Novel Effect of Mineralocorticoid Receptor Antagonism to Reduce Proinflammatory Cytokines and Hypothalamic Activation in Rats With Ischemia-Induced Heart Failure

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Abstract—Blocking brain mineralocorticoid receptors (MRs) reduces the high circulating levels of tumor necrosis factor (TNF)-α in heart failure (HF) rats. TNF-α and other proinflammatory cytokines activate neurons in the paraventricular nucleus (PVN) of hypothalamus, including corticotropin-releasing hormone (CRH) neurons, by inducing cyclooxygenase (COX)-2 activity and synthesis of prostaglandin E₂ by perivascular cells of the cerebral vasculature. We tested the hypothesis that systemic treatment with a MR antagonist would reduce hypothalamic COX-2 expression and PVN neuronal activation in HF rats. Rats underwent coronary ligation to induce HF, confirmed by echocardiography, or sham surgery, followed by 6 weeks treatment with eplerenone (30 mg/kg per day, orally) or vehicle (drinking water). Eplerenone-treated HF rats had lower plasma TNF-α, interleukin (IL)-1β and IL-6, less COX-2 staining of small blood vessels penetrating PVN, fewer PVN neurons expressing Fra-like activity (indicating chronic neuronal activation), and fewer PVN neurons staining for TNF-α, IL-1β, and CRH than vehicle-treated HF rats. COX-2 and CRH protein expression in hypothalamus were 1.7- and 1.9-fold higher, respectively, in HF+vehicle versus sham+vehicle rats; these increases were attenuated (26% and 25%, respectively) in HF+eplerenone rats. Eplerenone-treated HF rats had less prostaglandin E₂ in cerebrospinal fluid, lower plasma norepinephrine levels, lower left ventricular end-diastolic pressure, and lower right ventricle/body weight and lung/body weight ratios, but no improvement in left ventricular function. Treatment of HF rats with anticytokine agents, etanercept or pentoxifylline, produced very similar results. This study reveals a previously unrecognized effect of MR antagonism to minimize cytokine-induced central neural excitation in rats with HF. (Circ Res. 2006;99:758-766.)

Key Words: congestive heart failure ■ aldosterone ■ cytokines ■ cyclooxygenase-2 ■ nervous system

In congestive heart failure (HF), the appearance of proinflammatory cytokines in the circulation is a marker of disease severity and a harbinger of adverse outcome. We recently reported that chronic intracerebroventricular administration of spironolactone, a mineralocorticoid receptor (MR) antagonist, prevents the rise in plasma tumor necrosis factor (TNF)-α in a rat model of ischemia-induced HF. In the present study, we examined the potential impact of oral administration of a selective MR antagonist, eplerenone, on blood-borne cytokines and on cytokine-driven central neural mechanisms that may contribute to the progression of HF.

An important function of blood-borne cytokines is to inform the brain of inflammation, infection, or injury of peripheral tissues. However, cytokines are too large to readily cross the blood brain barrier, so the mechanisms by which the cytokine signal is conveyed centrally are still not fully understood. The leading hypothesis, based primarily on acute studies, is that the cytokines activate receptors on perivascular and endothelial cells of the blood brain barrier to induce cyclooxygenase (COX)-2 activity and the synthesis of prostaglandin E₂ (PGE₂). PGE₂ enters the brain where it may directly or indirectly activate hypothalamic neurons mediating neuroendocrine and sympathetic functions. PGE₂ may also induce cytokine synthesis within the brain itself.

The primary brain indicator of “cytokine stress” is upregulation of the expression of corticotropin-releasing hormone (CRH). An increase in CRH in neurons of the paraventricular nucleus of the hypothalamus (PVN) signifies activation of the hypothalamic/pituitary/adrenal (HPA) axis, which is manifest peripherally by increases in circulating glucocorticoids and catecholamines and in sympathetic nerve activity. This mechanism may be particularly important in HF, in which the PVN plays a major role in regulation of extracellular fluid volume and sympathetic drive.
We hypothesized that COX-2 and CRH expression would be increased in the PVN region of rats with ischemia-induced HF and that treatment with the MR antagonist eplerenone would reduce circulating proinflammatory cytokines and the expression of the central indicators of cytokine stress. We compared the effects of eplerenone with those of 2 specific anticytokine agents, the synthetic antibody complex etanercept, which binds circulating TNF-α, and the cytokine synthesis inhibitor pentoxifylline. The results suggest that blood-borne cytokines contribute significantly to the activation of PVN neurons in rats with ischemia-induced HF and that an orally administered MR antagonist can reduce circulating cytokine levels and thereby minimize their influence on the central nervous system. These findings suggest a new approach to anticytokine therapy in HF and so have important clinical implications.

