Central mineralocorticoid receptor blockade improves volume regulation and reduces sympathetic drive in heart failure

JOSEPH FRANCIS,1,2 ROBERT M. WEISS,1,2 SHUN-GUANG WEI,1,2 ALAN KIM JOHNSON,3 TERRY G. BELTZ,3 KATHY ZIMMERMAN,1 AND ROBERT B. FELDER1,2

1Research Service, Department of Veterans Affairs Medical Center, and Departments of Internal Medicine and Psychology, University of Iowa, Iowa City, Iowa 52242

Received 12 March 2001; accepted in final form 6 August 2001

Francis, Joseph, Robert M. Weiss, Shun-Guang Wei, Alan Kim Johnson, Terry G. Beltz, Kathy Zimmerman, and Robert B. Felder Central mineralocorticoid receptor blockade improves volume regulation and reduces sympathetic drive in heart failure. Am J Physiol Heart Circ Physiol 281: H2241–H2251, 2001.—The mineralocorticoid (MC) receptor antagonist spironolactone (SL) improves morbidity and mortality in patients with congestive heart failure (CHF). We tested the hypothesis that the central nervous system actions of SL contribute to its beneficial effects. SL (100 ng/h for 28 days) or ethanol vehicle (VEH) was administered intracerebroventricularly or intraperitoneally to rats with CHF induced by coronary artery ligation (CL) and to SHAM-operated controls. The intracerebroventricular SL treatment prevented the increase in sodium appetite and the decreases in sodium and water excretion observed within a week of CL in VEH-treated CHF rats. Intraperitoneal SL also improved volume regulation in the CHF rats, but only after 3 wk of treatment. Four weeks of SL treatment, either intracerebroventricularly or intraperitoneally, ameliorated both the increase in sympathetic drive and the impaired baroreflex function observed in VEH-treated CHF rats. These findings suggest that activation of MC receptors in the central nervous system plays a critical role in the altered volume regulation and augmented sympathetic drive that characterize clinical heart failure.

myocardial infarction; renin-angiotensin system; aldosterone; spironolactone; rat

IN CONGESTIVE HEART FAILURE (CHF), the activity of the renin-angiotensin-aldosterone (Aldo) system and of the sympathetic nervous system is augmented (25), resulting in volume retention and peripheral vasoconstriction. Drug therapies for CHF that have proven most successful in large clinical trials have targeted these peripheral effects of neurohumoral activation (8, 17, 22). One potential therapeutic target that has not received much attention until recently is Aldo, which acts on mineralocorticoid (MC) receptors in tissues throughout the body. The recently completed Randomized Aldactone Evaluation Study (19) specifically addressed the role of Aldo in clinical CHF. The study was stopped early because of a compelling reduction in morbidity and mortality in treated and stable CHF patients who received a daily dose of the MC antagonist spironolactone (SL). The mechanism of this beneficial effect of SL is not known, but in that study it could not be attributed to its well-known diuretic effect.

In CHF, the circulating levels of Aldo are high (30), even in patients treated with an angiotensin-converting enzyme inhibitor (18). Recent studies have described detrimental effects of high circulating levels of Aldo on the heart, vasculature, and kidneys (3, 7, 15). Surprisingly, the effects that high circulating levels of Aldo might have on the brain have received very little attention in the heart failure literature. However, studies in normal animals have demonstrated that activation of forebrain MC receptors by Aldo or its precursor deoxycorticosterone acetate increases sodium appetite (31), increases the binding of ANG II to AT1 receptors in forebrain regions that mediate thirst and sympathetic drive (6), and increases the production (10, 24) and release (24) of arginine vasopressin. Activation of central MC receptors also increases arterial pressure (9) and facilitates the central effects of ANG II on arterial pressure and thirst (28). Blocking central MC receptors reduces arterial pressure and induces a diuresis and sodium excretion (20), the latter influence on kidney function probably mediated via the renal nerves. Thus in normal animals physiological levels of Aldo act on MC receptors in critical brain centers that regulate fluid balance and sympathetic drive, the two critical homeostatic systems that are deranged in heart failure. Short-term stimulation of these central nervous system mechanisms with Aldo mimics the pathophysiology of CHF. Yet their relative contribution of the overall imbalances in fluid regulation and sympathetic drive in CHF is virtually unknown.

The present study was designed to elucidate the relative contribution of the central effects of Aldo to the altered cardiovascular homeostasis of rats with experimentally induced CHF. This study provides the first evidence to suggest that the central nervous system

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpheart.org 0363-6135/01 $5.00 Copyright © 2001 the American Physiological Society
influences of Aldo play a dominant role in perpetuating the CHF syndrome.

METHODS

Animals

Adult male Sprague-Dawley rats (3–4 mo old), weighing 300–325 g, were obtained from Harlan Sprague Dawley, Indianapolis, IN. They were housed in temperature-controlled (23 ± 2°C) and light-controlled (lights on between 0700 and 1900) animal quarters and were provided with rat chow ad libitum. The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

All rats were subjected to three or four survival surgeries and one echocardiography session. After each procedure, animals recovered from anesthesia under observation in the laboratory before returning to the metabolic cages. Surgery was performed using sterile technique, and animals were treated for postoperative pain with buprenorphine (0.1 mg/kg sc) initially 12 h later and then as needed.

