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Paraventricular nucleus corticotrophin releasing hormone contributes to sympathoexcitation via interaction with neurotransmitters in heart failure

Yu-Ming Kang,

Department of Physiology and Pathophysiology, Xi'an Jiaotong University School of Medicine, Xi'an 710061, China

Ai-Qun Zhang,

Institute of Hepatobiliary Surgery, General Hospital of Chinese People's Liberation Army, Beijing, China

Xiu-Fang Zhao,

Department of Internal Medicine, General Hospital of Chinese People's Armed Police Forces, Beijing, China

Jeffrey P. Cardinale,

Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

Carrie Elks,

Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

Xi-Mei Cao,

Department of Physiology, Shanxi Medical University, Taiyuan, China

Zhen-Wen Zhang, and

Department of Internal Medicine, General Hospital of Chinese People's Armed Police Forces, Beijing, China

Joseph Francis

Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA

Yu-Ming Kang: ykang@mail.xjtu.edu.cn; Joseph Francis: jfrancis@lsu.edu

Abstract

Recent studies indicate that systemic administration of tumor necrosis factor (TNF)- α induces increases in corticotrophin releasing hormone (CRH) and CRH type 1 receptors in the hypothalamic paraventricular nucleus (PVN). In this study, we explored the hypothesis that CRH in the PVN contributes to sympathoexcitation via interaction with neurotransmitters in heart failure (HF). Sprague–Dawley rats with HF or sham-operated controls (SHAM) were treated for 4 weeks with a continuous bilateral PVN infusion of the selective CRH-R1 antagonist NBI-27914 or vehicle. Rats with HF had higher levels of glutamate, norepinephrine (NE) and tyrosine hydroxylase (TH), and lower levels of gamma-aminobutyric acid (GABA) and the 67-kDa isoform

of glutamate decarboxylase (GAD67) in the PVN when compared to SHAM rats. Plasma levels of cytokines, NE, ACTH and renal sympathetic nerve activity (RSNA) were increased in HF rats. Bilateral PVN infusions of NBI-27914 attenuated the decreases in PVN GABA and GAD67, and the increases in RSNA, ACTH and PVN glutamate, NE and TH observed in HF rats. These findings suggest that CRH in the PVN modulates neurotransmitters and contributes to sympathoexcitation in rats with ischemia-induced HF.

Keywords

Corticotrophin releasing hormone; Neurotransmitters; Hypothalamic paraventricular nucleus; Sympathetic nervous system; Heart failure

Introduction

Stress is an important risk factor in the development and progression of cardiovascular disease. Acute myocardial infarction can be induced by stress in susceptible patients or animals. One well-studied stress system is the hypothalamo–pituitary–adrenal (HPA) axis. In rats with HF, the HPA axis is activated, likely by increased circulating and/or brain proinflammatory cytokines (PICs). The physiological marker of HPA axis activation is increased corticotrophin releasing hormone (CRH) in the hypothalamic paraventricular nucleus (PVN). The cell bodies of CRH-producing neurons are located in the PVN. The CRH is the principal hormone involved in the HPA axis activation, with CRH receptors (CRH-R) being the primary site for HPA axis induction. Previous studies demonstrated that centrally administered CRH elicits cardiovascular and autonomic responses [53]. Recent studies indicate that circulating PICs act upon the CRH neurons in the PVN [9, 12, 13, 38]. However, the mechanisms by which these PICs activate the sympathetic nervous system are not clear. Most of the CRH neurons in the PVN are involved in neuroendocrine functions [41]. Even though preautonomic and neuroendocrine CRH neurons co-mingle in the PVN, they might be differentially regulated. CRH acts upon CRH type 1 receptors (CRH-R1) and can induce autonomic responses in reaction to the initial stimuli. Under normal physiological conditions, CRH-R1 have only a scant presence in the PVN [32]. The levels of CRH-R1 in the PVN are upregulated under conditions of stress or in the presence of increased CRH [20, 29, 39].

