Hypertension

NERVOUS SYSTEM

Inhibition of Brainstem Endoplasmic Reticulum Stress Rescues Cardiorespiratory Dysfunction in High Output Heart Failure

Hugo S. Díaz, David C. Andrade, Camilo Toledo, Karla G. Schwarz, Katherin V. Pereyra, Esteban Díaz-Jara, Noah J. Marcus, Rodrigo Del Rio

ABSTRACT: Recent evidence shows that chronic activation of catecholaminergic neurons of the rostral ventrolateral medulla is crucial in promoting autonomic imbalance and cardiorespiratory dysfunction in high output heart failure (HF). Brainstem endoplasmic reticulum stress (ERS) is known to promote cardiovascular dysfunction; however, no studies have addressed the potential role of brainstem ERS in cardiorespiratory dysfunction in high output HF. In this study, we assessed the presence of brainstem ERS and its potential role in cardiorespiratory dysfunction in an experimental model of HF induced by volume overload. High output HF was surgically induced via creation of an arterio-venous fistula in adult male Sprague-Dawley rats. Tauroursodeoxycholic acid (TUDCA), an inhibitor of ERS, or vehicle was administered intracerebroventricularly for 4 weeks post-HF induction. Compared with vehicle treatment, TUDCA improved cardiac autonomic balance (LF_{HRV}/HF_{HRV} ratio, 3.02±0.29 versus 1.14±0.24), reduced cardiac arrhythmia incidence (141.5±26.7 versus 35.67±12.5 events/h), and reduced abnormal respiratory patterns (Apneas: 11.83±2.26 versus 4.33±1.80 events/h). TUDCA administration (HF+Veh versus HF+TUDCA, $P<0.05$) attenuated cardiac hypertrophy (HW/BW 4.4±0.3 versus 4.0±0.1 mg/g) and diastolic dysfunction. Analysis of rostral ventrolateral medulla gene expression confirmed the presence of ERS, inflammation, and activation of renin-angiotensin system pathways in high output HF and showed that TUDCA treatment completely abolished ERS and ERS-related signaling. Taken together, these results support the notion that ERS plays a role in cardiorespiratory dysfunction in high output HF and more importantly that reducing brain ERS with TUDCA treatment has a potent salutary effect on cardiac function in this model. **(*Hypertension*. 2021;77:718–728. DOI: 10.1161/HYPERTENSIONAHA.120.16056.)**

• **Data Supplement**

Key Words: apnea ■ chemoreceptor cells ■ endoplasmic reticulum stress ■ heart failure ■ sympathetic nervous system

Heart failure (HF) affects more than 26 million people worldwide,[1] and its prevalence is expected to double by 2030.[2] In clinical populations, HF without reduced ejection fraction has similar prevalence and mortality as HF with reduced ejection fraction, however, the mechanisms underlying morbidity and mortality in HF without reduced ejection fraction are diverse and poorly understood.[1] In both HF subsets, sympathoexcitation and respiratory disorders are associated with worse prognosis.[3–6] Previous work in volume overload HF models (no reduction in ejection fraction) has shown that C1 catecholaminergic neurons in the rostral ventrolateral medulla (RVLM), a major site for sympathetic nervous system (SNS) integration,[3] become chronically hyper-activated,[7] and their selective ablation reduces autonomic, respiratory, and cardiac dysfunction.[8,9] These results suggest that targeting the central nervous system in high output HF can improve cardiorespiratory function and HF outcomes. The molecular mechanisms underlying hyper-activation of C1 neurons in high output HF are incompletely understood, however, local renin-angiotensin system activation in the brain (bRAS) has been shown to play a major role in the pathophysiology of low output HF.[10] Increased bRAS activity in low output HF has been observed in areas of the brainstem associated

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Novelty and Significance

What Is New?

- The present study shows that brainstem endoplasmic reticulum stress drives cardiorespiratory alterations in high output heart failure (HF), and that pharmacological inhibition of endoplasmic reticulum stress markedly reduced cardiorespiratory dysfunction in HF.

What Is Relevant?

- Our findings support the therapeutic potential of endoplasmic reticulum stress inhibitors in the management of human high output HF.

Summary

HF is a multifactorial disease that currently lacks effective treatments. Chronic sympathoexcitation and breathing disorders have both been linked to disease progression and poor prognosis. In this study, we present the novel finding that inhibition of brain endoplasmic reticulum stress in compensated high output HF restores cardiac autonomic balance, normalizes resting breathing patterns, and significantly improves cardiac function. In conclusion, our results indicate promise for the use of endoplasmic reticulum stress inhibitors in the treatment of high output HF.

Nonstandard Abbreviation and Acronyms

bRAS	renin-angiotensin system activation in the brain
ERS	ndoplasmic reticulum stress
HF	heart failure
HF_{HRV}	high frequency component of heart rate variability
HRV	heart rate variability
LF_{HRV}	low frequency component of heart rate variability
MAPK	mitogen-activated protein kinase
RTN	retrotrapezoid nucleus
RVLM	rostral ventral lateral medulla
SNS	sympathetic nervous system
TUDCA	tauroursodeoxycholic acid

with SNS control,[11–15] and increased bRAS activity promotes increased neuron firing,[16,17] increased production of microglial cytokines,[18,19] and increased astrocytic angiotensin II processing.[20,21] This, in turn, results in activation of a positive feedback loop that perpetuates SNS and cardiorespiratory dysfunction in low output HF.[3,10] Interestingly, augmented plasma angiotensin II levels has also been reported in high output HF patients and is associated with sympathoexcitation and poorer prognosis.[5] Despite this evidence, no studies have directly addressed the role of bRAS in the setting of high output HF.