Procedures

 Intracerebroventricular cannula implantation. Rats were anesthetized with Equithesin-like anesthetic cocktail (consisting of 0.97 g pentobarbital sodium and 4.25 g chloral hydrate/100 ml distilled water) at a dose of 0.33 ml/100 g body wt. The animals were secured in a Kopf stereotaxic apparatus with the skull leveled between the bregma and lambda. A stainless steel cerebral 23-gauge cannula (16 mm long) was implanted into the third ventricle using the stereotaxic coordinates of 1.0 mm caudal to bregma, 1.5 mm lateral to midline, and 8.7 mm ventral to the dura. The cannulas were fixed to the cranium using dental acrylic resin and small screws. Metal tubing (30 gauge) was used as an obturator to keep the cannula patent. Animals were allowed to recover for a week and then they were adapted to the metabolic cages.

Induction of CHF or SHAM. Heart failure was induced by occluding the left anterior descending coronary artery. Rats were anesthetized with ketamine + xylazine (90 mg/kg and 10 mg/kg ip, respectively), endotracheally intubated, and mechanically ventilated with room air, respiratory rate 50–55/min, tidal volume 2.5 ml. Under sterile conditions, a left thoracotomy was performed to expose the heart. The pericardium was opened, and the heart was exteriorized. The left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium. The heart was returned to the chest cavity, the lungs were reinfated, and the chest incision was closed. SHAM rats were treated in the same manner, but did not undergo coronary artery occlusion. After completion of the surgical procedures, rats were removed from the ventilator and extubated. Posturgical animals were given benzathine penicillin (30,000 units im) and lidocaine (2 mg im every 4 h for two doses).

Implantation of osmotic minipumps and infusion of drugs. SL (Sigma) was dissolved in absolute ethanol and diluted with sterile water (Sigma) to a final concentration of 0.4 mg/ml. The final ethanol concentration used in ethanol vehicle (VEH)-treated animals was 0.2% ethanol in 1-μl volume. Alzet miniosmotic pumps (model 2004, Alza; Palo Alto, CA) were filled with SL or VEH and attached to a flow moderator to obtain a continuous infusion of the drug. The pumps had an average flow rate of 0.25 μl/h and the final dose of drug was 100 ng/h infused for a period of 28 days. In previous studies by others, this dose of SL administered intracerebroventricularly (icv) for 12 days altered spatial learning (29) in rats; a single injection of 100 ng icv has also been shown to affect neuroendocrine function (21) and behavior (16).

At least 36 h before the intracerebroventricular infusion, the minipumps attached to Silastic tubing were filled with SL or its vehicle and then placed in sterile 0.9% saline at 37°C to ensure a constant pumping rate at the time of implantation. The pumps were placed subcutaneously behind the neck. The obturator was removed from the intracerebroventricular cannula, and the free end of the Silastic tubing was connected to the cannula and secured using dental acrylic. Intraperitoneal (ip) infusion of the same dose of SL was accomplished by implanting a minipump directly into the peritoneal cavity via a linea alba incision. In this case, the pumps were primed for only 4 h before implantation. The difference in priming time is related to differences in quantity of ambient fluid bathing the pump at the two implantation sites, per manufacturer guidelines.

Preparation for conscious sympathetic nerve recording and baroreflex testing. With the animal under pentobarbital anesthesia (60 mg/kg ip), the left femoral artery and vein were cannulated, and the catheters were tunneled to the back of the neck. The left kidney was exposed via a flank incision. A branch of the renal nerve was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve were covered with Presidant light dental acrylic (Coltene), and the electrodes were sutured to back muscle and then tunneled to the back of neck.

General Protocol

Animals were adapted to a metabolic cage environment for a period of 2 wk. During this time, they had ad libitum access to food, water, and 1.8% NaCl, and a normal 12:12-h light-dark cycle was maintained. These conditions were then continued for the duration of the study protocol. Weekly measurements were made of food, free water, and 1.8% NaCl intake, urine volume, and body weight. Urine samples were collected for the analysis of sodium content. At the end of the first week (week 0), after baseline samples were collected, animals were subjected to coronary artery ligation (CL) to induce CHF or identical surgery without CL (SHAM). The presence or absence of left ventricular dysfunction was ascertained by echocardiography within 24 h after CL. Alzet pumps were implanted within 24–36 h after CL for either intracerebroventricular or intraperitoneal infusions of SL (100 ng/h). After the metabolic cage protocol was completed, animals underwent sympathetic nerve recording and baroreflex testing in the conscious state.