Either HPA axis activation or CRH injection into the forebrain can increase both peripheral sympathetic nerve activity and circulating epinephrine and norepinephrine (NE). A number of excitatory and inhibitory neurotransmitters converge in the PVN to influence its neuronal activity. Among these neurotransmitters are glutamate, NE, and gamma-aminobutyric acid (GABA). Increased PICs in the PVN cause an imbalance in PVN neurotransmitters and contribute to sympathoexcitation in heart failure [21]. Despite the abundant evidence that cytokines respond to and drive the HPA axis, very few studies have examined the role of HPA axis activation in HF. Recent work indicates that myocardial infarction increases CRH in the PVN of HF rats [24, 25], and that blockade of PICs decreases sympathetic activity and downregulates the activation of CRH neurons in the PVN of HF rats [24, 25]. We recently found that central blockade of PICs restores of the balance between excitatory and inhibitory neurotransmitters in the PVN of HF rats [21]. The aim of this study was to determine the role of PIC-driven HPA and CRH activity in inducing sympathoexcitation via interaction with neurotransmitters in the PVN of HF rats.

Methods

Animals

All experimental procedures were conducted using adult male Sprague-Dawley rats (275–300 g). Rats were housed in light- and temperature-controlled (12 h light/dark cycle, $23 \pm 2^\circ\text{C}$, respectively) animal quarters and were fed rat chow and tap water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committees of Xi'an Jiaotong University and Louisiana State University. All procedures requiring use of animals were in compliance with the “National Institutes of Health Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Coronary ligation and cannula implantation

Rats underwent sterile surgery under anesthesia [90 mg/kg ketamine + 7.5 mg/kg xylazine, intraperitoneally (IP)] for induction of HF by ligation of the left anterior descending coronary artery, or the same surgery without vessel ligation (SHAM), as previously described [21, 22, 24, 25]. While still under anesthesia, each rat had two cannulae implanted, using stereotaxic coordinates [36], to facilitate bilateral infusions into the PVN [15]. Animals received buprenorphine (0.01 mg/kg, SC) immediately following surgery and 12 h post-operation.

Echocardiographic assessment of left ventricular function

Echocardiography was performed under ketamine (25 mg/kg, IP) sedation for assessment of left ventricular (LV) function as previously described [22, 25]. Ischemic zone (IZ) was estimated by planimetry of the region of the LV endocardial silhouette which demonstrated akinesis or dyskinesis, and expressed as a percentage of the whole (%IZ). From these measurements, LV ejection fraction (LVEF), and LV end-diastolic volume (LVEDV) were also determined.

Drug infusion

Within 24 h of coronary ligation or sham operation, each rat was anesthetized (60 mg/kg ketamine + 5 mg/kg xylazine, IP) and underwent subcutaneous implantation of osmotic mini-pumps (Alzet Model #1004). Mini-pumps were connected to the bilateral PVN cannulae for continuous infusion (0.11 $\mu\text{l/h/side}$) of the selective CRH-R1 antagonist NBI-27914 (5-chloro-4-[*N*-(cyclo-propyl)methyl-*N*-propylamino]-2-methyl-6-(2,4,6-trichlor-o-phenyl)amino-pyridine, Sigma) at a total dose of 10 $\mu\text{g/h}$, or vehicle, over a 4-week treatment period. Another set of HF and SHAM rats were treated with IP infusion of a similar dose of NBI-27914 or vehicle over a 4-week treatment period. NBI-27914 was dissolved in dimethyl sulfoxide (DMSO) and further diluted with artificial cerebrospinal fluid for PVN infusion or saline for IP infusion to the desired concentration.

Electrophysiological recordings and anatomical measurements

Arterial pressure (AP), heart rate (HR) and renal sympathetic nerve activity (RSNA) were measured as described previously [21, 23]. Maximum RSNA was measured using an intravenous bolus administration of sodium nitroprusside (SNP, 10 μg) [21, 35] at the end of the experiment, the background noise, defined as the signal-recorded postmortem, was subtracted from actual RSNA and expressed as percent of maximum (in response to SNP) [17]. The left ventricular end-diastolic pressure (LVEDP), the right ventricle (RV)/body weight (BW) ratio and lung/BW ratio were measured as described previously [21, 22, 25].

The survival rate between the first and second echo-cardiograms for each group was calculated by dividing the number of rats at the second echocardiography assessment by the number of rats at the first echocardiography assessment.