Endoplasmic reticulum stress (ERS) has received significant attention in the field of cardiovascular diseases[22] as it plays a pivotal role in regulating signaling pathways associated with bRAS activation. Indeed, angiotensin II administration promotes ERS-dependent oxidative stress and inflammation in central SNS control areas.[23,24] Conversely, ERS induction upregulates bRAS and production of reactive oxygen species in the RVLM, inducing systemic hypertension.[24] Downstream signaling targets of ERS include but are not limited to MAPKs (mitogen-activated protein kinases)[14,25] and Nuclear Factor-kappa B.[3,10,26] Tauroursodeoxycholic acid (TUDCA), a taurine derivate of ursodeoxycholic acid, has been shown to inhibit ERS.[22] Importantly, pretreatment of rats with intracerebroventricular administration of TUDCA before myocardial infarction to induce HF significantly reduces upregulation of bRAS in SNS control nuclei and reduces cardiac and autonomic dysfunction.[14] These promising results strongly suggest that targeting brain ERS may have therapeutic potential. However, to date no studies have examined the expression of ERS biomarkers in the brain nor the salutary potential of TUDCA to improve cardiorespiratory function in high output HF.

Considering that hyper-activation of RVLM C1 neurons is associated with increased activation of bRAS and production of reactive oxygen species,[7,27] we hypothesized that brain ERS plays a major role in pathophysiology of high output HF. We addressed this hypothesis by administering intracerebroventricular TUDCA in compensated volume overload HF rats and assessing cardiovascular and respiratory function during disease progression.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files. For extended methods please refer to supplemental material.

Animals

Twenty-two male Sprague-Dawley rats (250±12 g) were obtained from the Animal Facility at the Pontificia Universidad Católica de Chile. Animals were housed under controlled temperature and humidity conditions and had ad libitum access to water and food. At the end of the experiments, animals were humanely euthanized with an overdose of sodium pentobarbital (100 mg/kg intraperitoneally).

Overview of Experimental Procedures

Rats underwent surgery to induce high output HF (or sham surgery) and were subsequently allowed to recover. At 4 weeks postsurgery, cardiac function was assessed via echocardiography, and rats were then randomly assigned to the following experimental groups: Sham+vehicle (Sham+Veh, n=6), Sham+TUDCA (n=4), HF+Veh (n=6), and HF+TUDCA (n=6). Animals then underwent surgery for implantation of an osmotic minipump to deliver intracerebroventricular vehicle or TUDCA. Three weeks postosmotic mini-pump surgery (7 weeks post-Sham/HF surgery), animals were implanted with a radio-telemetry device for measurement of arterial pressure. Animals were allowed 1 week to recover from surgery before any physiological recordings were made (Figure 1A). At 8 weeks post-HF surgery or Sham, animals underwent echocardiography to assess cardiac function after TUDCA or vehicle treatment.

High Output HF

High output HF was induced by volume overload as previously described.[7,8,27–29]

Echocardiographic Assessment of Cardiac Function

Transthoracic echocardiography was performed as previously described.[7,8]

Intracerebroventricular TUDCA Administration

Four weeks post-HF or Sham surgery, rats were anesthetized (Ketamine:100 mg/kg; and xylazine:10 mg/kg) and fixed to a stereotaxic frame for implantation of an osmotic mini-pump containing TUDCA or Vehicle solution. TUDCA concentrations were adjusted to deliver 10 µg/day (0.25 µL/h) for 28 days. Postoperative analgesia and supportive care was provided via ketoprofen (1 mg, SC) and enrofloxacin (1 mg, SC), respectively.

Telemetry Implantation

At week 7 postsurgery, a pressure transmitter was surgically implanted.[8,28]

Invasive Cardiac Hemodynamic Assessment

At the conclusion of the experimental timeline, animals were anesthetized with α-chloralose and urethane (40 and 800 mg/kg, respectively) for intraventricular pressure-volume loops analysis.[7,8,27–29]

Assessment of Ventilatory Pattern and Chemoreflex Gain

Resting breathing and chemoreflex were recorded using whole-body plethysmography.[7–9] Breathing irregularity was visualized and quantified using Poincare plots.[7–9] Apneic episodes, hypopneas, sigh frequency, and postsigh apneas were scored as previously described.[7–9] Peripheral and central chemoreflex gain were determined from the hypoxic ventilatory response and hypercapnic ventilatory responses, respectively.[7–9]

Active Expiration Analysis

Increases in the ratio (E2/E1) between late expiration (E2) and early expiration (E1) was used as the indicator of active expiration.[28]

Cardiac Autonomic Function and Cardiorespiratory Coupling

Cardiac autonomic function was evaluated by analysis of heart rate variability (HRV), as previously described.[8,28] The magnitude of the square coherence function was used to determine cardiorespiratory coupling using tidal volume (V_T) and systolic blood pressure as inputs signals as described previously.[28] Coherence was assessed at the frequency of the maximum V_T spectral peak in the very low frequency domain.[28]

Arrhythmia Incidence

Arrhythmia index (events/h) was assessed in 60 minutes tachograms derived from the telemetry blood pressure signal. This technique cannot identify specific types of isolated arrhythmic events, but it can differentiate prolonged runs (sinus or ventricular tachycardias or bradycardias and atrial fibrillation) from isolated ectopic events (PVC or PACs).[7,8,18]

RVLM Gene Expression of ERS, bRAS, and Inflammatory Biomarkers

Rat brains were immediately removed after euthanasia, frozen in liquid nitrogen, and stored at −80 °C. The RVLM was punched bilaterally using a blunt 18-gauge needle attached to a syringe.[7] RNA isolation and cDNA synthesis were performed using the RNAqueous Micro (Ambion) and iScript (Promega) kits, respectively, according to manufacturer instructions. Gene expression of ERS, bRAS, and inflammatory biomarkers was assessed by real-time polymerase chain reaction. Primers nucleotide sequences are shown in Table S4 in the Data Supplement. β-Actin mRNA was quantified as an internal control for each sample and quantifications were performed using the $2^{-\Delta\Delta CT}$ method. We also analyzed protein expression levels of the ERS chaperone Binding immunoglobulin Protein. Micropunches were lysed in radioimmunoprecipitation assay buffer, and proteins were then electrophoresed in polyacrylamide gels (10%), transferred to polyvinylidene fluoride membranes (Millipore), and probed against BiP and GAPDH. The relative amount of protein of interest was calculated as the ratio of intensity of the band relative to the intensity of GAPDH.

