

Central mineralocorticoid receptor blockade decreases plasma TNF- α after coronary artery ligation in rats

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Francis, Joseph, Robert M. Weiss, Alan Kim Johnson, and Robert B. Felder. Central mineralocorticoid receptor blockade decreases plasma TNF- α after coronary artery ligation in rats. *Am J Physiol Regul Integr Comp Physiol* 284: R328–R335, 2003; 10.1152/ajpregu.00376.2002.—The Randomized Aldactone Evaluation Study (RALES) demonstrated a substantial clinical benefit to blocking the effects of aldosterone (Aldo) in patients with heart failure. We recently demonstrated that the enhanced renal conservation of sodium and water in rats with heart failure can be reduced by blocking the central nervous system effects of Aldo with the mineralocorticoid receptor (MR) antagonist spironolactone (SL). Preliminary data from our laboratory suggested that central MR might contribute to another peripheral mechanism in heart failure, the release of proinflammatory cytokines. In the present study, SL (100 ng/h for 21 days) or ethanol vehicle (Veh) was administered via the 3rd cerebral ventricle to one group of rats after coronary ligation (CL) or sham CL (Sham) to induce congestive heart failure (CHF). In Veh-treated CHF rats, tumor necrosis factor- α (TNF- α) levels increased during *day 1* and continued to increase throughout the 3-wk observation period. In CHF rats treated with SL, started 24 h after CL, TNF- α levels rose initially but returned to control levels by *day 5* after CL and remained low throughout the study. These findings suggest that activation of MR in the central nervous system plays a critical role in regulating TNF- α release in heart failure rats. Thus some of the beneficial effect of blocking MR in heart failure could be due at least in part to a reduction in TNF- α production.

myocardial infarction; tumor necrosis factor; aldosterone; spironolactone

INTEREST IN ALDOSTERONE (Aldo), a product of renin-angiotensin-aldosterone system activation in heart failure, has escalated (20) since the publication of the Randomized Aldactone Evaluation Study (RALES), which showed that heart failure patients treated with the mineralocorticoid receptor (MR) antagonist spironolactone (SL) had substantially decreased mortality and morbidity (56). The mechanism responsible for this beneficial effect of MR blockade was not determined, but it was not thought to be due to the well-known diuretic effect of SL. Aldo is present in high concentrations in patients with heart failure despite standard

treatment (55), and high concentrations of Aldo have detrimental effects on the heart (7), blood vessels (22), and kidneys (62).

Aldo also has central nervous system actions (38, 58, 65, 66, 73) that may promote the progression of heart failure. Aldo acts on central MR (1, 72) to increase sodium appetite (16), sympathetic drive (38), the production and release of arginine vasopressin (66), and binding of angiotensin II to its receptors (17). We recently demonstrated that blocking MR in the central nervous system of rats with ischemia-induced heart failure attenuated fluid accumulation and sympathetic drive, indicating that at least some of the adverse effects of circulating Aldo are mediated at a central nervous system level (32). Preliminary data (31) from our laboratory suggest yet another adverse influence of the central actions of Aldo in heart failure: stimulation of the production of proinflammatory cytokines.

Myocardial infarction (35) and heart failure (27) are characterized by high circulating levels of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), markers of immune system stimulation by injury, inflammation, or infection. Circulating pro-inflammatory cytokines activate the hypothalamic-pituitary-adrenal axis (13, 21, 23, 46, 61, 70). Sympathetic drive (63, 64) and the release of arginine vasopressin and corticosterone are increased. Because the pro-inflammatory cytokines and Aldo act on neurons in the same region of the brain to elicit similar peripheral responses, it is reasonable to consider central interactions that might regulate the activity of these two mediators.

We hypothesized that circulating Aldo, acting at the forebrain level, might alter the production and release of TNF- α . In the present studies, blood-borne TNF- α was measured in rats with ischemia-induced heart failure treated with a centrally administered MR antagonist or vehicle. The results suggest a novel influence of Aldo to promote cytokine production in heart failure.

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METHODS

Animals

These studies were performed in accordance with the American Physiological Society's "Guiding principles for research involving animals and human beings." 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), weighing 300–325 g, were obtained from Harlan Sprague Dawley, Indianapolis, IN. They were housed in temperature-controlled ($23 \pm 2^\circ\text{C}$) and light-controlled (lights on between 0700 and 1900) animal quarters and were provided with rat chow ad libitum.