ELISA studies

Plasma and tissue cytokine (TNF- α , IL-1 β and IL-6) levels were measured using ELISA (Biosource International Inc.) techniques, as previously described [21–23]. Plasma ACTH was measured using an ELISA kit (MD Biosciences) according to manufacturer instructions.

HPLC measurements of tissue neurotransmitter levels and circulating catecholamine levels

Tissue concentrations of glutamate and GABA were measured using HPLC with electrochemical detection (ECD-300, Eicom Corporation, Japan) and tissue NE concentration was measured using HPLC with electrochemical detection (HTEC-500, Eicom Corporation, Japan) as previously described [21]. Plasma NE and epinephrine were measured using HPLC as previously described [17, 18].

Tissue microdissection

Microdissection procedure was used to isolate the PVN, as previously described [21]. The PVN was punched with the help of a stereotaxic atlas [36]. The samples were stored at -70°C until analyzed for cytokines using ELISA and neurotransmitters using high performance liquid chromatography (HPLC).

Immunohistochemical studies

Transverse sections from brains were obtained from the region approximately 1.80 mm from the bregma. Immunohistochemical labeling was performed in floating sections as described previously [24, 25] to identify Fra-like protein (Fra-LI, a marker of chronic neuronal activation; sc-253, Santa Cruz Biotechnology), and CRH (Phoenix Pharmaceuticals). For each rat, the neurons positive for Fra-LI or CRH within the bilateral borders of the PVN were manually counted in three consecutive sections and an average value was reported. Neurons positive for Fra-LI or CRH within a window superimposed over the dorsal parvocellular (dpPVN), ventrolateral parvocellular (vlpPVN), and magnocellular (mPVN) subregions of the PVN and were counted similarly for data analysis.

Western blot

Measurement of tissue protein was performed as previously described [22–25, 30, 43]. Briefly, protein extracted from the PVN was used for measurements of tyrosine hydroxylase (TH, Abcam) and the 67-kDa isoform of glutamate decarboxylase (GAD67, Abcam) expression by western blot. Equal protein loading was determined by probing all blots with β -actin antibody (Santa Cruz Biotechnology) and normalizing their protein intensities to that of β -actin. The bands were analyzed using NIH Image J software.

Statistical analysis

All data are expressed as mean $\pm$ SEM. Data were analyzed by two-way ANOVA. Multiple testing was corrected for by using Tukey's test. The echocardiography data were analyzed with repeated measures ANOVA. A probability value of $P < 0.05$ was considered statistically significant.

Results

Echocardiography

Echocardiography performed within 24 h of coronary artery ligation revealed that HF rats had a lower LVEF, a higher LVEDV, and a higher LVEDV/M ratio than SHAM rats (Table 1). The %IZ, LVEF, LVEDV, and LVEDV/M ratio were equivalent among rats assigned to vehicle versus drug treatment. At 4 weeks, LVEF was higher in the HF rats that received NBI-27914 when compared with the HF rats that received vehicle (Table 1). However, there were no significant differences in LVEDV, LVEDV/mass ratio or %IZ between NBI-27914 and VEH-treated HF rats.

Functional/anatomical indicators of heart failure

Compared with SHAM rats, HF rats had higher LVEDP, RV/BW and lung/BW ratio. The NBI-27914-treated HF rats had significantly lower LVEDP and lung/BW ratios than vehicle-treated HF rats (Table 2). IP treatment with the same doses of NBI-27914 did not affect LVEDP, RV/BW or lung/BW ratio (Table 2).

NBI-27914 treatment improved the survival ($P < 0.05$) (HF + NBI-27914, 82.4%; HF + VEH, 70.0%) over the 4-week interval between the first and second echocardiograms.

Humoral indicators of heart failure

Humoral indicators of heart failure paralleled the PVN findings. Plasma levels of NE, EPI, ACTH, TNF- α , IL-1 β and IL-6 were all higher in HF rats than in SHAM rats. Bilateral PVN infusions of NBI-27914 attenuated the increases in plasma levels of these factors in HF rats (Table 3, and Fig. 1). However, the plasma levels were unaffected by IP treatment with the same dose of NBI-27914.