DATA ANALYSIS

Data are presented as mean±SE in text and tables. Median and interquartile ranges are shown in violin plots. Correlations were performed using Pearson analysis. Statistical significance of data was evaluated using 1-way ANOVA or 2-way ANOVA according to data structure, followed by corresponding post hoc analysis. The level of significance was defined as $P<0.05$.

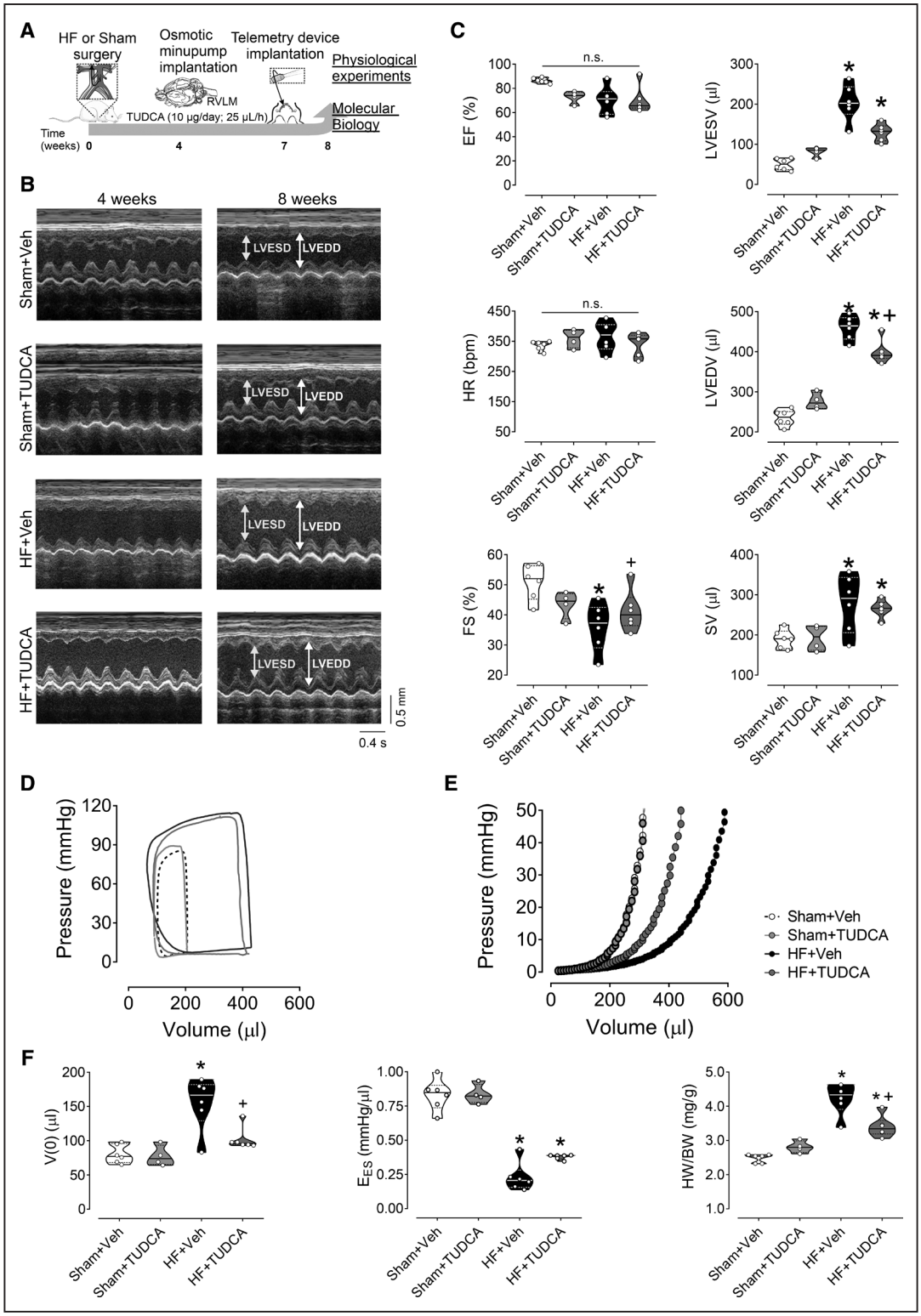

Figure 1. Tauroursodeoxycholic acid (TUDCA) attenuates cardiac dysfunction in heart failure (HF).
A, General description of experimental procedures. **B**, Representative echocardiographic images of the LV of Sham, Sham+Veh, Sham+TUDCA, HF+Veh, and HF+TUDCA rats at 4- and 8-weeks postinduction surgery. **C**, Summary data showing quantification of echocardiographic parameters. **D**, Representative pressure-volume loops in all experimental conditions. **E**, Exponential curves representing diastolic function as measured by the end diastolic pressure-volume relationship (EDPVR). **F**, Summary data from single-beat analysis of EDPVR (V0) and ESPVR (E_{ES}) and effect of TUDCA on cardiac hypertrophy (expressed as heart weight to body weight [HW/BW] ratio). Note that HF+TUDCA animals show a marked improvement in diastolic function and significantly reduced cardiac hypertrophy. Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). EF% indicates ejection fraction; FS%, fractional shortening; HR, heart rate; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; RVLM, rostral ventral lateral medulla; and SV, stroke volume. One-way ANOVA and Holm-Sidak posthoc analysis *P<0.05 vs Sham+Veh and +P<0.05 vs HF+Veh.

RESULTS

TUDCA Prevents Cardiac Hypertrophy and Diastolic Dysfunction in High Output HF

At experimental week 8, cardiac function was assessed via echocardiography (Figure 1B, Table S1). At this timepoint, HF+Veh rats display dilated cardiac chambers without change in ejection fraction compared with Sham rats. In HF rats, TUDCA treatment significantly reduced ($P<0.05$) left ventricular end systolic and end diastolic volumes (end systolic volume: 180.1±28.9 versus 135.5 180.1±8.0 µL; end diastolic volume: 452.4±11.8 versus 400.2±14.5 µL; HF+Veh versus HF+TUDCA) and prevented decreases in (fractional shortening: 36.0±3.2% versus 41.3±2.8%; HF+Veh versus HF+TUDCA), suggesting that TUDCA administration prevented worsening of cardiac function in HF. TUDCA had no deleterious effects on cardiac dimensions/function in Sham+TUDCA rats (Figure 1). Invasive hemodynamic analysis of cardiac function by single-beat PV loops (Figure 1D through 1F, Table S2) revealed that TUDCA treatment effectively prevented diastolic dysfunction in HF rats (Figure 1J and 1L). Also, no differences in resting heart rate nor in blood pressure were found between groups (Table S3). Additionally, TUDCA significantly attenuated increases in the cardiac hypertrophy index in HF rats (heart weight to body weight ratio, HW/BW: 4.35±0.27 versus 3.99±0.13 mg/g; HF+Veh versus HF+TUDCA, $P<0.05$).