Data Acquisition

Echocardiography. Echocardiography was performed using an Acuson (Mountain View, CA) Sequoia clinical imager fitted with a 5-MHz sector-array probe, which generates two-dimensional images at a rate of ~100/s. Animals were sedated with ketamine (25 mg/kg ip) to facilitate positioning for echocardiographic study. The anterior chest was shaved, and prewarmed acoustic coupling gel was applied. The animal was positioned in the left lateral recumbent position to optimize the windows for echocardiography. The probe was applied gently to the chest. Short-axis images were acquired parallel to the mitral valve plane to obtain the largest cross-sectional image of the left ventricle that does not contain the mitral valve. Long-axis views were obtained perpendicular to the mitral valve plane and were deemed optimal when the diastolic apex-to-base length was longest and when both
mitral and aortic valves were visible. Pulse-wave Doppler tracings were obtained with gates placed so as to interrogate mitral inflow. Images were written to a magneto-optical disk for subsequent off-line analysis.

Analysis of the stored images was performed off-line using proprietary software developed specifically for this purpose by the vendor (Acuson). Heart rate (HR) was determined from the Doppler tracings. Left ventricular end-diastolic volume, left ventricular end-systolic volume, left ventricular ejection fraction, left ventricular stroke volume, and left ventricular mass were computed using the area-length method (11). The portion of the left ventricle that displayed akinesis was planimetered electronically and expressed as a percentage of the total left ventricle silhouette to estimate the size of the ischemic zone. Only animals with large infarctions (ischemic zone ≥35%; range 35–66%) were used in the study.

Metabolic cage assessment of fluid balance. Ingestion of food, water, and 1.8% NaCl, body weight, urine volume, and urinary sodium content were measured twice weekly for two consecutive 24-h periods and an average value for each variable was reported for that time point. Urine sodium was measured using a sodium and potassium analyzer (NOVA Biomedical; Waltham, MA).

 Conscious sympathetic nerve recording and baroreflex testing. Sympathetic nerve recordings were made in the conscious, freely mobile state 4 h after recovery from anesthesia to implant bipolar electrodes on the renal nerve. The externalized recording electrodes were connected to a Grass P511 AC amplifier to record renal sympathetic nerve activity (RSNA). The externalized femoral artery catheter was connected to a strain-gauge transducer to record arterial pressure. HR was derived by computer analysis of the arterial pressure pulse frequency. After 20 min of stabilization, baseline arterial pressure, HR, and RSNA were recorded for 15 min. Baroreflex testing was performed using bolus intravenous injections of phenylephrine (2–10 μg/kg) and sodium nitroprusside (5–20 μg/kg). Arterial pressure and RSNA returned to baseline between interventions.

RSNA was passed to a Paynter filter (20-ms time constant; Bak Electronics; Germantown, MD) to rectify and integrate the raw signal. Arterial pressure, integrated RSNA, and windowed RSNA [counted spikes/s exceeding a voltage selected by a window discriminator (model DIS-1, Bak Electronics)] were passed to a CED 1401 Laboratory Interface (Cambridge Electronic Design; Cambridge, UK) linked to a personal computer. The integrated RSNA signal was further analyzed to determine the burst frequency (bursts/s) as an additional measure of sympathetic activity. A burst of RSNA activity was defined by the nadir of RSNA on either side, as identified by the computer on the basis of operator-assigned parameters. The raw RSNA signal and arterial pressure were also recorded on video cassette recorder tape using a PCM Recording Adaptor (A. R. Vetter; Rebersburg, PA) for analysis off-line.

Baseline values for HR, mean arterial pressure (MAP), windowed RSNA, integrated RSNA, and bursts of RSNA per second were determined for five sequential 2-min intervals, and those values were averaged to obtain a single value for each variable. Data from baroreflex testing were analyzed in 1-s time bins to construct baroreflex curves comparing responses to phenylephrine and sodium nitroprusside with steady-state control values over the 20 s before each test. A nonlinear regression program (SigmaPlot 4.01) was used to analyze the baroreflex curve: \( y = y_0 + a/(1 + \exp(-(x - x_0)/b)) \), where \( x \) is MAP; \( y \) is ΔHR or ΔRSNA; \( a \) is the range of ΔHR or ΔRSNA; \( b \) is the slope coefficient; \( x_0 \) is MAP at the midpoint range of ΔRSNA or ΔHR; and \( y_0 \) is the minimum of ΔRSNA or ΔHR. In each rat, raw data of MAP, ΔHR, and ΔRSNA were fit to the logistic function to generate parameters \( a, b, x_0, \) and \( y_0 \). The maximum gain of the baroreflex curve was defined as \( a/b \).

Measurement of left ventricular end-diastolic pressure. After sympathectomized recordings were completed, the animals were anesthetized with pentobarbital (50 mg/kg ip), the right carotid artery was exposed, and a PE-50 cannula attached to a pressure transducer was advanced through the carotid artery across the aortic valve into the left ventricular chamber. Pressure was recorded continuously while the cannula was positioned at a site within the left ventricle at which left ventricular pressure could be accurately recorded (the onset of the rapid rise in left ventricular pressure after atrial contraction could be observed) and left ventricular systolic pressure was not higher than aortic pressure on entering the left ventricle (there was no evidence of ventricular outflow obstruction by the cannula). Left ventricular pressure was then recorded for an interval of 2 min. A single left ventricular end-diastolic pressure value was determined by applying a horizontal cursor across the visually estimated end-diastolic pressure of five sequential left ventricular pressure wave forms.