CRH in the PVN

Compared with SHAM rats, HF rats had higher levels of CRH expression in the PVN as revealed by immunohistochemistry (Fig. 2). HF rats treated with NBI-27914 had fewer CRH-positive PVN neurons than vehicle-treated HF rats (Fig. 2).

PVN neurotransmitters

HF rats had higher levels of NE and glutamate, and lower levels of GABA in the PVN. Four-week bilateral infusions of NBI-27914 into the PVN prevented the decrease in PVN GABA and the increases in PVN glutamate and NE in HF rats (Fig. 3). However, IP treatment with the same dose of NBI-27914 did not alter NE, glutamate, or GABA in the PVN of HF rats.

TH and GAD67 protein expression in the PVN

Western blot showed that HF rats had higher TH levels and lower GAD67 levels in the PVN when compared with SHAM rats (Fig. 4). Bilateral PVN infusion of NBI-27914 for 4 weeks prevented the decrease in PVN GAD67, and the increases in TH in the PVN of HF rats (Fig. 4).

Fra-LI activity, an indicator of chronic neuronal activation, in the PVN

Compared with SHAM rats, Fra-LI activity was higher in the PVN of HF rats. Bilateral PVN infusions of NBI-27914 prevented the increases in Fra-LI of HF rats (Fig. 5). However, IP treatment with the same dose of NBI-27914 had no effect on the number of Fra-LI in the PVN.

PVN levels of proinflammatory cytokines

PVN levels of TNF- α , IL-1 β and IL-6 were higher in HF than in SHAM rats. PVN levels of TNF- α , IL-1 β and IL-6 were lower in HF rats that received bilateral PVN infusions of NBI-27914 (Table 3). IP treatment with the same dose of NBI-27914 had no effect on PIC levels in the PVN of HF rats.

Renal sympathetic nerve activity (RSNA)

At the conclusion of the study, HF rats exhibited higher renal sympathetic nerve activity (RSNA, % of max) when compared to SHAM rats. Bilateral PVN infusions of NBI-27914 inhibited RSNA in HF rats (Fig. 6). IP treatment with the same dose of NBI-27914 did not affect RSNA.

Discussion

The novel finding of this study is that CRH, possibly through a PIC activation mechanism, induces an imbalance between excitatory and inhibitory neurotransmitters in the PVN of HF rats, which contributes to sympathoexcitation. Treatment with PVN infusion of a CRH-R1 antagonist attenuated this imbalance and sympathoexcitation in HF rats. Similar doses of these blockers given peripherally did not restore neurotransmitter levels in the PVN of HF rats, suggesting that central nervous system cytokines modulate neurotransmitters, especially NE, in the PVN, and contribute to sympathoexcitation in heart failure post-MI.

One of the pathophysiological characteristics of HF is elevated sympathetic drive, which is a major factor contributing to the morbidity and mortality of HF patients. Recent evidence points to a central nervous system mechanism that contributes to the sympathetic abnormality typically observed in HF. The excitatory and inhibitory neurotransmitters converge in the PVN to influence its neuronal activity [46]. In the brain, the PVN is an important central integration site for sympathetic nerve activity [48, 49], as well as an important region for cardiovascular control and homeostasis [40, 44]. The primary controlling neurotransmitters include glutamate, NE, and GABA. It has been reported that functional glutamate receptors are expressed in the PVN [5, 34] and are involved in cardiovascular reflexes [1, 3]. It has also been shown that sympathetic hyperactivity in rats with HF is associated with increased NE in the resting PVN [47, 50], and it is well known that NE plays a critical role in the pathophysiologic process of HF [37, 48]. Furthermore, a large body of evidence suggests that GABA plays an important role in central sympathetic and cardiovascular regulation [7, 8, 45] and that it is the dominant inhibitory neurotransmitter within the PVN. Considerable evidence suggests that the PVN is one of the sites in which the cardiovascular effects of GABA are elicited. Previously, work from Patel's [52] laboratory demonstrated that inhibitory mechanisms of sympathetic regulation within the PVN via GABA were reduced in HF rats. Through a western blot approach, we also identified the neurons expressing GAD67, a marker used to identify GABAergic neurons in the PVN. Our results show that the expression of GAD67 in PVN neurons of HF rats was lower when compared with SHAM rats, and that this reduction was normalized in HF rats treated with bilateral PVN infusion of the selective CRH-R1 antagonist. Elevated excitatory neurotransmitters and decreased inhibitory neurotransmitters in the PVN are shown to contribute to sympathetic dysregulation in HF [2, 11].