Materials and Methods

Animals

Adult male Sprague–Dawley rats weighing 275 to 300 g were obtained from Harlan Sprague Dawley (Indianapolis, Ind). They were housed in temperature (23±2°C) and light-controlled animal quarters and were provided with rat chow ad libitum. 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.

General Experimental Protocol

Rats underwent surgery to induce HF, or they underwent sham operation (SHAM). Left ventricular (LV) function was assessed by echocardiography immediately after surgery to assign rats to treatment groups. Immediately thereafter, rats were placed on one of the chronic treatment regimens described below. A second echocardiogram was obtained near the end of the treatment protocol. At 6 weeks, rats were anesthetized for hemodynamic measurements and then euthanized to gather tissue, plasma, and cerebrospinal fluid (CSF) for further analysis.

Treatment Regimens

Treatment 1: MR Antagonism

HF and SHAM rats were treated with eplerenone (EPL) (30 mg/kg daily in drinking water) or vehicle (VEH) (drinking water alone). This treatment addressed the primary hypothesis that MR antagonism would reduce circulating proinflammatory cytokines and expression of the central indicators of cytokine stress.

Treatment 2: Cytokine Inhibition

HF and SHAM rats were treated with etanercept (ET) (1 mg/kg daily, IP, every third day) or pentoxifylline (PTX) (30 mg/kg daily, in drinking water) to determine whether the cytokine lowering alone is sufficient to account for the observed responses to EPL.

Treatment 3: Combined MR Antagonism and Cytokine Inhibition

HF and SHAM rats were treated with EPL (30 mg/kg daily in drinking water) and ET (1 mg/kg daily IP every third day) to determine whether the combination of direct MR antagonism and the indirect effect of MR antagonism to lower circulating cytokines would produce additive or facilitative responses.

Twelve animals were subjected to each drug treatment regimen, 6 for immunohistochemical studies and the remainder for molecular studies. The same group of SHAM+VEH rats served as the control for treatment with EPL and PTX, which were both administered orally. Full data were acquired for treatment 1, which addressed the primary study hypothesis. Partial data necessary to address specific issues were acquired for treatments 2 and 3.

Specific Methods

Induction of HF

Rats underwent surgery under ketamine/xylazine anesthesia (90 and 10 mg/kg IP, respectively) to induce HF by ligating the left anterior descending coronary artery, or the same surgery without ligating the vessel (SHAM), as previously described. Animals received benzathine penicillin (30000 U IM) and buprenorphine (0.1 mg/kg SC) immediately after surgery and 12 hours later.

Echocardiographic Assessment of LV Function

Echocardiography was performed under ketamine (25 mg/kg IP) sedation to assess LV function as previously described. Images were acquired with a Sonos 5500 (Philips Medical Systems, Bothell, Wash) fitted with an 8-MHz sector-array probe, which generates 2D images at a rate of ~100 per second. Short- and long-axis images of the left ventricle were analyzed. LV mass and volume were calculated using the area length method. Ischemic zone (IZ) was estimated by planimetry of the region of the LV endocardial silhouette which demonstrated akinesia or dyskinesia, expressed as a percentage of the whole (%IZ). Only animals with large infarctions (%IZ>39%) were used in the study. LV ejection fraction (LVEF), LV end-diastolic volume (LVEDV), and LV end-diastolic volume-to-mass (LVEDV/M) ratio, all indexes of severity of congestive HF, were reported.

Hemodynamic Measurements

Under pentobarbital anesthesia (50 mg/kg IP), a 1.4-French micromanometer-tipped catheter (SPR-671, Millar Instruments, Houston, Tex) was inserted via the right carotid artery and advanced to the aorta. Mean arterial pressure (MAP) and heart rate (HR) were measured. The catheter was then advanced into the left ventricle for measurement of LV end-diastolic pressure (LVEDP).