Anatomical assessment of heart failure. At the conclusion of the protocol, the hearts were arrested under anesthesia, and the heart and lungs were removed for examination. The presence or absence of ischemic injury, as indicated by left ventricle scarring, was determined by visual inspection. The heart-to-body weight and lung-to-body weight ratios were determined.

Verification of cannula placement and minipump function. At the end of the study, brains were removed to check the site of cannula implantation. Only animals with the cannula in the third ventricle region were used in the study. 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

Each value is expressed as mean ± SE. Changes in salt intake, water intake, urine volume, and urinary sodium among the four groups were analyzed by two-way repeated-measures ANOVA followed by post hoc Fisher’s least squares difference test. A nonlinear regression program (SigmaPlot, Jandel Scientific) was used to analyze the components defining the individual sigmoid curve fits of the baroreflex data (described above), and these values were averaged to construct representative baroreflex curves for each group relating changes in HR and RSNA to changes in MAP. The baseline values of RSNA, MAP, and HR and the range and maximal gain (\( G_{max} \)) values obtained from the individual baroreflex curve fits were analyzed using a one-way ANOVA followed by a Student’s \( t \)-test, with differences considered significant at \( P < 0.05 \).

RESULTS

Survival

Surgical mortality within 24 h of surgery was 30% in rats undergoing CL and 5% in rats undergoing SHAM CL. Among the intracerebroventricularly treated rats, 15 of 16 SHAM and 17 of 24 CHF rats completed the study protocol; among the intraperitoneally treated rats, 15 of 16 SHAM and 15 of 20 CHF rats completed the study protocol.
Characteristics of Study Groups

Compared with SHAM rats, CHF rats assigned to the two treatment regimens (intracerebroventricular or intraperitoneal) had reduced left ventricular ejection fraction and increased left ventricular end-diastolic volume (LVEDV, ml). These parameters were not different for congestive heart failure (CHF) rats or SHAM rats assigned to intracerebroventricular (icv; A) or intraperitoneal (ip; B) treatment. *P < 0.05, CHF vs. SHAM. SL, spironolactone; VEH, ethanol vehicle.

Volume Regulation

Behavioral effects. Sodium appetite (ingestion of hypertonic saline solutions) and thirst (water drinking) are behavioral responses to activation of volume regulatory centers in the central nervous system (4, 13, 14). Blood-borne ANG II activates AT1 receptors in forebrain structures to increase thirst and sodium appetite, and blood-borne Aldo activates MC receptors in amygdala to induce sodium appetite. Central osmoreceptors also influence these processes.

Figure 2 shows the effects of VEH versus SL treatment on sodium ingestion in the CHF and SHAM rats. Data from the intracerebroventricularly treated animals are shown in Fig. 2A. In the SHAM-VEH group, consumption of the 1.8% NaCl solution did not change over the duration of the protocol. In contrast, in the CHF-VEH rats, ingestion of 1.8% NaCl had increased within the first week after CL and remained high for the remainder of the protocol. As might be anticipated from the known effects of central MC receptors in normal animals, intake of 1.8% NaCl was reduced in the SHAM-SL group (at weeks 2 and 3). Notably, intake of 1.8% NaCl was reduced in the CHF-SL rats to a comparable level, but this was a striking reduction when compared with the CHF-VEH group. This reduction in sodium intake had already occurred when the first measurement was taken in week 1, and sodium intake in the CHF-SL group remained below that of both CHF-VEH and SHAM-VEH throughout the 4-wk protocol.

Data from the intraperitoneally treated rats are shown in Fig. 2B. Again, in the SHAM-VEH group,

