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Angiotensin II Type 1a Receptors in the Subfornical Organ Modulate Neuroinflammation in the Hypothalamic Paraventricular Nucleus in Heart Failure Rats

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Abstract—Inflammation in the hypothalamic paraventricular nucleus (PVN) contributes to neurohumoral excitation and its adverse consequences in systolic heart failure (HF). The stimuli that trigger inflammation in the PVN in HF are not well understood. Angiotensin II (AngII) has pro-inflammatory effects, and circulating levels of AngII increase in HF. The subfornical organ (SFO), a circumventricular structure that lacks an effective blood–brain barrier and senses circulating AngII, contains PVN-projecting neurons. We hypothesized that activation of AngII type 1a receptors ($AT_{1a}R$) in the SFO induces neuroinflammation downstream in the PVN. Male rats received SFO microinjections of an adeno-associated virus carrying shRNA for $AT_{1a}R$, a scrambled shRNA, or vehicle. One week later, some rats were euthanized to confirm the transfection potential and knockdown efficiency of the shRNA. Others underwent coronary ligation to induce HF or a sham coronary ligation (Sham). Four weeks later, HF rats that received the scrambled shRNA had increased mRNA in SFO and PVN for $AT_{1a}R$, inflammatory mediators and indicators of neuronal and glial activation, increased plasma AngII, tumor necrosis factor-α, norepinephrine and arginine vasopressin, and impaired cardiac function, compared with Sham rats that received scrambled shRNA. The central abnormalities were ameliorated in HF rats that received $AT_{1a}R$ shRNA, along with plasma norepinephrine and vasopressin. Sham rats that received $AT_{1a}R$ shRNA had reduced SFO $AT_{1a}R$ mRNA but no other changes compared with Sham rats that received scrambled shRNA. The results suggest that activation of $AT_{1a}R$ in the SFO upregulates the neuroinflammation in the PVN that contributes to neurohumoral excitation in HF.

Key words: forebrain, proinflammatory cytokines, renin-angiotensin system, sympathetic nerve activity, arginine vasopressin.

INTRODUCTION

Increased renin-angiotensin system (RAS) activity and inflammation in cardiovascular-related regions of the central nervous system contribute to the overactivity of neurohumoral systems that promote volume retention, cardiac remodeling and serious cardiac arrhythmias in systolic heart failure (HF). A primary central nervous system site involved in this process is the hypothalamic paraventricular nucleus (PVN), which contains both presympathetic and neuroendocrine neurons (Ferguson et al., 2008). Interventions that reduce RAS activity and inflammation in the PVN are uniformly successful in reducing sympathetic excitation and improving indices of volume regulation and cardiac hemodynamics in rats with HF (Zhang et al., 1999; Francis et al., 2001, 2004; Guggilam et al., 2008; Kang et al., 2008a,b, 2010; Yu et al., 2012; Huang et al., 2014). However, the mechanisms upregulating the activity of these two excitatory neurochemical systems in the PVN in HF are still poorly understood.

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Abbreviations: AAV, adeno-associated virus; ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptors; $AT_{1a}R$, angiotensin II type 1a receptors; AngII, angiotensin II; AVP, arginine vasopressin; BW, body weight; CD68, cluster of differentiation 68; CL, coronary artery ligation; CON, control; COX-2, cyclooxygenase-2; COX-1, cyclooxygenase-1; DBP, diastolic blood pressure; dP/dt_{max}, maximum rate of rise of LV pressure; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; HF, heart failure; HR, heart rate; IL-1β, interleukin-1 beta; IL-6, interleukin-6; %IZ, ischemic zone as a percent of left ventricular circumference; LPS, lipopolysaccharide; LV, left ventricle; LVEDP, left ventricular end diastolic pressure; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVPSP, left ventricular peak systolic pressure; NE, norepinephrine; NF-κB, nuclear factor κB; PGE_2, prostaglandin E2; PIC, proinflammatory cytokine; PVN, hypothalamic paraventricular nucleus; SFO, subfornical organ; TNF-α, tumor necrosis factor – alpha; TNFR1, tumor necrosis factor – alpha receptor 1; RAS, renin-angiotensin system; ROS, reactive oxygen species; RV, right ventricle; SBP, systolic blood pressure; shRNA, short hairpin RNA; Vol, volume.

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The present study sought to determine whether increased RAS activity in the subfornical organ (SFO) – a forebrain circumventricular organ that lacks an effective blood–brain barrier, senses circulating humoral factors in HF (McKinley et al., 2003; Ferguson, 2014) and projects to the directly to the PVN (Li and Ferguson, 1993; Kawano and Masuko, 2010) – contributes to the inflammatory response in the PVN in HF. In HF, angiotensin II (AngII) type 1 receptors (AT_1R) are upregulated in the SFO (Tan et al., 2004; Wei et al., 2008b), which is exposed to increased plasma levels of angiotensin II (AngII) in that setting (Huang and Leenen, 2009; Wang et al., 2014). Previous studies have shown that a slow-pressor infusion of AngII upregulates the expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and cyclooxygenase-2 (COX-2) in the PVN of normal rats (Yu et al., 2013b), and that chronic intracerebroventricular infusion of the AT_1R blocker losartan significantly reduces the expression of the TNF-α, IL-1β and IL-6 in the PVN of HF rats (Kang et al., 2008a), though neither of those studies considered what role AT_1R in the SFO might play in upregulating the expression of these neuroinflammatory mediators in the PVN.