TUDCA Prevents Autonomic Dysfunction and Reduces Arrhythmias in High Output HF

Autonomic dysfunction is directly related to cardiac arrhythmogenesis and cardiac dysfunction in HF.[3,30] We examined the effects of TUDCA administration on cardiac sympatho-vagal balance in HF rats using spectral analysis of HRV in conscious animals at rest. As shown in Figure 2, HF+Veh rats had significant shifts in the low frequency (LF_{HRV}) and high frequency (HF_{HRV}) components of HRV compared with Sham rats. HF+Veh rats showed higher LF_{HRV}/HF_{HRV} ratio (autonomic imbalance) compared with Sham animals, and TUDCA administration prevented changes in HRV in HF-TUDCA rats (LF_{HRV}/HF_{HRV}: 3.02±0.29 versus 1.14±0.24; HF+Veh versus HF+TUDCA, $P<0.05$). In addition, TUDCA treatment markedly reduced arrhythmia scores in HF rats relative to vehicle-treated HF rats (141.5±26.7 versus 35.7±12.5; HF+Veh versus HF+TUDCA, $P<0.05$). Most of the arrhythmic events detected in HF animals were related to premature ventricular contractions. Neither episodes of atrial fibrillation nor sustained ventricular arrhythmias were detected in HF+Veh or HF+TUDCA

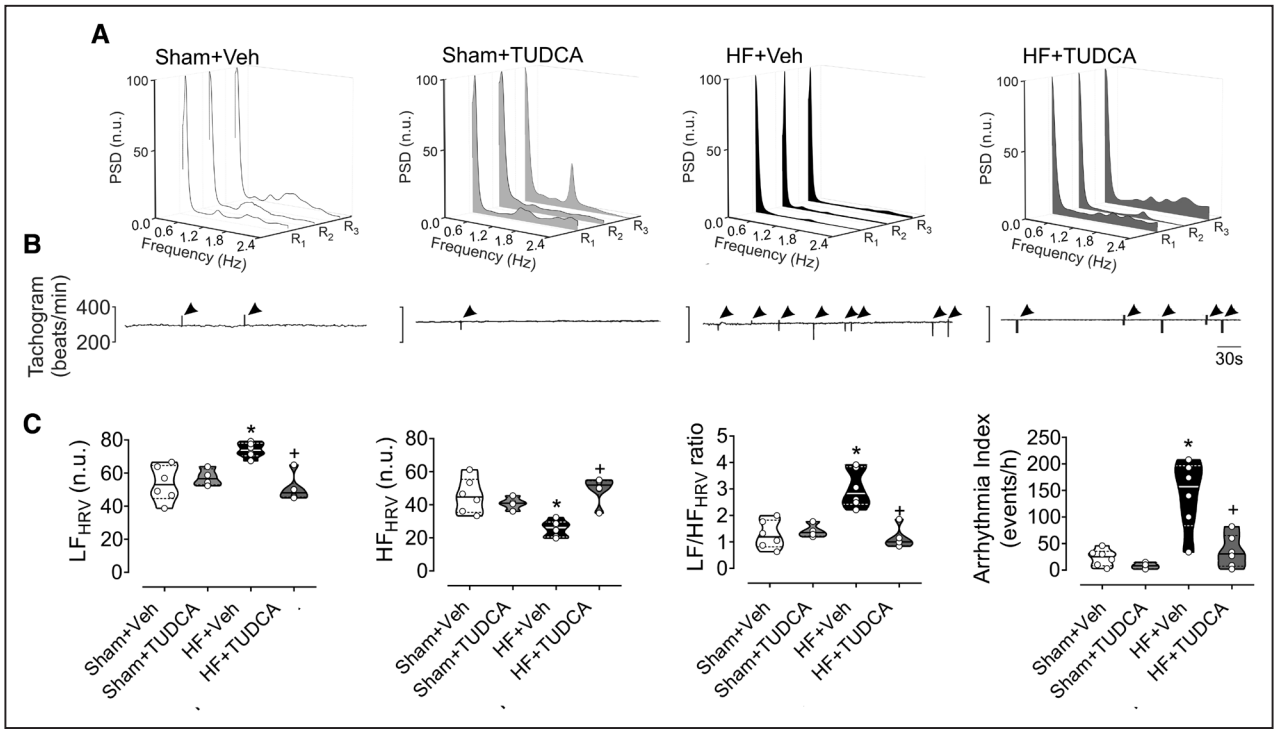

Figure 2. Tauroursodeoxycholic acid (TUDCA) prevents cardiac autonomic imbalance and arrhythmias in heart failure (HF). **A**, Representative heart rate variability (HRV) spectras obtained in 3 different rats from all experimental conditions. Note that HF+Veh rats displayed a marked increase in the low frequency HRV component (LF_{HRV}, 0.04–0.6 Hz) and TUDCA treatment markedly reduced LF_{HRV}. **B**, Representative heart rate tachograms obtained over 5 min in Sham+Veh, Sham+TUDCA, HF+Veh, and HF+TUDCA rats. **C**, Summary data showing the changes in LF_{HRV}, high frequency (HF_{HRV}, 0.6–2.4 Hz) and LF_{HRV}/HF_{HRV} ratio. Note that cardiac autonomic imbalance and cardiac arrhythmias were significantly improved following TUDCA treatment. Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). One-way ANOVA and Holm-Sidak posthoc analysis *$P<0.05$ vs Sham+Veh; +$P<0.05$ vs HF+Veh.

rats, and no mortality was observed in either group. TUDCA had no significant effect on autonomic function or arrhythmias in Sham-treated rats (Figure 2).