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

Procedures

Intracerebroventricular cannula implantation. The method for cannula implantation has been described before (32). Briefly, rats were anesthetized with an Equithesin-like anesthetic cocktail (consisting of 0.97 g pentobarbital sodium and 4.25 g of chloral hydrate/100 ml distilled water) at a dose of 0.33 ml/100g body wt and were secured to a stereotaxic apparatus with skull leveled between bregma and lambda. A stainless steel 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 below the dura, with the cannula directed at a 10° angle toward the midline. The cannulas were fixed to the cranium using dental acrylic and small screws. Metal tubing (30 gauge) was used as an obturator to keep the cannula patent.

Jugular catheterization. The procedure for jugular catheterization has been described before (33). Briefly, while the animals were under ketamine-xylazine (90 mg/kg and 10 mg/kg ip, respectively) anesthesia, a midline cervical incision was made and the jugular vein was isolated by blunt dissection. A Silastic catheter (Dow Corning, Midland, MD) was inserted into the vein and held in position by sutures. The free end of the catheter was externalized to the base of the skull. The catheter was flushed every day with bacteriostatic saline containing heparin (100 U/ml) and sealed with a piece of blunt steel tubing to prevent clogging.

Induction of congestive heart failure or sham coronary ligation. Heart failure was induced by the coronary artery ligation (CL) technique as described before (29, 32, 33). Briefly, 2 wk after the implantation of the 3rd ventricular cannula, heart failure was induced by ligating the left anterior descending (LAD) coronary artery. Under ketamine-xylazine anesthesia (90 and 10 mg/kg ip, respectively), animals were endotracheally intubated and mechanically ventilated and the LAD was ligated between the pulmonary outflow tract and the left atrium. Sham-ligated rats (Sham) underwent the same surgery but did not undergo coronary ligation. The heart was returned to the chest cavity, the lungs reinflated, and the chest incision closed. Postsurgically, animals were given benzathine penicillin (30,000 units IM) and buprenorphine (0.1 mg/kg sc) immediately after surgery and 12 h later.

Measurements of TNF- α Levels

Blood collection. Blood (0.75 ml) was collected into chilled EDTA tubes on the day before CL and subsequently on days 1, 3, 5, 7, 14, and 21 after CL. The blood samples were centrifuged at 4°C , and the plasma samples were separated and stored at -70°C until assayed for TNF- α . Red blood cells were suspended in an equal volume of heparinized saline and reinfused.

Plasma levels. Plasma TNF- α levels in rats were measured using an ultrasensitive rat TNF- α ELISA kit (Biosource International) according to manufacturer instructions. The details of methodology are described elsewhere (57) with modification. Briefly, a 96-well microplate was coated with an antibody specific for rat TNF- α . Sample (100 μl) was added in duplicate to the microplates and incubated for 2 h and then washed five times. Subsequently, 100 μl of biotinylated anti-TNF- α antibody solution was added and incubated for 45 min and then washed. Streptavidin-horseradish peroxidase conjugate solution (100 μl) was added and incubated for 45 min and washed. Finally, 100 μl of chromagen solution was added and incubated in the dark for 15 min. The reaction was stopped with HCl and read at 450 nm using an ELISA plate reader. Standard curves were made with rat TNF- α as a standard. The minimum detectable concentration of TNF- α was <0.1 $\mu\text{g/ml}$.

Echocardiographic assessment of left ventricular function. Within 24 h after CL or Sham, echocardiography was performed with rats under sedation using ketamine (25 mg/kg ip) to confirm the impairment or preservation of the left ventricular function. Details of this procedure are presented elsewhere (29, 32). Briefly, images were acquired with an Acuson (Mountainview, CA) Sequoia model 256 clinical echocardiograph imager fitted with an 8-MHz sector-array probe, which generates two-dimensional images at a rate of $\sim 100/\text{s}$. Short- and long-axis images of the left ventricle (LV) were analyzed. LV mass and volume were calculated using the area length method. Infarct size was estimated by planimetric measurement of the percentage of the LV that demonstrated systolic akinesis or dyskinesis. From these measurements, percent ischemic zone (%IZ), LV ejection fraction (LVEF), LV end-diastolic volume (LVEDV), and LV end-diastolic volume-to-mass ratio, all indexes of severity of CHF, were reported. After completion of two-dimensional imaging, pulse-wave Doppler interrogation of mitral inflow was performed to determine heart rate. Only animals with large infarctions (%IZ $\geq 35\%$; range 35–66%) were used in the study.