Collection of Blood, CSF, and Tissues

Rats were anesthetized with pentobarbital (50 mg/kg) for collection of CSF and then decapitated to collect trunk blood and brain, heart, and lung tissues. Trunk blood was collected in chilled EDTA tubes. Plasma samples were separated and stored at −80°C until assayed for TNF-α, interleukin (IL)-1β, IL-6, norepinephrine (NE), and aldosterone (ALDO) levels. The hypothalamus was removed as previously described. The heart was harvested, the ventricles were separated, and the right ventricle was weighed. The lungs were also harvested and weighed. Right ventricular (RV) and lung weights were expressed as a function of body weight (BW).

Biochemical Assays

Plasma and tissue cytokine levels were measured using ELISA (Biosource International Inc, Camarillo, Calif) techniques, as described before. PGE2 in CSF was measured using a high sensitivity kit (R&D Systems Inc, Minneapolis, Minn). The minimum detectable concentration of PGE2 is <8.25 pg/mL. ALDO was measured using a high-sensitivity kit (Alpha Diagnostic International Inc, San Antonio, Tex). The minimum detectable concentration of ALDO is 10 pg/mL. NE was measured using a high sensitivity kit (Rocky Mountain Diagnostics, Colorado Springs, Colo). The minimum detectable concentration of NE is 2.7 pg/mL.

Western Blot

Protein extracted from hypothalamus was used for measurement of COX-2 and CRH protein expression by Western blot. The bands were analyzed using NIH ImageJ software.

Immunohistochemistry

Immunohistochemical studies were performed to assess PVN neuronal activation and the expression of IL-1β, TNF-α, COX-2, and CRH in PVN. Expression of Fra-like (Fra-LI) (for family gene) activity was used as an indicator of chronic neuronal activation. A general avidin/biotin/peroxidase complex (ABC) procedure was
used. The neurochemical phenotype of Fra-LI-labeled (Santa Cruz, K-25, sc-253, 1:2000) neurons was determined using a double-staining protocol. Images were captured at ×10 magnification using a Diaphot 300 microscope (Nikon), and threshold intensity values for each section were set to allow for most of the positively labeled cells to be visualized. In each animal, Fra-LI or specific neuropeptide positive neurons within the borders of PVN bilaterally were counted in 2 representative 40-μm transverse sections approximately 1.80 mm from bregma. Manual counts were used to quantify the numbers of Fra-LI or specific neuropeptide positive PVN neurons. NIH ImageJ software was used to confirm the manual cell counts and to quantify the intensity of COX-2 expression in the PVN.

Statistical Analysis
All data are expressed as mean±SEM. The significance of differences between mean values was analyzed by repeated-measure ANOVA followed by post hoc Tukey test. A probability value of \( P<0.05 \) was considered to be statistically significant.

Results

Echocardiographic Characterization of the Study Groups
Echocardiography performed within 24 hours of coronary artery ligation revealed that HF rats had a lower LVEF, a higher LVEDV, and a higher LVEDV/M ratio than SHAM rats (Figure 1). The %IZ, LVEF, LVEDV, and LVEDV/M ratio were matched among rats assigned to VEH versus drug treatment.

Effects of Eplerenone

Functional/Anatomical Indicators of HF
The %IZ was 41.3±2.1 for HF rats assigned to VEH treatment, and 39.3±2.3 for HF rats assigned to EPL treatment. LVEDV and the LVEDV/M ratio increased similarly in HF+VEH and HF+EPL rats over the 6-week treatment interval. LVEDP was lower (\( P<0.05 \)) in HF+EPL (17.8±2.9 mm Hg) than HF+VEH (25.8±1.4 mm Hg) but still higher (\( P<0.05 \)) than SHAM+VEH (4.2±0.7 mm Hg). There were no significant differences in MAP (in mm Hg: HF+EPL, 102.6±7.4; HF+VEH, 103.7±4.4; SHAM+VEH, 106.9±4.2) or heart rate (in beats/sec: HF+VEH, 5.4±0.2; HF+EPL, 5.6±0.2; SHAM+VEH, 5.5±0.2) attributable to EPL treatment. Right ventricle/BW ratio was 53.3% lower and wet lung/BW ratio was 29.5% lower in HF+EPL than HF+VEH rats (Figure 2). EPL treatment appeared to improve survival (HF+EPL, 83.3%; HF+VEH, 70.6%) over the 6-week interval between the first and second echocardiograms, but some animals did not survive the second echocardiography session.