TUDCA Prevents Disordered Breathing and Increased Central Chemoreflex Sensitivity in HF

Breathing disorders (ie, apneas and hypopneas) and irregular breathing patterns are important pathophysiological hallmarks in HF[4] and are associated with enhanced ventilatory chemoreflex gain.[4,7,28,29] As shown in Figure 3A through 3D, HF+Veh rats had irregular ventilatory frequency and amplitude compared with Sham rats (breath-to-breath SD2: 53.14±2.64 versus 74.47±4.50; coefficient of variation of V_T: 6.74±2.11% versus 12.68±1.05%; Sham+Veh versus HF+Veh, $P<0.05$) and a significantly higher apnea/hypopnea index (4.67±1.31 versus 11.83±2.26 events/h; Sham+Veh versus HF+Veh, $P<0.05$). Also, apnea/hypopnea index was significantly lower in HF+TUDCA rats compared with HF+Veh treated rats (apnea/hypopnea index: 4.33±1.80 versus 11.83±2.26 events/h; HF+TUDCA versus HF+Veh, $P<0.05$). No changes in apnea duration, sigh frequency, and postsigh apneas were found between experimental groups (Figure 3D, Table S4).

In addition, HF+Veh animals had a larger hypercapnic ventilatory response compared with Sham animals, with no differences between groups observed for the hypoxic ventilatory response (Figure 4). These results are in agreement with previous findings of increased chemoreflex gain and breathing instability in high output HF.[7,28,29] In addition, TUDCA administration prevented increases in chemoreflex drive in HF rats. Indeed, a ≈2-fold difference in hypercapnic ventilatory response was found in HF+TUDCA rats compared with HF+Veh rats (hypercapnic ventilatory response: 9.29±1.41 versus 4.75±0.85 mL/min/F_iCO_2%; HF+Veh versus HF+TUDCA, $P<0.05$). TUDCA had no significant effect on hypoxic ventilatory response in any group (Figure 4). No changes in resting ventilation in normoxia were found between groups (Table S3).

TUDCA Prevents Brain ERS and Blunts RVLM-Mediated Respiratory-Sympathetic Coupling

At the completion of the experimental protocol, animals were humanely euthanized, and micro punches from the brainstem containing the RVLM were extracted and prepared for real-time quantitative polymerase chain reaction analysis of biomarkers of ERS, bRAS activation, and

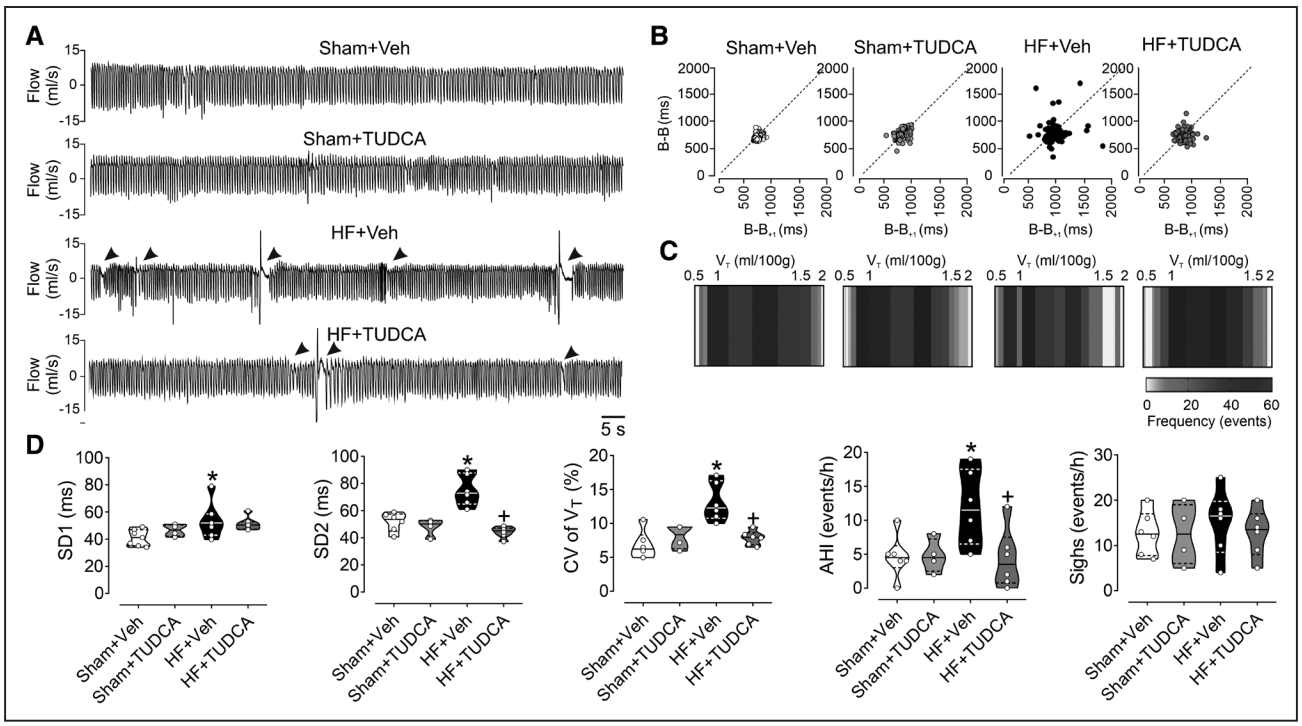

Figure 3. Tauroursodeoxycholic acid (TUDCA) normalizes irregular breathing in heart failure (HF).
A, Representative traces of resting breathing obtained in one animal from each experimental group. Arrowheads represent breathing disturbances (ie, apnea, hypopnea). **B**, Representative Poincaré plots of the breath-to-breath (BB) interval variability from Sham+Veh, Sham+TUDCA, HF+Veh, and HF+TUDCA rats. **C**,Representative frequency histogram analysis of tidal volume (V_T) amplitude oscillation in one rat per experimental condition. **D**, Summary data showing the effects of TUDCA on short-term (SD1) and long-term (SD2) variability of the BB interval. **F**, Summary data showing V_T coefficient of variation (CV of V_T), as well as the incidence of apneas and hypopneas and sighs during 1 h of resting breathing recordings. Note that TUDCA reduced breathing variability, apnea incidence in HF. Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). AHI indicates Apnea/Hypopnea Index. One-way ANOVA and Holm-Sidak posthoc analysis *$P<0.05$ vs Sham+Veh; +$P<0.05$ vs HF+Veh.