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR, beats/min</th>
<th>LV Mass, g</th>
<th>LVEDV/LV Mass, ml/g</th>
<th>Cardiac Output, ml/min</th>
<th>Infarct Zone, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM-VEH</td>
<td>8</td>
<td>376.2 ± 7.3</td>
<td>0.823 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>116.9 ± 3.0</td>
<td>0</td>
</tr>
<tr>
<td>SHAM-SL</td>
<td>7</td>
<td>384.3 ± 12.8</td>
<td>0.85 ± 0.06</td>
<td>0.42 ± 0.03</td>
<td>115.5 ± 9.3</td>
<td>0</td>
</tr>
<tr>
<td>CHF-VEH</td>
<td>8</td>
<td>391.6 ± 21.1</td>
<td>0.934 ± 0.09</td>
<td>1.16 ± 0.19*</td>
<td>87.3 ± 7.3</td>
<td>49.57 ± 4.77*</td>
</tr>
<tr>
<td>CHF-SL</td>
<td>9</td>
<td>370.7 ± 13.5</td>
<td>0.937 ± 0.09</td>
<td>1.22 ± 0.11*</td>
<td>98.5 ± 12.1</td>
<td>46.63 ± 2.95*</td>
</tr>
<tr>
<td>IP Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM-VEH</td>
<td>7</td>
<td>384.4 ± 30.6</td>
<td>0.854 ± 0.06</td>
<td>0.44 ± 0.03</td>
<td>109.2 ± 8.9</td>
<td>0</td>
</tr>
<tr>
<td>SHAM-SL</td>
<td>7</td>
<td>387.9 ± 10.4</td>
<td>0.835 ± 0.03</td>
<td>0.42 ± 0.04</td>
<td>113.1 ± 4.5</td>
<td>0</td>
</tr>
<tr>
<td>CHF-VEH</td>
<td>7</td>
<td>374.4 ± 22.1</td>
<td>0.975 ± 0.06</td>
<td>0.82 ± 0.07*</td>
<td>93.70 ± 6.6</td>
<td>47.43 ± 3.4*</td>
</tr>
<tr>
<td>CHF-SL</td>
<td>8</td>
<td>376.1 ± 21.4</td>
<td>0.915 ± 0.08</td>
<td>0.86 ± 0.18*</td>
<td>101.2 ± 18.1</td>
<td>45.54 ± 5.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. HR, heart rate; LV, left ventricular; LVEDV, LV end-diastolic volume; ICV, intracerebroventricular; IP, intraperitoneal; VEH, ethanol vehicle; SL, spironolactone; CHF, congestive heart failure. *P < 0.05 compared with SHAM groups.
sodium intake did not change during the study. CHF-VEH had the anticipated increased sodium intake, beginning at week 1 and lasting throughout the 4-wk protocol. Interestingly, the SHAM-SL rats had a reduction in sodium intake at weeks 1 and 2 compared with the SHAM-VEH group, suggesting an early central influence of the intraperitoneally administered SL. The CHF-SL rats had an early increase in intake of 1.8% NaCl in week 1, like the CHF-VEH rats, but this normalized to the level of SHAM-VEH by week 2.

As shown in Figure 3, free water intake was not different in any of the treatment groups at baseline and only minor and inconsistent variations from SHAM-VEH were seen over the duration of the study protocol. There were no significant differences between the CHF-VEH and SHAM-VEH groups. These data should be considered in conjunction with the data regarding the ingestion of 1.8% sodium chloride (Fig. 2), which might contribute small quantities (1–2 ml, <10%) to the total water intake.

Renal effects. Changes in renal handling of sodium and water effected by SL may be mediated by local renal MC receptors or indirectly by MC receptor influences on central nervous system mechanisms regulating sympathetic drive (4, 9, 20) and arginine vasopressin release (10, 24).

Figures 4 and 5 show the effects of VEH versus SL treatment on renal retention of sodium and water in the CHF and SHAM rats. Data for the intracerebroventricularly treated animals are shown in the left panels. Both urine sodium (Fig. 4) and urine volume (Fig. 5) were substantially reduced early in the CHF-VEH rats week 1 after CL. Intracerebroventricular treatment with SL prevented these reductions in urine

Fig. 2. Ingestion of 1.8% saline (ml/day) solution in CHF and SHAM rats treated with intracerebroventricular (A) or intraperitoneal (B) SL or VEH. The VEH-treated SHAM rats in both treatment groups had no change in sodium intake. The VEH-treated CHF rats in both groups had increased sodium intake throughout the protocol. Intracerebroventricular SL treatment (A) reduced sodium intake in both SHAM and CHF rats. Intraperitoneal SL (B) reduced sodium intake in the CHF rats in the third week of treatment. *P < 0.05 vs. SHAM-VEH; †P < 0.05 CHF-VEH vs. CHF-SL. Time 0, baseline values before CL. n = No. of rats.

Fig. 3. Ingestion of water (ml/day) in CHF and SHAM rats treated with intracerebroventricular (A) or intraperitoneal (B) SL or VEH. Water intake was not different in any of the treatment groups at baseline and these were minor and inconsistent differences from SHAM-VEH over the duration of the study protocol. n = No. of rats.
sodium and urine volume; in fact, these values were higher in the SHAM-VEH than the CHF-SL rats. Urine sodium and urine volume were not affected by SL treatment in the SHAM rats.

Data for the intraperitoneally treated animals are shown in the right panels. Again, the CHF-VEH rats had a substantial reduction in urine sodium (Fig. 4) and urine volume (Fig. 5). The CHF-SL rats also had reduced urine sodium and urine volume in weeks 1 and 2 after CL, but there was normalization of urine sodium and urine volume in weeks 3 and 4, with urine volume in CHF-SL exceeding that in SHAM-VEH at week 3. There was no change from baseline in either variable during the protocol in the SHAM-VEH rats. The SHAM-SL rats did not differ from SHAM-VEH rats.