Humoral Indicators of HF
Plasma aldosterone (ALDO), norepinephrine (NE), and proinflammatory cytokines levels were higher in HF than in SHAM rats. EPL treatment of HF rats had no effect on plasma ALDO levels (Figure 3A). Plasma NE levels were lower in HF+EPL than in HF+VEH (Figure 3B) but higher than in SHAM+VEH. HF+EPL rats had substantially lower plasma TNF-α (Figure 3C), IL-1β (Figure 3D), and IL-6 (in pg/mL: HF+EPL, 71.2±13.6 versus HF+VEH 114.7±11.3, \( P<0.05 \); SHAM+VEH, 39.7±5.8) levels than VEH-treated HF rats, but IL-1β and IL-6 levels were still higher in HF+EPL than SHAM+VEH.

Indicators of Central Neural Activation in HF

Fra-LI Activity
Expression of Fra-LI activity increased in the PVN of HF+VEH compared with SHAM rats (Figures 4, 5B, and 6 through 8). Compared with HF+VEH, HF+EPL rats had fewer Fra-LI-positive PVN neurons (Figure 5B).

Cyclooxygenase-2
Intense staining for COX-2 was observed in small vessels penetrating the PVN region, in close proximity to PVN neurons expressing Fra-LI activity, in HF+VEH compared with SHAM rats (Figure 4A and 4B). Confocal microscopy confirmed a vascular distribution of the COX-2 staining, and suggested a more specific localization of COX-2 to perivascular cells (Figure 4C). The intensity of COX-2 immunofluorescence in PVN (Figure 4D) and COX-2 protein expression in the hypothalamus (Figure 4E) were both significantly
higher in HF + VEH than in SHAM rats and were both attenuated in the HF + EPL group.

**Prostaglandin E2**
PGE2, a marker of cytokine-induced COX-2 activity, was higher in the CSF of HF than of SHAM rats (Figure 5A). CSF levels of PGE2 were lower in HF + EPL rats but still significantly higher than in SHAM rats.

**Corticotropin-Releasing Hormone**
Immunohistochemistry revealed increased CRH expression in PVN neurons of HF rats (Figure 6A and 6B) compared with SHAM rats (Figure 6D). CRH protein expression in hypothalamus was 1.9-fold higher in HF than SHAM rats (Figure 6E). HF rats treated with EPL had fewer PVN neurons positive for CRH (Figure 6C and 6D) and less hypothalamic CRH expression (Figure 6E) than HF + VEH rats. Double-labeling revealed that CRH-positive neurons in HF + VEH rats were distributed among Fra-LI–positive neurons, with 42.7% of Fra-LI–positive neurons also positive for CRH in HF + VEH rats. Only 15.5% of Fra-LI–positive neurons were positive for CRH in HF + EPL rats.

**Proinflammatory Cytokines**
Immunohistochemistry revealed TNF-α (Figure 7A and 7B) and IL-1β (Figure 8A and 8B) expression in PVN neurons of HF rats. Both cytokines were expressed in more PVN neurons in HF + VEH than SHAM rats (Figure 7D and 8D). Hypothalamic levels of IL-1β and TNF-α, measured by ELISA, were also higher in HF than in SHAM rats (Figure 7E and 8E). HF rats treated with EPL had fewer PVN neurons positive for TNF-α (Figure 7C and 7D) and IL-1β (Figure 8C and 8D) and less hypothalamic TNF-α (Figure 7E) and IL-1β (Figure 8E) than HF + VEH rats. Double-labeling revealed that IL-1β– and TNF-α–positive neurons in HF + VEH rats were distributed among Fra-LI–positive neurons, with 40.1% of Fra-LI–positive neurons also positive for IL-1β (14.8%) and TNF-α (15.9%).