Implantation of osmotic mini-pumps and infusion of drugs. The procedure for implantation of mini-pumps has been described before (32). Briefly, 36 h before the infusion, the mini-pumps attached to Silastic tubing were filled with SL or its vehicle and then placed in sterile 0.9% saline at room temperature to ensure a constant pumping rate at the time of implantation. The pumps were then 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.

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 vehicle-treated animals was 0.2% ethanol in 1- μl volume. Alzet mini-osmotic pumps (model 2004, Alza, Palo Alto, CA) were filled with SL or the ethanol vehicle and attached to a flow modulator to obtain a continuous infusion of the drug. The pumps had an average flow rate of 0.25 $\mu\text{l/h}$, and the final

dose of drug infused was 100 ng/h for a period of 21 days. Recently, we demonstrated that this dose of SL, administered into the 3rd cerebral ventricle, attenuated fluid accumulation and sympathetic drive in rats with ischemia-induced heart failure; the same dose administered intraperitoneally had no effect on these variables during the first 2 wk of administration (30). Other investigators have shown that this dose of SL alters spatial learning (74) and neuroendocrine functions (52) in rats.

Anatomical assessment of heart failure. At the conclusion of the protocol, the hearts were arrested under pentobarbital sodium anesthesia (100 mg/kg body wt ip) and heart and lungs were removed for examination. The presence or absence of ischemic injury, as indicated by left ventricular scarring, was determined by visual inspection. The heart-to-body weight and lung-to-body weight ratios were determined.

Verification of cannula placement and mini-pump function. At the end of the study, brains were removed to check the site of cannula implantation. The osmotic mini-pumps 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 a mean \pm SE. Changes in TNF- α levels between the four groups were analyzed by two-way repeated-measure ANOVA followed by post hoc Fischer's least significant difference test. Echocardiographic parameters were analyzed using one-way ANOVA followed by Fischer's least significant difference test.

RESULTS

Echocardiographic Assessment

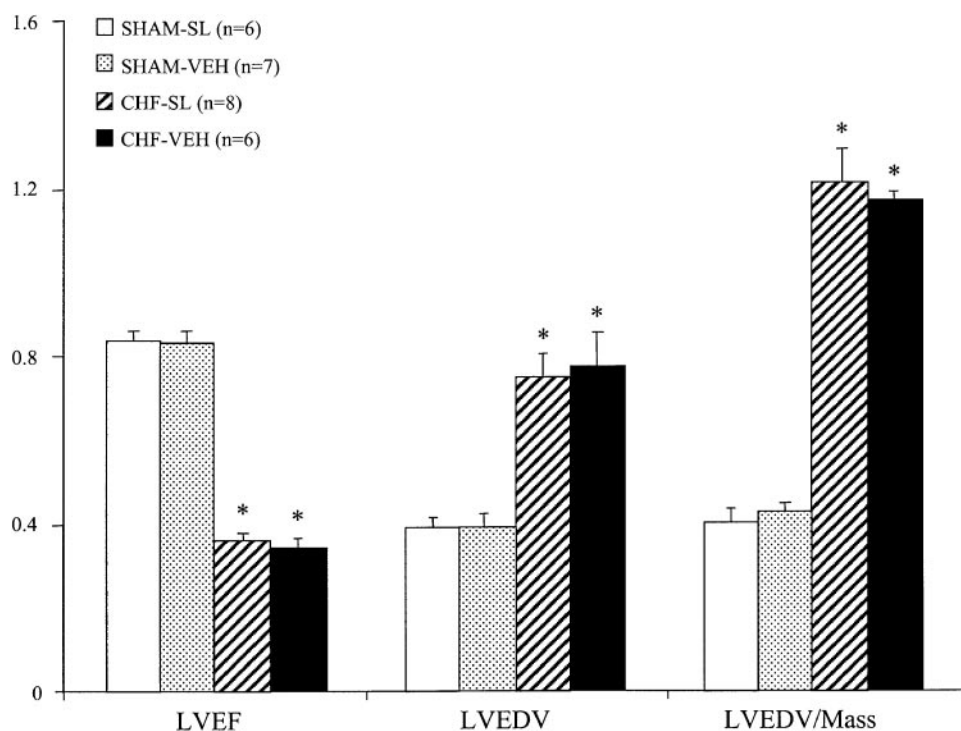
Compared to Sham rats, CHF rats assigned to the two treatment groups had reduced LVEF and increased LVEDV (Fig. 1). The estimated infarct size was not different in the CHF rats assigned to the