Proinflammatory cytokines, including TNF- α , IL-1 β and IL-6 [6, 8, 21, 27, 28], are released into the circulation post-MI [26, 51]. In HF rats, TNF- α , which appears quickly in the cytokine cascade [9], increases in the blood, brain and heart within minutes after an acute MI and continues to rise over the ensuing weeks [14]. IL-1 β has a similar pattern of early appearance post-MI [16]. The PVN is particularly sensitive to the influences of

inflammatory stress and peripheral cytokine production. Blood-borne and brain cytokines are shown to stimulate COX-2 for the eventual generation of prostaglandin E₂ (PGE₂), which acts centrally to increase sympathetic drive [24, 25] and to induce expression of CRH [31] in the PVN neurons that mediate the HPA axis's stress response. Within the PVN, the parvocellular CRH neurons are specifically activated by peripheral administration of IL-1 β [4]. Central nervous system signaling by blood-borne cytokines activates CRH-producing neurons in the PVN. In rats, deafferentation of the hypothalamus and lesions of the PVN blocked the plasma ACTH response to IL-1 β [12]. These findings suggest that PICs contribute to HPA axis activation, such that the early stage PICs act to increase CRH, which then causes an imbalance between excitatory and inhibitory neurotransmitters and initiating sympathoexcitation in these HF rats. This is shown by our finding that a decrease in plasma ACTH after blockade of CRH and this was accompanied by modulation of neurotransmitters within the PVN, thereby affecting the typical negative feedback system at several levels of the HPA axis activation contributing to the attenuated sympathoexcitation in heart failure.

The endocrine (i.e., glucocorticoid) response of the HPA axis to an acute cytokine challenge depends upon the noradrenergic activation of CRH-containing neurons in the PVN. CRH can excite PVN neurons and elicit a sympathoexcitatory response. ICV infusion of CRH caused significant increases in PVN neuronal activity and RSNA [53]. Our results in this study suggest that sympathoexcitatory effects of CRH in PVN may be a primary mechanism of HF progression after MI. Due to the different distributions and functions of CRH in the brain [10, 19, 42], it is difficult to observe identical responses to CRH antagonists. However, this study has clearly demonstrated that CRH is involved in the regulation of sympathetic activity; this is based upon the effect of bilateral PVN infusion of the CRH antagonist NBI-27914 on blocking the RSNA response to HF. We have also previously demonstrated that PICs were increased in the PVN in HF rats [21, 22], and elevated PICs in the PVN can cause an imbalance in PVN neurotransmitters and contribute to sympathoexcitation in HF [21]. Moreover, high levels of circulating cytokines post-MI have additional effects on the brain that may promote the development of HF. Central nervous system signaling by blood-borne cytokines activates CRH producing neurons in the PVN, where TNF- α , IL-1 β , and IL-6 all share a common property of activating the HPA axis [9, 12, 33, 49] and increasing sympathetic nerve activity [54]. The present study suggests that a HF-induced increase in CRH in the PVN causes an imbalance in PVN neurotransmitters and contributes to sympathoexcitation in HF rats.

In summary, the results of the present study indicate that elevated brain PICs in heart failure increase CRH neuronal activity and CRH-R1 expression in the PVN. This increased CRH then causes an imbalance between excitatory and inhibitory neurotransmitters in the PVN neuronal tissue, thereby contributing to sympathoexcitation in HF rats. Central blockade of CRH restored these alterations in the PVN of HF rats. Though further investigations are needed to determine the mechanisms by which these interactions occur, as well as an extended follow-up period to assess the potential long-term effect of CRH modulation on HF mortality, these findings outline a possible therapeutic approach whereby central inhibition of CRH and a restoration of neurotransmitter imbalance may be beneficial for the treatment of heart failure.