**Effects of Anti-Cytokine Agents**
The effects of PTX and ET on HF rats were quite similar to those of EPL. Six weeks of treatment with PTX or ET had no effect on echocardiographic indices of HF (Figure 1) or on
plasma aldosterone levels (Figure 3A) in the HF rats. HF rats treated with PTX or ET, like those treated with EPL, had lower right ventricle/BW and lung/BW ratios (Figure 2), lower plasma TNF-α (Figure 3C), IL-1β (Figure 3D), and NE (Figure 3B) levels, less COX-2 expression in penetrating vessels of the PVN (Figure 4D), lower CSF levels of PGE2 (Figure 5A), fewer Fra-LI–positive PVN neurons (Figure 5B), and fewer PVN neurons expressing CRH (Figure 6D). Survival over the interval from first to second echocardiogram was 75% in HF rats treated with either PTX or ET.

Effects of Combined Treatment
The effects of concomitant treatment with EPL and ET on HF rats resembled the effects of EPL or ET alone (Figure 1 to 8). Trends toward lower levels of IL-1β in plasma (Figure 3D) and hypothalamus (Figure 8E), less COX-2 fluorescence in PVN microvessels (Figure 4D), and fewer PVN neurons expressing CRH were noted, but these differences were small and not statistically significant. Survival also seemed slightly better in the combined treatment group, at 92%, but a statistically significant difference among groups was not demonstrated.

Discussion
The important new findings of this study are: (1) in rats with ischemia-induced HF, there is a pronounced increase in COX-2 expression, signifying local production of PGE2, in microvessels penetrating the PVN; (2) the expression of CRH, TNF-α, and

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**Figure 4.** Immunohistochemistry for COX-2 expression in the PVN of hypothalamus. A, Double-labeling for COX-2 (blue) and Fra-LI activity (black dots), an indicator of chronic neuronal excitation, in a coronal section of the PVN of a HF rat. B, High-power view of the section shown in A, demonstrating the vascular distribution of COX-2 among Fra-LI–positive PVN neurons. C, Confocal microscopic section from PVN, showing endothelial cells (blue) and COX-2 (bright red), with an apparent localization of COX-2 in perivascular rather than endothelial cells. D, Effects of treatment with EPL, PTX, ET, and EPL+ET on fluorescent intensity of COX-2 in PVN of HF and SHAM rats. E, Western blot for COX-2 protein in hypothalamus of EPL- and VEH-treated HF and SHAM rats. *P<0.05 vs control (SHAM+treated or SHAM+VEH); †P<0.05, HF+treated vs HF+VEH.

**Figure 5.** A, Effect of treatment with EPL, PTX, ET, and EPL+ET on CSF level of PGE2, an index of cyclooxygenase activity in the cerebral vessels, in HF and SHAM rats. B, Effects of treatment with EPL, PTX, ET, and EPL+ET on numbers of Fra-LI–positive neurons in PVN of HF and SHAM rats. *P<0.05 vs control (SHAM+treated or SHAM+VEH); †P<0.05, HF+treated vs HF+VEH.
IL-1β is upregulated among chronically activated (Fra-LI–positive) PVN neurons; (3) treatment with the selective MR antagonist eplerenone lowers circulating cytokines and substantially reduces these central manifestations of inflammation and stress; (4) lowering cytokines with etanercept and pentoxifylline has effects closely resembling those of eplerenone; (5) combined treatment with eplerenone and etanercept reveals no additive effect, suggesting that the effects of eplerenone on central...
markers of inflammation and stress are largely attributable to the reduction in circulating cytokines.

Induction of COX-2 expression in endothelial and perivascular cells of the blood/brain barrier is a well-recognized response to acute cytokine stress. Activation of the HPA axis by this mechanism plays a key role in regulating peripheral cytokine production and terminating the inflammatory/stress response. In HF, cytokines circulate at high levels for long periods of time. The present study suggests that one outcome of chronic cytokine stress in HF is sustained COX-2 expression in the microvasculature of the PVN. Consequences may include increased PGE2 in the CSF, chronic activation of PVN neurons, chronic activation of the HPA axis, cytokine synthesis within the brain itself, and increased sympathetic nerve activity. All were present in HF rats, and all were ameliorated by lowering circulating cytokines.

We previously reported that central administration of spironolactone, another MR antagonist, prevented the rise in TNF-α in this same HF model. Because TNF-α appears early in the cytokine cascade, we suggested that other proinflammatory cytokines might also be reduced. Data from the present study support that concept. Orally administered eplerenone lowered circulating levels of three proinflammatory cytokines that activate the HPA axis: TNF-α, IL-1β and IL-6. At least with regard to TNF-α and IL-1β, the response to treatment with eplerenone emulated the responses to the TNF-α synthesis inhibitor pentoxifylline and the TNF-α binding agent etanercept. As previously reported and as shown here, the effects of these anticytokine agents are not confined to a reduction of TNF-α. We did not measure the effect of PTX and ET on IL-6.