Sympathetic Drive and Baroreflex Regulation

Baseline sympathetic nerve activity. Measurements of sympathetic nerve activity were made in conscious rats after 4 wk of intracerebroventricular or intraperitoneal SL or VEH, at a time when SL treatment by either route had normalized the indices of volume regulation. SL has known central (9) and peripheral (26) effects on sympathetic regulation.

Figure 6 shows representative tracings from two CHF rats, one treated with intracerebroventricular VEH (A) and the other with intracerebroventricular SL (B). MAP is similar in the two rats, but the rectified and integrated RSNA is substantially less in the CHF-SL rat. A rate meter recording of windowed spike frequency of RSNA is also shown; this measurement
was used for determination of changes in RSNA during baroreflex testing (see below) in individual animals not for across-group comparisons.

Analysis of the group data indicated that HR was higher ($P < 0.05$) in the CHF-VEH than the SHAM-VEH rats in both treatment groups (ip: 443 ± 13 vs. 417 ± 12 beats/min; icv: 447 ± 10 vs. 412 ± 14 beats/min). SL had no effect on HR in the SHAM rats, but HR was lower in the CHF-SL rats in both treatment groups (ip: 423 ± 10 vs. 443 ± 13 beats/min; icv: 421 ± 13 vs. 447 ± 10 beats/min). MAP tended to be lower in the CHF-VEH than the SHAM-VEH rats in both treatment groups (ip: 104.7 ± 7.1 vs. 116.4 ± 5.3 mmHg, $P = 0.073$; icv: 107.6 ± 5.1 vs. 121.0 ± 6.3 mmHg, $P = 0.058$), but these changes did not achieve statistical significance. MAP tended to be higher in the CHF groups treated with SL by either route (ip: 112.5 ± 4.4 vs. 104.7 ± 7.1 mmHg, $P = 0.082$; icv: 114.3 ± 7.1 vs. 107.6 ± 5.1 mmHg, $P = 0.084$), but these trends were also not statistically significant.

Although the validity of comparisons of sympathetic nerve activity across groups of animals is debatable, our data analysis indicates that integrated RSNA was significantly higher ($P < 0.05$) in the CHF-VEH than the SHAM-VEH rats (ip: 15.7 ± 0.7 vs. 11.8 ± 0.8 mV; icv: 15.6 ± 0.4 vs. 11.1 ± 0.6 mV). Treatment with SL by either route had no effect on integrated RSNA in the SHAM rats. In contrast, CHF rats treated with SL by either route had lower ($P < 0.05$) RSNA (ip: 12.1 ± 0.6 vs. 15.7 ± 0.7 mV; icv: 11.7 ± 0.4 vs. 15.6 ± 0.4 mV).

Burst frequency within the integrated voltage signal was also higher ($P < 0.05$) in the CHF than the SHAM rats (ip: 6.7 ± 0.2 vs. 4.7 ± 0.2 bursts/s; icv: 6.8 ± 0.4 vs. 4.4 ± 0.2 bursts/s) and was lower ($P < 0.05$) in the CHF rats treated with SL by either route (ip: 5.4 ± 0.3 vs. 6.7 ± 0.2 burst/s; icv: 5.3 ± 0.4 vs. 6.8 ± 0.4 bursts/s). In the SHAM rats, burst frequency was not affected by either route of SL administration.

The baseline windowed RSNA (spikes/s) was higher ($P < 0.05$) in the CHF-VEH than the SHAM-VEH rats (ip: 207.9 ± 7.3 vs. 157.8 ± 6.0 spikes/s; icv: 204.8 ± 7.8 vs. 150.8 ± 5.1 spikes/s). Windowed RSNA was not different in the SHAM-SL and SHAM-VEH rats. In contrast, windowed RSNA was lower ($P < 0.05$) in the CHF rats treated with SL by either route (ip: 173.0 ± 6.4 vs. 207.9 ± 7.3 spikes/s; icv: 168.9 ± 7.3 vs. 204.8 ± 7.8 spikes/s).

**Baroreflex responses.** Figure 7 shows the sigmoid curve fits relating changes in HR and RSNA (as spikes/s) to increasing and decreasing arterial pressure with phenylephrine and nitroprusside, respectively. Both intracerebroventricular (Fig. 7, A and C) and intraperitoneal treatment (Fig. 7, B and D) of the CHF rats with SL resulted in improvement of the blunted baroreflex regulation of HR and RSNA. In both cases, however, baroreflex function remained blunted compared with the SHAM rats. SL treatment had no effect on the baroreflex in the SHAM groups.

As shown in Fig. 8, $G_{max}$ and range values derived from the logistic curve fits for HR and RSNA were diminished in the CHF rats compared with the SHAM controls. SL treatment improved $G_{max}$ and range for HR and RSNA in the CHF rats, but did not restore them to normal (SHAM-VEH) levels. SL treatment had no effect on $G_{max}$ and range for HR or RSNA in SHAM rats.