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Abbreviations

AP	Arterial pressure
ANOVA	Analysis of variance
BW	Body weight
CRH	Corticotrophin releasing hormone
dpPVN	Dorsal parvocellular PVN
ECD	Electrochemical detection
EPI	Epinephrine
ELISA	Enzyme-linked immunosorbent assay
GABA	Gamma-aminobutyric acid
GAD67	67-kDa isoform of glutamate decarboxylase
GLU	Glutamate
HF	Heart failure
HPA	Hypothalamo–pituitary–adrenal axis
HPLC	High performance liquid chromatography
HR	Heart rate
IZ	Ischemic zone
IP	Intraperitoneal injection
ICV	Intracerebroventricular injection
LV	Left ventricle
LVEF	LV ejection fraction
LVEDV	LV end-diastolic volume
LVEDP	Left ventricular end-diastolic pressure
mPVN	Magnocellular PVN
MI	Myocardial infarction
NE	Norepinephrine
PVN	Hypothalamic paraventricular nucleus
PICs	Pro-inflammatory cytokines
PGE₂	Prostaglandin E ₂
RSNA	Renal sympathetic nerve activity
RV	Right ventricle
SHAM	Sham-operated control
SNP	Sodium nitroprusside
TNF	Tumour necrosis factor
TH	Tyrosine hydroxylase
vlpPVN	Ventrolateral parvocellular PVN

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Fig. 1.

Plasma ACTH, NE and epinephrine (EPI) were higher in HF rats than in SHAM rats. Bilateral PVN infusions of NBI-27914 attenuated the increases in plasma ACTH, NE and EPI of HF rats. * $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; † $P < 0.05$ HF + NBI-27914 versus HF + vehicle

Fig. 2.

Immunohistochemistry for CRH expression in the PVN of hypothalamus. CRH expression in the PVN was lower in the NBI-27914-treated HF rats than in vehicle-treated HF rats. $*P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; $^{\dagger}P < 0.05$ HF + NBI-27914 versus HF + vehicle

Fig. 3. PVN levels of norepinephrine (NE), glutamate and GABA in heart failure (HF) and sham operated (SHAM) rats treated for 4 weeks with bilateral PVN infusion of NBI-27914 or vehicle. Bilateral PVN infusions of the selective CRH-R1 antagonist NBI-27914 prevented, the decrease in PVN GABA, and the increases in PVN glutamate and NE observed in HF rats. IP treatment with the same dose of NBI-27914 did not alter NE, glutamate, and GABA in the PVN of HF rats. * $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; † $P < 0.05$ HF + NBI-27914 versus HF + vehicle

Fig. 4.

Western blot of TH and GAD67 in the PVN showed that HF rats had higher levels of TH and lower levels of GAD67 when compared with SHAM rats. Bilateral PVN infusions of NBI-27914 decreased expression of TH, and increased GAD67 expression in the PVN of HF rats. $*P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; $^{\dagger}P < 0.05$ HF + NBI-27914 versus HF + vehicle

Fig. 5.

The effects of bilateral PVN infusion of NBI-27914 on neuronal activity in the PVN of rats with ischemia-induced HF. Fos-LI activity (*black dots*), an indicator of chronic neuronal excitation, increased in the PVN of HF rats when compared with SHAM rats. Fos-LI activity was lower in the NBI-27914-treated HF rats than in vehicle-treated HF rats. * $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; † $P < 0.05$ HF + NBI-27914 versus HF + vehicle

Fig. 6.

Renal sympathetic nerve activity (RSNA) was increased in HF rats compared with SHAM rats. RSNA of HF rats treated with bilateral PVN infusion of NBI-27914 was lower than vehicle-treated HF rats. * $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle; † $P < 0.05$ HF + NBI-27914 versus HF + vehicle

Table 1Echocardiographic measurements ($n = 14$)

