HF rat and one SHAM rat died before the designated time point. Among the rats assigned to IP treatment groups, one ENL-treated and one VEH-treated HF rat died by the 2nd wk after ligation. Thus, over the time course of this study, ENL treatment had no apparent influence on survival. Characteristics of Study Groups

HF rats had reduced LVEF and increased LVEDV compared with the SHAM rats (Table 1). However, percent infarct zone, LVEF, and LVEDV did not differ between HF rats assigned to either (IP or ICV) ENL treatment protocol. HR was higher in HF than in SHAM rats but did not differ between those animals assigned to treatment with VEH vs. ENL.

Volume Regulation

Untreated HF rats exhibited an increased appetite for sodium compared with SHAM rats (Fig. 1), as previously reported in this model (14, 16, 17). Treatment with ICV ENL normalized sodium appetite in the HF rats within a few days (Fig. 1A), so that sodium ingestion in the HF + ENL rats was not different from that of SHAM + VEH and SHAM + ENL rats. IP ENL dramatically increased sodium appetite in the HF (HF + ENL) group and tended to increase it in the SHAM (SHAM + ENL) group as well compared with the VEH-treated controls (Fig. 1B). This general finding is consistent with the known effect of peripheral ACE inhibition to induce an increase in ANG I, which acts centrally to stimulate sodium appetite (49). The exaggerated effect of IP ENL on sodium consumption in the HF group could be consistent with an augmented ACE activity in the circumventricular organs (47) vs. an excessive increase in blood-borne ANG I.

RESULTS

Survival

Ten of 40 rats (25%) undergoing coronary artery ligation died within 24 h of surgery. All 24 rats undergoing sham coronary ligation survived the procedure. Among the rats assigned to ICV treatment groups, one ENL-treated and one VEH-treated HF rat died before the designated time point. Among the rats assigned to IP treatment groups, one ENL-treated and one VEH-treated HF rat died by the 2nd wk after ligation. Thus, over the time course of this study, ENL treatment had no apparent influence on survival.

Characteristics of Study Groups

HF rats had reduced LVEF and increased LVEDV compared with the SHAM rats (Table 1). However, percent infarct zone, LVEF, and LVEDV did not differ between HF rats assigned to either (IP or ICV) ENL treatment protocol. HR was higher in HF than in SHAM rats but did not differ between those animals assigned to treatment with VEH vs. ENL.

Table 1. Echocardiographic measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR, beats/min</th>
<th>LVEDV, ml</th>
<th>LV Mass, g</th>
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<tr>
<td>SHAM + VEH</td>
<td>6</td>
<td>384 ± 26</td>
<td>0.432 ± 0.03</td>
<td>0.77 ± 0.06</td>
<td>0.80 ± 0.03</td>
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<td>393 ± 26</td>
<td>0.426 ± 0.03</td>
<td>0.84 ± 0.08</td>
<td>0.83 ± 0.02</td>
<td>0</td>
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<td>441 ± 15*</td>
<td>1.008 ± 0.03*</td>
<td>0.76 ± 0.02</td>
<td>0.36 ± 0.02*</td>
<td>53.6 ± 2.3*</td>
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<tr>
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<td>7</td>
<td>425 ± 10*</td>
<td>0.97 ± 0.04*</td>
<td>0.78 ± 0.04</td>
<td>0.39 ± 0.02*</td>
<td>55.1 ± 1.1*</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>SHAM + VEH</td>
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<td>385 ± 21</td>
<td>0.387 ± 0.02</td>
<td>0.85 ± 0.06</td>
<td>0.81 ± 0.04</td>
<td>0</td>
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<td>SHAM + ENL</td>
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<td>389 ± 22</td>
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<td>HF + VEH</td>
<td>7</td>
<td>430 ± 11*</td>
<td>0.937 ± 0.03*</td>
<td>0.76 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>54.2 ± 2.5*</td>
</tr>
<tr>
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<td>0.78 ± 0.02</td>
<td>0.37 ± 0.01*</td>
<td>53.2 ± 1.4*</td>
</tr>
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</table>

Values are means ± SE. SHAM, sham operation; VEH, vehicle; ENL, enalaprilat; HF, heart failure; HR, heart rate; LVEDV, left ventricular (LV) end-diastolic volume; LVEF, LV ejection fraction; ICV, intracerebroventricular; IP, intraperitoneal. *P < 0.05 vs. SHAM.