CHF+Veh vs. CHF+SL (%IZ: 49.15 ± 5.04 vs. $46.91 \pm 3.3\%$) treatment groups. These animals also had comparable LVEF (0.35 ± 0.03 vs. 0.36 ± 0.02), LVEDV (771.15 ± 61.8 vs. $751.9 \pm 87.9 \mu\text{l}$), and LVEDV/mass (M) ratios (1.22 ± 0.29 vs. 1.17 ± 0.21). The two sham CHF groups also had comparable values of LVEF (0.84 ± 0.03 vs. 0.84 ± 0.02 ; Sham+Veh vs. Sham+SL), LVEDV (334.65 ± 27.67 vs. $329.78 \pm 21.82 \mu\text{l}$), and LVEDV/M (0.43 ± 0.02 vs. 0.40 ± 0.05), and these values were significantly ($P < 0.05$) different from those of the CHF groups. However, the heart rate during the echocardiography under sedation was comparable across all groups.

Effects of Central Infusion of MR Antagonist on Plasma TNF- α Levels

Figure 2 shows the effect of continuous 3rd ventricular infusion of SL vs. Veh beginning 24 h after CL on plasma TNF- α levels in Sham and CHF rats. The baseline TNF- α levels (pg/ml) in the four groups studied were comparable ($P > 0.05$) across groups (CHF+Veh 2.39 ± 0.64 , CHF+SL 2.82 ± 0.93 , Sham+Veh 2.41 ± 0.54 , Sham+SL 2.22 ± 0.74). In the Sham groups, TNF- α levels remained at or around baseline during the entire study. In contrast, in the CHF+Veh group TNF- α levels increased within 24 h of CL (24.57 ± 2.6) and remained elevated (25.02 ± 6.6 , 47.72 ± 16.45 , 58.48 ± 14.59 , 116.33 ± 14.55 , and 156.39 ± 19.03 on days 3, 5, 7, 14, and 21 after CL, respectively) throughout the study. The TNF- α levels in CHF+SL group also increased within 24 h (32.21 ± 6.3) but tended to decrease (19.74 ± 6.64 vs. 25.02 ± 6.6 CHF+SL vs. CHF+Veh) on day 3, returned to baseline by day 5 (3.59 ± 1.35), and then remained low

Fig. 1. Echocardiographic measures of left ventricular (LV) function in rats with congestive heart failure (CHF) induced by coronary artery ligation (CL) and sham-operated (Sham) control rats, obtained 24 h after CL or sham CL and before initiating treatment with spironolactone (SL) or ethanol vehicle (Veh). Compared with Sham rats assigned to SL (Sham-SL) or Veh (Sham-Veh), CHF rats assigned to SL (CHF-SL), or Veh (CHF-Veh) treatment had decreased LV ejection fraction (LVEF), increased LV end-diastolic volume (LVEDV, ml) and increased LVEDV-to-mass ratio (LVEDV/Mass). Pretreatment values for the Sham and CHF rats assigned to either treatment were nearly identical. * $P < 0.05$, CHF vs. Sham subjected to same treatment.