To address this question, we used an adeno-associated viral (AAV) vector carrying an shRNA specific for $AT_{1a}R$, the AT_1R subtype that mediates the effects of AngII in the SFO and other cardiovascular autonomic regions of the brain (Lenkei et al., 1997). We microinjected the $AT_{1a}R$ shRNA into the SFO prior to the induction of HF and measured the effects of $AT_{1a}R$ knockdown in the SFO on mRNA for TNF-α, IL-1β, COX-2, and markers of neuronal and glial activation in the SFO and PVN, on plasma AngII, TNF-α, norepinephrine (NE), arginine vasopressin (AVP), and on indices of cardiac remodeling and hemodynamics. The results reveal that activation of $AT_{1a}R$ in the SFO of HF rats promotes the expression of the inflammatory mediators in the PVN that contribute to neurohumoral excitation in HF.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats weighing 250–300 g were purchased from Envigo/Harlan (Indianapolis, IN, USA). Animals were housed in temperature-(23 ± 2 °C) and light-controlled animal care facility, and standard rat chow and water were given ad libitum. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa. All efforts were made to minimize the number of animals used and their suffering.

Experimental protocols

Sixty-seven rats underwent SFO microinjection of an AAV vector carrying green fluorescent protein (GFP) and either shRNA against $AT_{1a}R$ (AAV-$AT_{1a}R$ shRNA, $n = 27$) or a scrambled control shRNA (AAV-CON shRNA, $n = 31$), or vehicle (Veh, artificial cerebrospinal fluid, $n = 9$).

One week later, some rats that had received SFO microinjection of Veh ($n = 9$), AAV-$AT_{1a}R$ shRNA ($n = 9$) or AAV-CON shRNA ($n = 9$) were euthanized to verify the transfection potential (by immunofluorescent imaging, $n = 3$/group) and the knockdown efficiency (by RT-PCR, $n = 6$/group) of the AAV-$AT_{1a}R$ shRNA.

The remaining rats that had received AAV-$AT_{1a}R$ shRNA or AAV-CON shRNA underwent either coronary artery ligation (CL) to induce HF or a sham operation (Sham), resulting in 4 experimental groups: HF + AAV-$AT_{1a}R$ shRNA ($n = 11$), HF + AAV-CON shRNA ($n = 15$), Sham + AAV-$AT_{1a}R$ shRNA ($n = 7$), Sham + AAV-CON shRNA ($n = 7$).

Left ventricular function was evaluated by echocardiogram within 24 h of CL or sham operation. Four weeks later, a second echocardiogram was performed to determine treatment effects. At the end of the 5-week protocol, rats from each treatment group were anesthetized for invasive assessment of cardiac hemodynamics and then euthanized while still under anesthesia to collect brain and blood for real-time PCR and ELISA. The heart and lungs were also harvested and weighed to assess peripheral indicators of HF.

Six rats died within 24 h of coronary ligation (HF + AAV-$AT_{1a}R$ shRNA: $n = 2$; HF + AAV-CON shRNA: $n = 4$). Three more rats died between 24 h and the end of the protocol (HF + AAV-$AT_{1a}R$ shRNA: $n = 1$; HF + AAV-CON shRNA: $n = 2$). Five rats with a small myocardial infarction on initial echocardiogram (ischemic zone, as defined below, ≤30%) were excluded from further study (HF + AAV-$AT_{1a}R$ shRNA: $n = 2$; HF + AAV-CON shRNA: $n = 3$). No Sham rats died prior to completing the protocol. The final animal numbers that were used for data analysis were as follows: $n = 7$ rats for Sham + AAV-CON shRNA and Sham + AAV-AT1aR shRNA; $n = 6$ rats for HF + AAV-CON shRNA and HF + AAV-AT1aR shRNA.

SFO microinjections

SFO microinjection was performed as previously described (Moreau et al., 2012; Wei et al., 2015). Briefly, rats were anesthetized (ketamine 100 mg/kg + xylazine 10 mg/kg IP) and positioned in a stereotaxic apparatus (Kopf Instruments; Tujunga, CA, USA). A longitudinal skin incision was made to expose the skull, and a small hole was drilled at stereotaxic coordinates 0.8 mm caudal and 1.7 mm lateral to bregma. A 30-gauge guide cannula was inserted and advanced at a 25° angle to the vertical meridian to a position 5 mm ventral to the cortical surface, approaching the SFO at an angle to avoid the sagittal sinus. A 35-gauge injection cannula connected via calibrated polyethylene tubing to a 1-μl Hamilton microsyringe was inserted into the guide cannula and extended 0.5 mm beyond the tip of the guide cannula. $AT_{1a}R$ shRNA (0.3 μl of 1.1×10^{12} genomic particles/ml) or CON shRNA AAV vector, both carrying GFP (Genedetect, Sarasota, FL, USA), was then injected into the SFO over 15–30 s. The injection cannula was left at the

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