Fig. 1. Effects of chronic third cerebral ventricle [intracerebroventricular (ICV)] or intraperitoneal (IP) infusion of enalaprilat (ENL) or vehicle (VEH) on consumption of 1.8% NaCl after coronary artery ligation (CL) to induce heart failure (HF) or after sham operation (SHAM). A: ingestion of NaCl increased over baseline (Pre) in HF + VEH rats, beginning 1 day after CL. ICV ENL blunted initial increase in sodium consumption, and by day 4 sodium intake in HF + ENL rats was not different from that in SHAM + VEH or SHAM + ENL rats. ICV ENL had no effect on SHAM rats. B: ingestion of NaCl increased dramatically over baseline (Pre) in HF + VEH rats beginning 1 day after CL (note change in y-axis scale compared with A). Sodium consumption increased dramatically in HF rats treated with IP ENL, a response opposite to that of treatment with ICV ENL (A). Sodium ingestion also tended to increase in SHAM rats treated with IP ENL. *P < 0.05, HF + ENL vs. SHAM + VEH. †P < 0.05, HF + VEH vs. HF + ENL. ‡P < 0.05, SHAM + VEH vs. SHAM + ENL. Brackets indicate significant difference between all data points. Pre, baseline; CF. days 1–28, 1–28 days after CL. Long arrow, CL; short arrow, start of ENL infusion.
Consumption of tap water decreased transiently (on day 1) after coronary ligation in the HF + VEH rats (Fig. 2). Otherwise, there were no significant changes in water ingestion in HF or SHAM rats treated with ICV ENL or VEH. Among the IP treatment groups, there were significant variations in water ingestion in the HF + VEH rats (Fig. 2A), and the HF + ENL rats experienced significant increases in water ingestion at multiple time points (Fig. 2B). There were no significant changes in water consumption in the SHAM groups over the course of the experimental protocol.

Sodium excretion was reduced in HF rats (HF + VEH), as shown in Fig. 3 and as previously reported (14, 16, 17). Treatment of HF rats with ICV ENL normalized urinary sodium compared with SHAM + VEH and SHAM + ENL (Fig. 3A), but treatment with IP ENL had no effect on sodium excretion in the HF rat (Fig. 3B).

Similarly, urine volume was reduced in HF rats (HF + VEH), as shown in Fig. 4 and as previously reported (14, 16, 17). Treatment of HF rats with ICV ENL normalized urine volume compared with SHAM + VEH and SHAM + ENL (Fig. 4A). In marked contrast, IP treatment of the HF rat with ENL (HF + ENL) dramatically increased urine volume (Fig. 4B). The mechanisms for the observed treatment effects on urine volume were not explored in this study. It is possible that IP ENL exerted local effects on renal blood flow and diuresis, perhaps mediated by the reduced breakdown of bradykinin (34). The remote effects of ICV ENL on renal reabsorption of water were likely mediated by a reduction in the central ANG II influences on RSNA (9) or by a reduction in ANG II-induced release of arginine vasopressin (28, 33).

Consistent with our prior experience with this model (17), the HF rats did not gain weight; on the contrary, the HF rats had lower body weights than the comparably treated SHAM rats at most time points (Table 2). The HF rats treated with ICV VEH or ENL had significantly lower body weights than the comparably treated SHAM rats at all time points, and their weights had barely returned to baseline at the completion of the experimental protocol.
ICV ENL had lower HR and a small but statistically significant reduction in RSNA compared with VEH-treated HF rats. These measurements were obtained from conscious rats 4–6 h after recovery from anesthesia. The stress of recent surgery likely increased baseline sympathetic drive and, therefore, may have minimized differences between the HF and SHAM rats. Nevertheless, clearly discernable and statistically significant differences in HR and RSNA were observed between HF and SHAM rats and between HF rats treated with ICV VEH and with ENL.

Chronic treatment of HF rats with ICV ENL also improved baroreflex regulation of HR and RSNA measured under these same conditions. In HF rats treated with ICV VEH, compared with VEH-treated SHAM rats, baroreflex function was impaired, as indicated by reduced range and maximum gain of baroreflex regulation of HR and RSNA (Fig. 6A). Chronic treatment of HF rats with ICV ENL improved both indexes of protocol. It is unclear why body weights of the IP-treated HF and IP-treated SHAM rats were similar at the conclusion of the protocol. As previously reported (17), HF rats consume less rat chow than SHAM rats. Although food consumption was not measured in this study, the weights of the HF animals likely reflect the balance between reduced food intake and volume retention. However, the HF rats do not show overt signs of volume retention, such as peripheral edema.