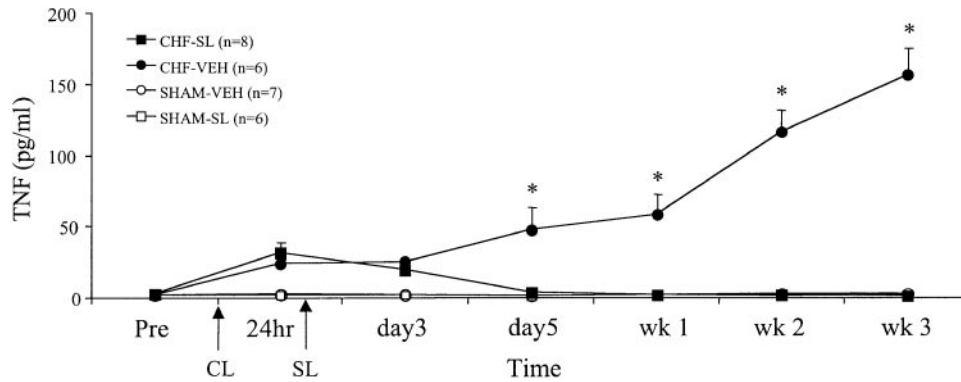


Fig. 2. Plasma tumor necrosis factor (TNF)- α levels in rats with CHF induced by CL and in Sham-operated control rats treated with continuous infusion of Veh or SL into the 3rd cerebral ventricle for 21 days. TNF- α levels were comparable in the 4 groups at baseline, before CL or sham CL. In the Veh-treated CHF rats (CHF-Veh), TNF- α levels increased within 24 h after CL and remained elevated throughout the study. In the SL-treated CHF rats (CHF-SL), there was a similar early increase in TNF- α within 24 h (before treatment was started), but TNF- α was reduced to baseline level by day 4 of SL treatment (day 5 after CL) and remained low throughout the rest of the study. In the SL and Veh-treated Sham rats (Sham-SL and Sham-Veh) the TNF- α levels remained at or around baseline throughout the study. * $P < 0.05$ SL vs. Veh in the CHF rats.

(2.02 ± 1.51 , 2.33 ± 0.3 , and 1.30 ± 0.84 on days 7, 14, and 21 post-CL, respectively) throughout the study.

Anatomical Assessment of Heart Failure

In the CHF rats, gross examination of the heart revealed a dense scar on the left ventricular wall. At the conclusion of the study, there was no difference in the body weight (g) among the four groups of rats studied. CHF+Veh (354 ± 12.4), CHF+SL (347 ± 11.4), Sham+Veh (362 ± 10.8), and Sham+SL (368 ± 13.6). The heart weight-to-body weight ratio was higher ($P < 0.05$) in both CHF groups compared with Sham animals: CHF+Veh vs. Sham+Veh, 5.27 ± 0.3 vs. 3.98 ± 0.5 ; CHF+SL vs. Sham+SL, 4.9 ± 0.4 vs. 4.01 ± 0.2 . In the CHF+SL group (vs. CHF+Veh) there was a trend toward a decreased heart weight-to-body weight ratio that did not achieve statistical significance ($P = 0.18$). The lung weight-to-body weight ratio was also higher ($P < 0.05$) in the CHF group compared with Sham animals: CHF+Veh vs. Sham+Veh, 10.25 ± 0.5 vs. 7.38 ± 0.5 ; CHF+SL vs. Sham+SL, 8.64 ± 0.4 vs. 7.21 ± 0.6 . Compared with CHF+Veh group, intracerebroventricularly treated CHF+SL had reduced ($P < 0.05$) lung weight-to-body weight ratio.

Verification of Cannula Placement and Pump Function

At the end of the study, brains were removed and sectioned to verify the cannula location. Only animals with the cannula in the third ventricle were used in this study. The osmotic mini-pumps were dysfunctional in four rats, as indicated by residual volume of drug in the pump (2 rats) or defects in the connection to the flow modulator (1 rat) or the intracerebroventricular cannula (1 rat). Data from these animals were not included in the statistical analysis.

DISCUSSION

This study describes a previously unrecognized central interaction between the renin-angiotensin-aldosterone system and the immune system in heart failure. The results suggest that Aldo acts centrally to stimulate the production of circulating proinflammatory cytokines, which are present in high concentrations in the setting of a large myocardial infarction (35) and heart failure (27). The possibility that MRs modulate the activity of immune system mediators has important clinical implications.