**Left Ventricular End-Diastolic Pressure**

Left ventricular end-diastolic pressure was checked under anesthesia just before euthaniasia. Left ventricular end-diastolic pressure was higher ($P < 0.05$) in the CHF-VEH than in the SHAM-VEH rats in both treatment groups (ip: 18.7 ± 3.0 vs. 5.1 ± 1.8 mmHg; icv: 17.8 ± 2.0 vs. 4.9 ± 1.2 mmHg). Treatment with SL had no significant effect on left ventricular end-diastolic pressure in CHF or SHAM rats, although there was a trend for left ventricular end-diastolic pressure to be somewhat lower in the SL-treated CHF rats (ip: 15.1 ± 3.0, $P = 0.187$; icv: 13.6 ± 1.9; $P = 0.118$).

**Anatomical Assessment**

There was no difference in body weight between the CHF-VEH vs. SHAM-VEH rats in either treatment group (ip: 326.7 ± 8.7 vs. 320.8 ± 16.9 g; icv: 304.8 ± 14.9 vs. 322.0 ± 7.1 g). Treatment with SL by either route had no effect on body weight in the SHAM or the CHF rats. Heart weight-to-body weight ratio was higher ($P < 0.05$) in the CHF-VEH than the SHAM-VEH rats in both treatment groups (ip: 5.4 ± 0.2 vs. 4.0 ± 0.2; icv: 5.6 ± 0.4 vs. 3.9 ± 0.2) as was the lung weight-to-body weight ratio (ip: 10.4 ± 0.4 vs. 7.4 ± 0.5; icv: 11.2 ± 0.9 vs. 7.3 ± 0.6). Compared with CHF-VEH, intracerebroventricularly treated CHF-SL rats had reduced ($P < 0.05$) lung weight-to-body weight ratio (11.2 ± 0.9 vs. 8.5 ± 0.5), and there was a trend ($P = 0.12$) toward decreased heart weight-to-body weight ratio (4.8 ± 0.2). With intraperitoneal SL treatment of the CHF rats, there was a...
trend toward lower lung weight-to-body weight ratio (8.9 ± 1.2, \(P = 0.27\)) and heart weight-to-body weight ratio (5.0 ± 0.4, \(P = 0.39\)) that did not reach statistical significance.

DISCUSSION

This study reports the novel finding that MC receptors in the central nervous system play a dominant role in the pathophysiology of ischemia-induced heart failure in the rat. Blockade of central MC receptors has a pronounced salubrious influence on the derangements of volume regulation and sympathetic drive that lead to overt heart failure after myocardial injury.

MC receptors are present in numerous tissues in which SL might induce an improvement in the manifestations of CHF by blocking the effects of circulating or intrinsically produced Aldo (3, 7, 15). One important site of Aldo action, which has received little attention in the heart failure literature, is the brain. In normal animals, activation of MC receptors in the forebrain (1, 5) induces both behavioral and physiological effects (13, 14) that favor volume expansion and complement its direct influences on the kidneys (5, 20, 23, 27, 28). Aldo stimulates sodium appetite through its actions on MC receptors in medial amygdala (31), increases the binding of ANG II to \(AT_1\) receptors in the subfornical organ that mediate drinking behavior (6), and increases the production of AVP in magnocellular neurons of paraventricular nucleus and supraoptic nucleus, promoting free water retention. Aldo also acts on central MC receptors to increase sympathetic drive (9) and to increase the dipsogenic and pressor responses to the central actions of ANG II (28).

The global systemic impact of central MC receptor activation on the progression of CHF is demonstrated here for the first time. The role of central MC receptors was selectively examined by delivering SL directly into the third cerebral ventricle in the immediate vicinity of these forebrain structures that regulate fluid balance and sympathetic drive (4, 14). The immediate and
dramatic beneficial effects of intracerebroventricular SL on volume regulation were not observed with intraperitoneal infusion of the same dose of SL. The increase in sodium ingestion and the renal retention of sodium and water seen early after CL in the VEH-treated CHF rats were abolished. Both the behavioral response and the renal response to intracerebroventricular SL can be explained by altered central MC receptor activity. In a recent acute study in conscious rats (20), intracerebroventricular bolus injection of a selective MC receptor antagonist lowered arterial pressure and induced a natriuresis and diuresis dependent on intact renal nerves. These findings are consistent with ours, and suggest the beneficial effects of central MC blockade on renal sodium and water handling may be largely due to a reduction in RSNA originating in the brain. However, our findings do not exclude an additional potential contributing influence of reduced AVP (antidiuretic hormone) production and release secondary to central MC receptor blockade.

To our knowledge, only one other study (2) has addressed the potential mechanism of the beneficial effects of SL in the rat model of ischemic heart failure. That study examined the effects of chronic oral treatment with SL alone or in combination with an angiotensin-converting enzyme inhibitor on urine output and found a diuretic effect of combination treatment but no independent effect of SL. In that study, SL was administered in the drinking water; because SL is not soluble in water, and no independent measure of adequacy or effectiveness of the SL dosing was described, the negative finding for SL alone is difficult to interpret. In addition, the assessment of urine output in that study was made over a single 24-h period after 12 wk of SL or combination therapy. At the latest time point (week 4) in our study, both urine sodium and urine output had returned to control (SHAM-VEH) values in the animals treated systemically with SL. Thus, after 4 wk of treatment, SL had induced a normalization of renal sodium and water handling in CHF rats—in effect, a central “diuretic” effect.