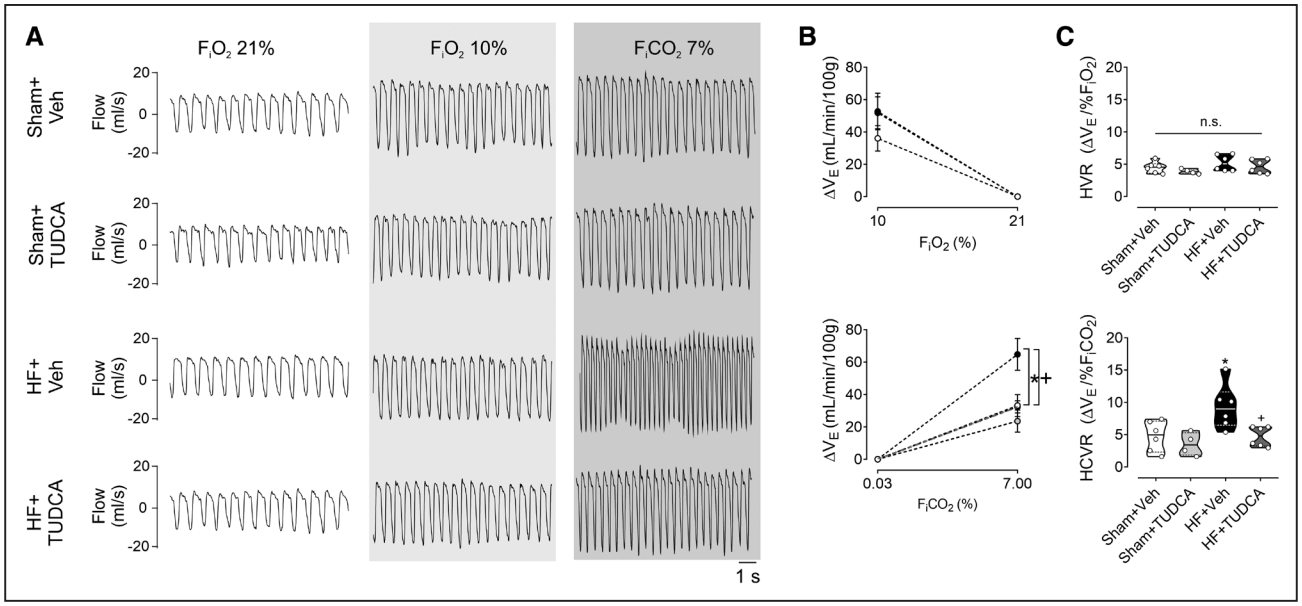

Figure 4. Tauroursodeoxycholic acid (TUDCA) reduces high central chemoreflex gain in heart failure (HF).
A, Representative ventilatory traces from one animal from each experimental group during chemoreflex studies. **B**, Changes in minute-ventilation (V_E) after hypoxic and hypercapnic challenges. **C**, Summary data showing the gain of the hypoxic ventilatory response (HVR) and the ventilatory response to hypercapnia (HCVR). Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). One-way ANOVA and Holm-Sidak posthoc analysis *P<0.05 vs Sham+Veh; +P<0.05 vs HF+Veh.

inflammation (Figure 5B through 5C; See Table S5 for primers). Compared with Sham rats, HF+Veh rats showed increased RVLM expression (P<0.05) of: ERS biomarkers BiP, CCAAT-enhancer-binding protein Homologous Protein, and spliced form of X-box binding protein 1 (3.2-,3.1-, and 4.3-fold increase versus Sham, respectively); bRAS activation markers AT1 and gp91^{phox} (6.8- and 9.0-fold increase versus Sham, respectively); and proinflammatory cytokines Tumor Necrosis Factor-α and Interleukin-1β (6.4- and 2.2-fold increase versus Sham, respectively). TUDCA treatment prevented increases in ERS, bRAS, and inflammatory biomarker expression in the RVLM of HF-treated rats (Figure 5).

We have previously observed respiratory-sympathetic coupling in high output HF rats,[7,28,29] and other studies have shown that enhanced ventilatory chemoreflex gain is closely linked to increased coupling of respiratory and cardiovascular function and active expiration at rest.[31,32] Based on these previous findings, we became interested in the possibility that TUDCA treatment could ameliorate pathological respiratory coupling in HF rats. To address this possibility, we first determined if active expiration was affected in HF+TUDCA rats (Figure 6A and 6B). Compared with HF-vehicle rats, HF- TUDCA rats had significantly lower measures of active expiration as quantified by late-to-early ratio (E2/E1) in the ventilatory expiration phase (E2/E1: 0.94±0.07 versus 0.74±0.05; HF+Veh versus HF+TUDCA, P<0.05). Based on a higher coherence between tidal volume oscillation and systolic blood pressure, we determined that respiratory-sympathetic coupling was present in HF rats versus Sham rats, and we observed that TUDCA treatment in HF effectively eliminated respiratory-sympathetic coupling (Figure 6C). In addition, the relationship between respiratory-sympathetic coupling and active expiration in HF rats (r=0.814, P=0.048) was blunted in HF-TUDCA rats (r=0.475, P=0.341, Figure 6D).

DISCUSSION

Sympathoexcitation is associated with cardiorespiratory dysfunction and higher mortality rates in HF, independent of its cause.[5] SNS activity is controlled by a diffuse network of autonomic nuclei[3] including the hypothalamic paraventricular nucleus,[12,13] the nucleus of the solitary tract,[11] the RVLM,[7–9] and sensory circumventricular organs.[25] Recently, we have shown that RVLM C1 neurons play a central role in the regulation of cardiac sympathetic activity in volume overload HF rats,[7] and that specific ablation of RVLM C1 neurons improves cardiac and autonomic function in this model.[8,9] Furthermore, we have found that specific ablation of central chemoreceptor neurons in the retrotrapezoid nucleus (RTN) normalizes respiratory dysfunction.[28] Taken together, these studies suggest that brainstem structures associated with cardiorespiratory regulation play important roles in key pathophysiological aspects of high output HF.