**Sympathetic Drive and Baroreflex Regulation**

At 4 wk after coronary artery ligation, VEH-treated HF rats had higher HR and increased RSNA, measured as integrated voltage or as the pulse-triggered waveform average of the integrated voltage signal within the cardiac cycles over a 2-min sampling interval, than VEH-treated SHAM rats (Fig. 5). ICV ENL treatment of the SHAM rats had no effect on mean arterial pressure, HR, or RSNA. However, HF rats treated with

### Table 2. Body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SHAM + VEH</td>
<td>6</td>
<td>352 ± 5</td>
<td>344 ± 4</td>
<td>360 ± 3</td>
<td>378 ± 4*</td>
</tr>
<tr>
<td>SHAM + ENL</td>
<td>7</td>
<td>350 ± 4</td>
<td>346 ± 5</td>
<td>358 ± 5</td>
<td>372 ± 3*</td>
</tr>
<tr>
<td>HF + VEH</td>
<td>6</td>
<td>351 ± 5</td>
<td>327 ± 4*</td>
<td>339 ± 2*†</td>
<td>356 ± 2†</td>
</tr>
<tr>
<td>HF + ENL</td>
<td>7</td>
<td>347 ± 4</td>
<td>324 ± 5*</td>
<td>330 ± 5*†</td>
<td>347 ± 5†</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM + VEH</td>
<td>5</td>
<td>351 ± 2</td>
<td>361 ± 2*</td>
<td>375 ± 2*</td>
<td>387 ± 2*</td>
</tr>
<tr>
<td>SHAM + ENL</td>
<td>7</td>
<td>352 ± 2</td>
<td>362 ± 2*</td>
<td>371 ± 2*</td>
<td>386 ± 2*</td>
</tr>
<tr>
<td>HF + VEH</td>
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<td>351 ± 2</td>
<td>341 ± 4†</td>
<td>357 ± 7†</td>
<td>379 ± 6*</td>
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<tr>
<td>HF + ENL</td>
<td>7</td>
<td>355 ± 3</td>
<td>337 ± 3†</td>
<td>363 ± 3†</td>
<td>387 ± 7*</td>
</tr>
</tbody>
</table>

*Values are means ± SE in grams. †P < 0.05 vs. baseline. ‡P < 0.05 vs. SHAM receiving the same treatment.

Fig. 5. Effect of ICV ENL or VEH on baseline mean arterial pressure (A, MAP), heart rate (B, HR), and renal sympathetic nerve activity (RSNA) as integrated voltage (C) and as area under the curve (D) of the pulse-triggered waveform average of the integrated voltage signal, in SHAM and HF rats after CL. HF + ENL rats (n = 6) had lower HR and less RSNA than HF + VEH rats (n = 6). ENL had no effect on these variables in SHAM rats (n = 6) compared with SHAM + VEH rats (n = 5). *P < 0.05, VEH vs. ENL. ‡P < 0.05, HF + VEH vs. SHAM + VEH.
baroreflex function, indicating an overall improvement in sympathetic responses to hemodynamic stress. This improvement is illustrated by the logistic curve-fit data for HF rats treated with ICV ENL and ICV VEH (Fig. 6B). In contrast, IP ENL treatment of HF rats had no effect on resting mean arterial pressure, HR, or RSNA or on baroreflex function (Fig. 7).

DISCUSSION

Recent reports (10, 14, 16, 17, 23–25, 54, 55) suggest that altered neural mechanisms in the brain contribute substantially to the autonomic dysfunction in HF. Notable findings in HF animals that implicate the brain renin-angiotensin system include increased brain ACE activity (47), increased AT1 receptor binding densities in the brain (47, 53) with impressive responses to central AT1 receptor blockade (10), increased dipsogenic responses to ANG II (44), and reduced sympathetic dysfunction and cardiac remodeling after myocardial infarction in transgenic rats that lack brain angiotensinogen (51). The present study provides striking new physiological evidence supporting the hypothesis that the brain renin-angiotensin system contributes importantly to the volume accumulation and sympathetic dysregulation that characterize established HF. In this model of ischemia-induced HF, chronic ICV infusion of an ACE inhibitor normalized sodium appetite and the renal handling of sodium and water and significantly improved sympathetic regulation. Peripheral administration of the same low dose of ACE inhibitor to control for effects that might be attributed to leakage from the cerebrospinal fluid into the circulation had no beneficial effects on these parameters.

Within the brain, ACE is normally present in two separate domains, defined by the blood-brain barrier. High concentrations, exceeding those found in the lungs, are present in circumventricular organs (7), such as the area postrema and the subfornical organ, where the blood-brain barrier is absent. Blood-borne ANG I has easy access to these regions, where ACE converts ANG I to ANG II, which can then act on AT1 receptors (30). This mechanism has been tapped for years by investigators interested in the role of angiotensin in thirst and sodium appetite, e.g., volume depletion with a diuretic combined with peripheral low-dose ACE inhibition induces a prominent increase in blood-borne ANG I, which is converted centrally to ANG II (50). High doses of ACE inhibitors, administered peripherally, can inhibit ACE activity in these circumventricular organs (32, 50).