Although the significance of the Aldo component of the altered renin-angiotensin-Aldo system in established CHF was brought into stark perspective by the recently published Randomized Aldactone Evaluation Study trial (19), the influence of SL at earlier stages of CHF has not been clinically tested. With regard to that issue, our finding that centrally administered SL interferes with the regulatory mechanisms promoting volume retention early after myocardial infarction may have important clinical implications. A testable hypothesis that emerges from this study is that early MC receptor blockade in the clinical setting of acute myocardial infarction may slow the progression from left ventricular dysfunction to overt heart failure.

In the present study, a single dose of SL was chosen for continuous infusion based on previous work demonstrating that intracerebroventricular administration of this dose effected changes in central endocrine systems (16, 21, 29); the same dose was administered systemically as a control. Interestingly, the intraperitoneal dose decreased sodium appetite in the SHAM rats within the first week, suggesting a central nervous system effect of the intraperitoneally administered SL in these animals with presumably normal circulating levels of Aldo. In contrast, a significant effect on the CHF rats, with presumably much higher circulating levels of Aldo, was not seen until week 3. The lack of effect of the systemically administered SL in the first 2 wk of the study suggests a central site of action of the intracerebroventricular SL in CHF rats. The fact that volume regulation in the intraperitoneally treated CHF-SL “control” group resembled the intracerebroventricularly treated CHF-SL group by protocol week 3 suggests that the intraperitoneally administered SL ultimately bound to a sufficient number of central MCs.
nervous system MC receptors to effect a response. The observation that sodium appetite normalized at the same late time point as renal sodium and water excretion supports that interpretation. The pharmacokinetics of SL in CHF (12) with the prolonged plasma half-life of its active metabolites and the slow induction of cellular responses by activation of cytosolic MC receptors (23) may contribute to this delayed effect of the systemically administered drug.

The reduction in RSNA in the intraperitoneally treated CHF-SL rats 4–5 wk after CL provides further support for the view that SL exerts its effects predominantly via a neural action. A reduction in intravascular volume induced by a renal diuretic effect of MC receptor blockade might result in an increase of sympathetic drive, but a decrease implies a central inhibition of sympathetic outflow. Such an effect might result from the gradual accumulation of the active metabolites of SL at central MC receptors regulating sympathetic drive through mechanisms already suggested or, alternatively, from blockade of MC receptors at peripheral baroreceptor endings where Aldo has been shown to blunt baroreceptor afferent activity (26). In the present study, overall baroreflex regulation was improved to a similar degree after 4 wk of treatment whether SL was administered centrally or intraperitoneally. Although the specific site of action of SL within the baroreflex pathway was not determined, the equal effectiveness of the intracerebroventricular SL treatment suggests a central nervous system mechanism. However, because baroreflex testing was done late in the experimental protocol, we cannot exclude a leak from cerebrospinal fluid into the peripheral circulation.

Finally, the potential effects of central and peripheral administration of SL on myocardial function must be considered in the interpretation of these results. Recent studies have demonstrated that activation of MC receptors in myocardium by Aldo induces myocardial fibrosis that can be prevented by treatment with SL (3, 15). It is conceivable that the administration of SL early after CL reduced these adverse effects of Aldo on heart muscle, leading to less impairment of cardiac function and better tissue perfusion in the SL-treated animals. However, the similarity of the late responses to the intracerebroventricular and intraperitoneal SL treatments in the CHF groups, and the very small dose of intraperitoneally administered SL would seem to argue against that possibility. Another potential mechanism by which SL might benefit left ventricular function in CHF is by lowering systemic vascular resistance as a result of the reduction in sympathetic drive. On the basis of the findings of the present study, the possibility that treatment with SL improves left ventricular function sufficiently to alter neurohumoral drive and thus the development of overt heart failure warrants further study.

In summary, the present study demonstrates a dramatic role for MC receptors within the central nervous system in the pathophysiology of CHF. The magnitude of this central influence of the MC component of the renin-angiotensin-Aldo system in CHF was previously unrecognized. In the rat model of ischemia-induced heart failure, the central regulatory mechanisms sensitive to MC receptor blockade are activated early in heart failure. Our results argue strongly that early intervention to block these receptors may have a significant impact on the onset and subsequent progression of the heart failure syndrome.

Support for this project was provided by a Veterans Administration Merit Review (to R. B. Felder), National Heart, Lung, and Blood Institute Grant RO1-HL-63915 (to R. B. Felder), American Heart Association Grant-in-Aid 96-010430 (to R. M. Weiss), and National Heart, Lung, and Blood Institute Cardiovascular Interdisciplinary Research Fellowship Grant HL-07121 (to J. Francis).

REFERENCES