	HF + NBI-27914	HF + vehicle	SHAM + NBI-27914	SHAM + vehicle
Measurements at baseline				
LVEDV (ml)	$0.72 \pm 0.08^*$	$0.71 \pm 0.07^*$	0.36 ± 0.05	0.39 ± 0.05
LVEDV/Mass	$1.05 \pm 0.08^*$	$1.08 \pm 0.09^*$	0.54 ± 0.05	0.59 ± 0.06
LVEF	$0.35 \pm 0.04^*$	$0.37 \pm 0.05^*$	0.82 ± 0.05	0.84 ± 0.07
IZ (%)	48 ± 4	46 ± 4	–	–
Measurements at 4 weeks				
LVEDV (ml)	$1.35 \pm 0.07^{*,\ddagger}$	$1.40 \pm 0.08^{*,\ddagger}$	0.32 ± 0.03	0.35 ± 0.04
LVEDV/Mass	$1.61 \pm 0.12^{*,\ddagger}$	$1.70 \pm 0.15^{*,\ddagger}$	0.56 ± 0.05	0.61 ± 0.06
LVEF	$0.34 \pm 0.03^{*,\ddagger}$	$0.24 \pm 0.03^{*,\ddagger}$	0.83 ± 0.07	0.80 ± 0.06
IZ (%)	46 ± 4	50 ± 5	–	–

Values are mean $\pm$ SEM

SHAM sham-operated control, HF heart failure, LVEDV left ventricular end-diastolic volume, LVEF left ventricular ejection fraction, IZ% percent ischemic zone

* $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle;

‡ $P < 0.05$ HF + NBI-27914 versus HF + vehicle;

‡ $P < 0.05$, 4 weeks versus 24 h value

Table 2

Hemodynamic and anatomical measurements ($n = 7$)

Measurements at 4 weeks	RV/BW (mg/g)	Lung/BW (mg/g)	HR (beats/min)	MAP (mmHg)	PP (mmHg)	LVEDP (mmHg)
HF + NBI-27914	0.71 ± 0.09 [‡]	5.1 ± 0.6 [‡]	328 ± 14	93 ± 8	33 ± 4	7.61 ± 1.57 [‡]
HF + vehicle	1.10 ± 0.12 [*]	10.2 ± 0.7 [*]	334 ± 14	91 ± 7	35 ± 5	20.26 ± 1.90 [*]
SHAM + NBI-27914	0.56 ± 0.06	4.3 ± 0.4	323 ± 12	101 ± 8	36 ± 4	6.56 ± 1.42
SHAM + vehicle	0.64 ± 0.07	4.8 ± 0.5	326 ± 13	103 ± 9	38 ± 5	7.01 ± 1.46

Values are mean ± SEM

SHAM sham-operated control, HF heart failure, BW body weight, RV right ventricular, HR heart rate, MAP mean arterial pressure, PP pulse pressure, LVEDP left ventricular end-diastolic pressure

^{*} $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle;

[‡] $P < 0.05$ HF + NBI-27914 versus HF + vehicle

Table 3Proinflammatory cytokines in the PVN and plasma ($n = 7$)

Measurements at 4 weeks	PVN (pg/mg protein)			Plasma (pg/ml)		
	TNF- α	IL-1 β	IL-6	TNF- α	IL-1 β	IL-6
HF + NBI-27914	3.9 $\pm$ 0.4 [†]	20.2 $\pm$ 2.7 [†]	25.2 $\pm$ 2.4 [†]	14.4 $\pm$ 1.3 [†]	63.4 $\pm$ 6.1 [†]	41.5 $\pm$ 4.3 [†]
HF + vehicle	7.2 $\pm$ 0.6*	50.2 $\pm$ 4.9*	63.2 $\pm$ 6.0*	34.1 $\pm$ 3.3*	112.5 $\pm$ 10.2*	94.5 $\pm$ 9.2*
SHAM + NBI-27914	3.2 $\pm$ 0.4	17.9 $\pm$ 1.6	20.3 $\pm$ 1.9	11.2 $\pm$ 1.2	54.3 $\pm$ 4.7	34.2 $\pm$ 3.8
SHAM + vehicle	3.4 $\pm$ 0.4	19.1 $\pm$ 2.4	23.4 $\pm$ 2.0	12.5 $\pm$ 1.2	58.4 $\pm$ 5.2	38.3 $\pm$ 4.1

* $P < 0.05$ versus SHAM + NBI-27914 or SHAM + vehicle;[†] $P < 0.05$ HF + NBI-27914 versus HF + vehicle