ACE is present in lower but still significant concentrations inside the blood-brain barrier (5, 7), most notably in hypothalamic and brain stem regions involved in autonomic regulation. In these areas, which are impermeable to blood-borne peptides, the brain renin-angiotensin system (3, 29) is the source of ANG I. Recent work suggests that the brain renin-angiotensin system is overactive in HF (47, 51). Although the specific mechanisms that induce increased activity of the brain renin-angiotensin system are not known, blockade of ouabain-like activity was effective in reducing ACE and AT1 receptor affinity (47).

The present study deals specifically with the role of brain ACE activity as a contributing factor to the overactivity of the brain renin-angiotensin system in HF rats. It demonstrates that the typical manifestations of central AT1 receptor stimulation,
ACE activity and increase circulating ANG I but are insufficient to inhibit the higher concentrations of ACE in the circumventricular organs may have the paradoxical effect of stimulating the central production of ANG II and activating AT1 receptors in those regions (32, 50). Although this mechanism has not yet been examined in animals or humans with heart failure, there is clearly a theoretical basis for optimizing the dose of ACE inhibitors in a timely fashion.

Rats and humans have localized regions of ACE activity sequestered within the blood-brain barrier (48). Depending on their lipophilic properties and possibly on active membrane transport (39), chronically administered ACE inhibitors may also access these sites of ACE activity inside the blood-brain barrier (4). To what extent does ACE activity within these protected brain regions contribute to the autonomic dysregulation in human HF, and what benefits might be derived by specifically targeting ACE activity within the blood-brain barrier for therapeutic intervention? These are questions that demand further investigation.

Several limitations must be considered in the interpretation of the data from this study. First among these is the potential contribution of changes in LV function to the improvement in RSNA and baroreflex control. Prior work in rats has demonstrated that chronic central infusion of the ACE inhibitor captopril can reduce cardiac remodeling in HF (44). Because a posttreatment echocardiogram was not obtained at the conclusion of this protocol, we cannot exclude the possibility that the improvement in sympathetic regulation in HF rats treated with ICV ENL was linked to improved LV function; if so, both beneficial effects might be ascribed to inhibition of brain ACE activity. Second, the measurements of sympathetic nerve activity were obtained shortly after a surgical procedure, introducing the possibility that postoperative stress might have minimized the actual differences between groups in baseline sympathetic activity. Third, the mechanism for the beneficial effect of ICV ENL on renal function was not determined in this study. Although there is clear evidence that manipulations of the renin-angiotensin system within the brain can effect changes in kidney function (2, 8, 16, 20, 28, 38) via the renal sympathetic nerves (9, 38), the possibility that central ACE inhibition might have reduced circulating levels of arginine vasopressin must also be considered (27, 28, 33).

Finally, the “control” data from IP ENL, in the same low dose infused ICV, which was meant to validate the central origin of the ICV effects, unexpectedly induced a unique set of responses of their own. However, these results differ dramatically from the responses of the HF rats to ICV ENL and therefore support the interpretation that the ICV effects were centrally mediated. The augmentation of thirst and sodium appetite induced by IP ENL in the HF rats (vs. SHAM) may simply reflect blockade of a more active peripheral renin-angiotensin system, resulting in the higher blood levels of ANG I; alternatively, or in addition, the increase in brain ACE activity in this model of HF (47) may confer an enhanced sensitivity to this forebrain AT1 receptor-mediated behavioral response. The induction of increased urine volume is more difficult to explain but might be related to an enhanced dependence of renal vasoconstriction on circulating ANG II or possibly a vasodilatory effect of bradykinin as a by-product of ACE inhibition.

Fig. 7. Effects IP ENL (n = 6) or VEH (n = 6) on baseline hemodynamic variables (A) and indexes of baroreflex regulation (B) in HF rats. IP ENL had no effect on baseline HR, MAP, RSNA, or baroreflex regulation of HR or RSNA in these HF rats.
In summary, the present study provides compelling evidence that exaggerated activity of the brain renin-angiotensin system contributes substantially to the autonomic dysregulation observed in chronic HF. In fact, treatment of central ACE alone, with no other intervention, completely normalized indexes of volume regulation and substantially improved sympathetic regulation. Current clinical therapy with ACE inhibitors, directed toward the peripheral renin-angiotensin system, may not adequately treat the higher concentrations of ACE in the central nervous system; by increasing circulating ANG I, such treatment may actually exaggerate autonomic abnormalities in HF by providing substrate for the relatively untreated ACE in circumventricular organs of the brain. In experimental HF, ACE activity has been shown to be increased within the blood-brain barrier as well. It remains to be determined whether modifying ACE inhibitors to facilitate their access to ACE activity has been shown to be increased within the circumventricular organs of the brain. In experimental HF, HF by providing substrate for the relatively untreated ACE in treatment may actually exaggerate autonomic abnormalities in central nervous system; by increasing circulating ANG I, such inadequately treat the higher concentrations of ACE in the regulation. Current clinical therapy with ACE inhibitors, di-

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