To our knowledge, no previous study has demonstrated a relationship between the central influences of the renin-angiotensin-aldosterone system and circulating cytokines in heart failure animals. However, the anatomical and physiological substrates to support such an interaction are well documented. Blood-borne angiotensin II and Aldo, produced by the peripheral renin-angiotensin-aldosterone system, act on their receptors in the forebrain to induce increases in thirst and sodium appetite (8, 42, 48), sympathetic drive (38, 44), and the production and release of AVP (9, 66) and CRF (11, 41). Aldo may facilitate the effects of angiotensin II on these forebrain processes (17). Circulating cytokines, products of immune system activation (13), also act on the forebrain (60, 70) to induce increases in sympathetic drive (5, 63) and in the production of AVP (12) and CRF (43, 70), albeit likely via production of a secondary mediator, PGE₂ (5, 23, 50, 70), and an ascending pathway from hindbrain (24). Although these two central nervous system mechanisms serve different purposes under more ordinary circumstances, the renin-angiotensin-aldosterone system acting to restore intravascular volume and pressure (8), the immune system to restrain the peripheral immune response (25), both are activated in heart failure and they appear to influence the same general populations of fore-

brain neurons regulating sympathetic drive, AVP, and CRF.

The activity of neurons in the paraventricular nucleus of the hypothalamus (PVN), an important relay nucleus for the central influences of the immune system (60) and of the renin-angiotensin-aldosterone system (48) on peripheral mechanisms, is increased in heart failure (53, 71). In normal rats, PVN neurons can be activated by blood-borne angiotensin II (47) and by blood-borne cytokines (24). Within the PVN, it is likely that the renin-angiotensin-aldosterone system and the immune system share common mediators. For example, angiotensin II induces the release of norepinephrine in PVN (68), and the circulating cytokines have the same effect via PGE₂-mediated activation of catecholaminergic neurons projecting from medulla oblongata to PVN (23). The intrinsic brain renin-angiotensin-aldosterone system (10), which is active in this region of the brain, may participate in these interactions. Aldo has been shown to increase the binding of angiotensin II to angiotensin type I receptors in the PVN (17), and, in another setting (cultured neonatal cardiac myocytes), Aldo has been shown to upregulate the expression of angiotensin-converting enzyme (39), which catalyzes the conversion of angiotensin I to angiotensin II. Thus the available data suggest that the immune system and the renin-angiotensin-aldosterone system may share common central pathways and neurotransmitter systems. However, the mechanism by which Aldo interacts centrally with the immune system, e.g., whether the interaction is direct or via facilitation of angiotensin II effects, remains to be determined. In the context of the present study, it is of interest that therapeutic agents that block the production of angiotensin II can inhibit the induction of cytokine production by lipopolysaccharide (54) and that selective angiotensin type I receptor antagonism lowers the circulating levels of cytokines in patients with heart failure (69).

The present study did not attempt to identify the tissue source of cytokines regulated by central MRs. Immediately after myocardial infarction in the rat, TNF- α increases, likely due to the cardiac injury and the production of TNF- α by myocytes, fibroblasts, and inflammatory cells in the heart (14, 34, 35). The brain is another potential source of TNF- α production that can be stimulated by blood-borne TNF- α and by prostaglandins (49). A recent study (28) from our laboratory using real-time RT-PCR and immunohistochemistry demonstrated that the mRNA for TNF- α increases in the hypothalamus within 30 min after coronary artery ligation. Thus it may be that TNF- α released from heart signals the brain to produce TNF- α . Of course, lymphoid tissues also produce circulating cytokines, and it is well known that the proinflammatory cytokines augment their own production. Thus the sources of TNF- α later in heart failure are likely ubiquitous. Under normal conditions, cytokine signaling to the brain, with resulting increases in AVP, cortisol, and sympathetic drive, acts to suppress the peripheral immune response (40, 60). In heart failure, immune

system production of cytokines, like renin-angiotensin-aldosterone system production of angiotensin II, persists despite mechanisms of feedback regulation. From the present data, one might speculate that persistent high levels of Aldo, acting centrally, provide an overriding continuing stimulus to cytokine production.