Activation of bRAS in SNS control nuclei has been proposed to play a pivotal role in the pathophysiology of high output HF[3,11]; however, it is still unclear how this translates to autonomic dysfunction. We propose that ERS represents an important connection between bRAS activation, reactive oxygen species formation, cytokine production, and related sympathoexcitation in the setting of volume overload HF.

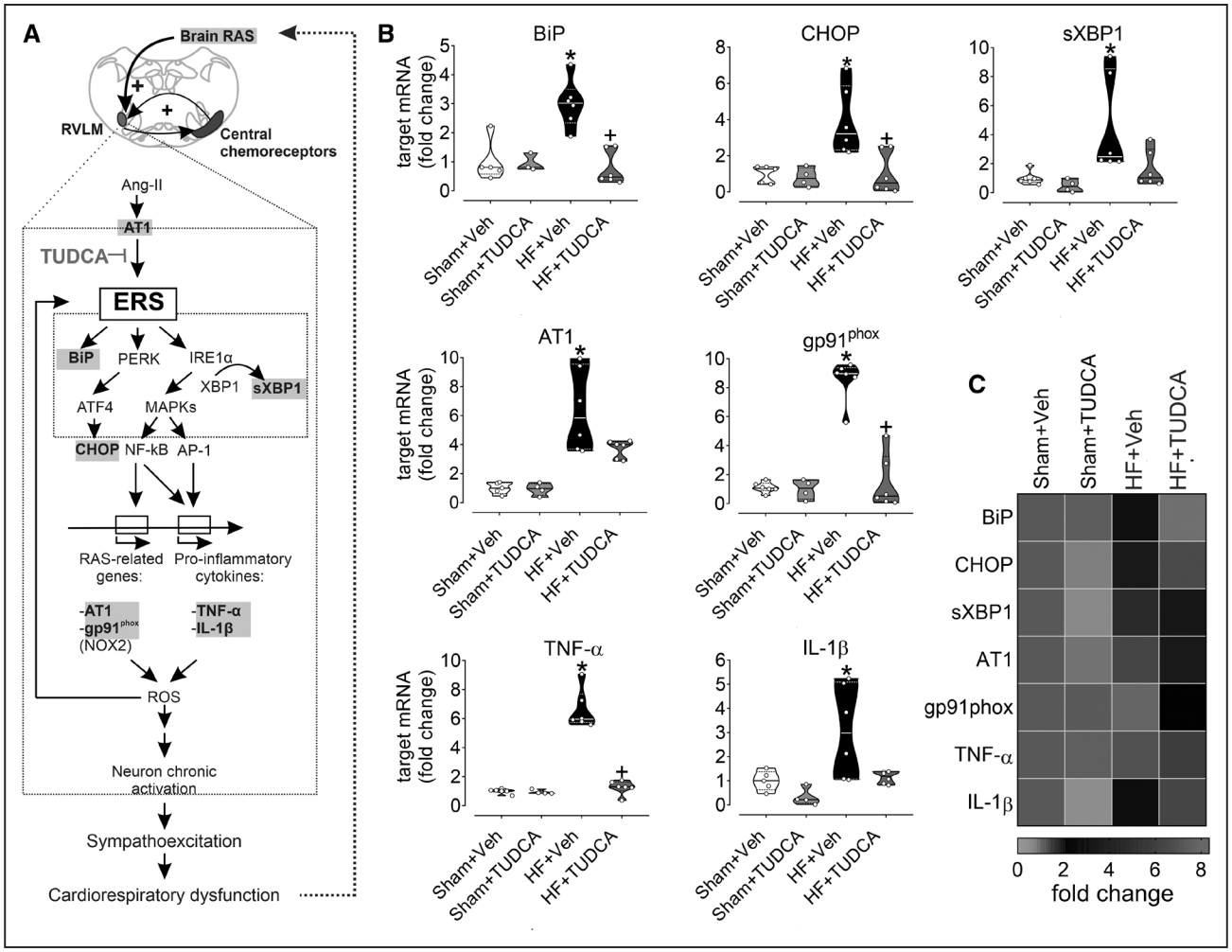

Figure 5. Tauroursodeoxycholic acid (TUDCA) prevents endoplasmic reticulum stress (ERS) signaling in the RVLM in heart failure (HF). **A**, Schematic depicting hypothesized signaling pathways responsible for chronic RVLM neuronal activation and consequent autonomic imbalance in HF. Components of this signaling cascade that were measured are highlighted in gray. **B**, mRNA expression levels of ERS, bRAS, and neuroinflammatory biomarkers taken from RVLM micropunches. **C**, Heatmap summary data showing the effects of TUDCA on mRNA expression profiles. Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). One-way ANOVA and Holm-Sidak posthoc analysis *P<0.05 vs Sham+Veh; +P<0.05 vs HF+Veh. AP-1 indicates activator protein 1; BiP, binding immunoglobulin Protein; CHOP, CCAAT-enhancer-binding protein Homologous Protein; IL-1β, interleukin-1β; MAPK, mitogen-activated protein kinase; NF-Kb, nuclear factor-kappa B; NOX2, nicotinamide adenine dinucleotide phosphate oxidase 2; PERK, protein kinase R-like endoplasmic reticulum kinase; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; TNF-α, tumor necrosis factor-α.

Importantly, these interrelationships have been shown in low output HF models.[14,23,25] In this study, we show for the first time that ERS is present, and that downstream targets of ERS signaling cascades are upregulated in the RVLM in a model of high output HF. We provide compelling evidence that preventing ERS via intracerebroventricular administration of TUDCA has a powerful salutary effect on cardiac dysfunction and reduces the incidence of cardiac arrhythmias. Furthermore, we show that TUDCA treatment reduces central chemoreflex gain, disordered breathing patterns, and respiratory-sympathetic coupling. Our results make a compelling case that reducing ERS in the central nervous system is an effective strategy to improve cardiovascular and respiratory outcomes in high output HF.