A limitation of this study is the inability to identify the location or locations at which eplerenone acts to reduce circulating cytokines. Multiple mechanisms have been proposed to explain activation of the immune system in HF, but the source of blood-borne cytokines remains obscure. In the context of our previous work demonstrating that circulating cytokines are increased in normal rats by activating brain MR and decreased in HF rats by blocking brain MR, we may speculate that orally administered eplerenone crosses the blood/brain barrier to block brain MR and modulates blood-borne cytokines by that mechanism. However, because eplerenone was administered orally in this study, we cannot exclude a direct inhibition of cytokine synthesis release or by peripheral tissues.

An intriguing finding of this study is the ability of the MR antagonist and anticytokine treatments to minimize the appearance of cytokines in PVN neurons of HF rats. Because PGE2 can stimulate cytokine synthesis, this observation raises the possibility that PGE2 generated by cells of the blood-brain barrier signals the brain to upregulate cytokine synthesis, reflecting inflammatory conditions in the periphery. The significance of the appearance of proinflammatory cytokines in PVN neurons is presently unknown. However, cytokines are potent stimuli for activation of the nuclear transcription factor kappa B, which may generate a variety of inflammatory/excitatory mediators within the brain parenchyma.

The appearance of PGE2 and CRH in the brain of the HF rat has important implications for autonomic regulation. Both stimulate the sympathetic nervous system. When injected into...
the forebrain region, PGE\textsubscript{2} elicits a prominent sympathoexcitatory response.\textsuperscript{24,39} A recent in vitro study suggested that PGE\textsubscript{2} induces excitation of preautonomic (ie, presynaptic) and neuroendocrine PVN neurons by hyperpolarizing GABA\textsubscript{ergic} neurons that inhibit their activity.\textsuperscript{12} CRH is also pathetically nerve activity, and improves baroreflex regulation.\textsuperscript{20}

Several additional points deserve comment. First, we observed no measurable effect of eplerenone on echocardiographic indices of left ventricular remodeling. A possible explanation is the low dose of eplerenone (30 mg/kg per day) used in this study. Studies of ventricular remodeling after myocardial infarction typically use 100 mg/kg per day.\textsuperscript{9,16,38} Even at that dose, in studies in which comparable data were obtained, improvements in measures of left ventricular function are relatively small and are often demonstrated after longer treatment intervals.\textsuperscript{16,38} In any case, it is clear from the present study that the effect of eplerenone to reduce inflammatory markers in the brain and circulation in HF cannot be attributed to improved cardiac performance. Second, it is conceivable that some experimental findings that have been attributed to aldosterone blockade per se, in HF and in other settings, may in fact have resulted from the unrecognized cytokine-lowering effect of MR antagonism.

Finally, it is important to note that the present study focused on a single facet of the central effects of MR antagonism: the impact of cytokine lowering on central mediators of inflammation and stress. Aldosterone has well-known direct actions on MR inside the blood/brain barrier that regulate sodium appetite\textsuperscript{4} and sympathetic drive.\textsuperscript{23} In rats with ischemia-induced HF, a centrally administered MR antagonist improves volume regulation, reduces renal sympathetic nerve activity, and improves baroreflex regulation.\textsuperscript{20} A reduction in LV remodeling after myocardial infarction has been attributed to the reduction in sympathoexcitatory drive following blockade of brain MR.\textsuperscript{25} We did not specifically address the effect of eplerenone on these actions of brain MR. With regard to the variables we did measure, we observed no additive treatment effect of combining eplerenone with a cytokine-lowering agent.

The present study demonstrates that an orally administered MR antagonist can lower circulating cytokines in rats with ischemia-induced HF and, by so doing, can profoundly reduce cytokine-induced expression of inflammatory and sympathoexcitatory mediators in the brain. This novel effect of MR antagonism may have implications for the treatment of HF and other clinical conditions characterized by chronic inflammation, immune activation, or stress.

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**Disclosures**

None.

**References**


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