The interpretation of the present findings assumes a localized effect of intracerebroventricularly administered SL on the central nervous system. In a previous study of heart failure rats (32), we demonstrated that SL, administered centrally in the same dose used in the present study, reduced the augmented sympathetic drive and alleviated the increased sodium appetite and the renal sodium and water retention, the cardinal manifestations of congestive heart failure in rats (33). The central effects on volume regulation occurred early, within the first week of intracerebroventricular SL (32). In contrast, systemic (intraperitoneal) administration of this same dose of SL had no effect on volume regulation during the first 2 wk of administration. A similar remote influence of central MR on renal function, likely mediated by renal sympathetic nerves, has been described in normal rats (15, 58). In this perspective, it is reasonable to postulate that neural or humoral signals regulated by central MR might act on lymphoid or other peripheral tissues to modulate cytokine production.

An alternative explanation of our findings might be that changes in peripheral neural or humoral signaling induced by centrally administered SL might slow the progression of left ventricular dysfunction in heart failure and thus secondarily affect circulating TNF- α levels. This explanation seems unlikely, because the reduction in TNF- α was rapid and complete. Preliminary echocardiographic data from our laboratory (unpublished data) indicate that left ventricular function is not altered during the first 2 wk of intracerebroventricular or intraperitoneal infusion of SL at the same dose used in the present study. Other investigators have demonstrated that left ventricular function was unchanged in rats with myocardial infarction after 1 mo of systemic treatment with a higher dose of SL (18).

A caveat to be considered in the interpretation of these results is that SL was used in this study because it is the only MR antagonist with proven benefit on mortality and morbidity in heart failure, but it is not the most selective of the MR antagonists. Moreover, the interactions between Aldo and corticosterone with their receptors in the brain are complex. MRs bind both Aldo and corticosterone with high affinity, and, under basal conditions, the MRs are largely occupied by corticosterone (38). Interestingly, in heart failure both Aldo (36) and cortisol (2) are increased, and under these circumstances their relative influences on central nervous system neurons remain to be determined. The MRs in the periventricular neurons of the hypothalamic region are said to be relatively protected from the effects of corticosterone by the colocalization in target cells of 11- β -hydroxysteroid dehydrogenase, which degrades corticosterone but not Aldo (26, 37, 38). It has been suggested that access by Aldo to only small

numbers of MR can initiate an effect (37). Because the competitive MR antagonist SL has a substantially higher binding affinity for MR than for glucocorticoid receptors (15), it seems likely that the centrally effects of a low dose of SL would be mediated predominantly by blocking the effect of Aldo on MR. Further studies with more selective MR antagonists would help to exclude an effect mediated by SL binding to other steroid receptors.

Circulating levels of TNF- α correlate with severity of heart failure (27) and predict adverse outcomes (19, 59). Circulating TNF- α can induce many adverse effects that mimic the findings of heart failure. These include increases in sympathetic drive, AVP, and corticosterone, as described above, as well as left ventricular dysfunction and left ventricular remodeling (6), increased activity of reactive oxygen species (51), and of renal release of renin (3). The RENAISSANCE trial, using the fusion protein etanercept to bind circulating TNF- α in patients with heart failure, was recently discontinued because of an apparent lack of efficacy (45). At present, cytokines remain an untreated deleterious factor in heart failure. MR blockade may help fill this void. Our results suggest that one mechanism for the beneficial effect of SL on morbidity and mortality in the RALES study may have been a centrally mediated reduction in circulating TNF- α . However, TNF- α levels were not measured in that study. Although SL was administered intraventricularly in the present study to selectively examine a central nervous system mechanism, it is anticipated that systemically administered SL would ultimately have an influence on receptors in the central nervous system, as it appeared to do in a previous study from our laboratory (32). Thus the dramatic influence of MR blockade in CHF may in fact reflect the reduction of a coexisting injurious humoral factor, TNF- α .

In summary, we demonstrated a novel interaction between the renin-angiotensin-aldosterone system and the immune system in heart failure. Our data suggest that Aldo, acting independently or via facilitation of central angiotensin II effects, stimulates central nervous system mechanisms that augment cytokines production and/or impair feedback regulation of peripheral cytokines. Although the mechanisms for this interaction remain to be determined, it may have important implications for the clinical treatment of heart failure. If Aldo stimulates cytokine production via a central mechanism, and cytokines in turn can act peripherally to stimulate renin (3, 4) and Aldo release (4), then central MR blockade may interrupt a detrimental feedforward circuit that contributes to the pathogenesis of heart failure.

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