ERS is a normal biological response to a variety of stimuli, including but not limited to bRAS activation.[23,24,26,33] Expression levels of BiP, CHOP, and sXBP1 increase during ERS induction and thus serve as reliable biomarkers of ERS.[34] Studies performed in low output HF suggest that ERS can contribute to bRAS-induced neuroinflammation through MAPK-related signaling pathways,[14,25] and while bRAS activation in rats with high output HF has been shown[27] its effects on regulation of autonomic and cardiorespiratory function is unknown. Accordingly, we propose that in high output HF, ERS play a pivotal role in altering neuronal function via effects on bRAS activity and increased production of reactive oxygen species and proinflammatory cytokines (Figure 5A). Our finding that treatment with TUDCA reduced RVLM expression of AT1 receptor, gp91^{phox}, TNF-α, and IL-1β in volume overload HF rats supports this hypothesis. Future investigations should specifically focus on further elucidating

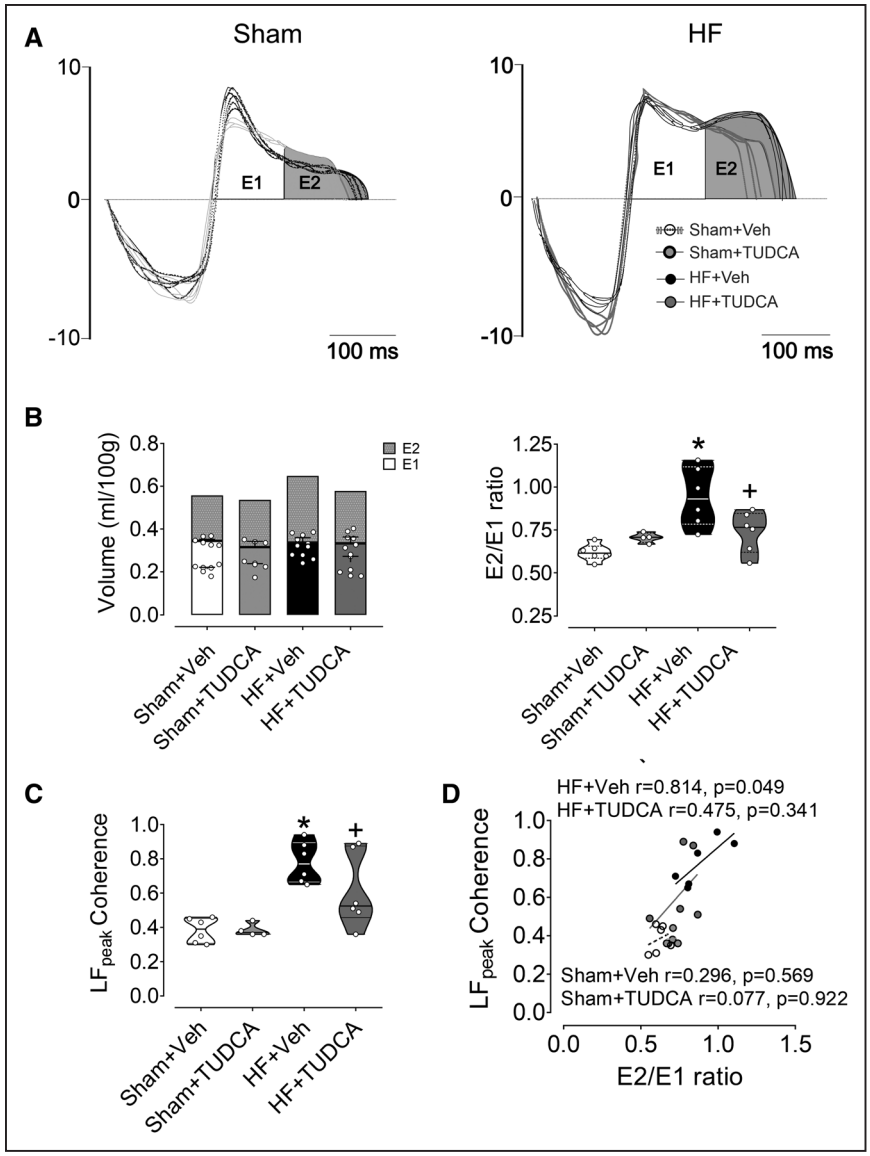

Figure 6. Tauroursodeoxycholic acid (TUDCA) abolishes cardiorespiratory coupling in heart failure (HF). **A**, Representative respiratory flow traces from Sham+Veh, Sham+TUDCA, HF+Veh, and HF+TUDCA rats showing expiratory phases. **B**, Summary data showing quantification of active expiration. **C**, Summary data displaying the magnitude of coherence between V_T oscillations and SBP signals. **D**, Cardiorespiratory coupling (vLF peak coherence) significantly correlates with active expiration (E2/E1) in HF rats and this correlation was lost after TUDCA treatment. Violin plots show data as median±quartiles. n: Sham (6), Sham+TUDCA (4), HF (6), HF+TUDCA (6). LF indicates low frequency. One-way ANOVA and Holm-Sidak posthoc analysis *P<0.05 vs Sham+Veh; +P<0.05 vs HF+Veh.

ERS signaling pathways and their downstream targets. Due to the method of drug delivery in our study (ie, intracerebroventricular administration), we cannot rule out the possibility that multiple cell types are affected by ERS. Future studies are needed to more specifically identify all cell types affected by ERS and more discretely identify cell populations within this group that contribute to cardiorespiratory dysfunction in high output HF.

Disordered breathing patterns in HF are linked to increases in chemoreflex gain independent of HF cause.[4,7,28,29] We have previously shown that central chemoreflex gain is a major source of breathing instability, while peripheral chemosensitivity is relatively unchanged in the volume overload model of high output HF.[7,28] There are several putative sites for central chemoreception in the brain; however, the RTN accounts for ≈80% of central chemoreflex function.[4,32,35] RTN chemoreceptor cells project to the central pattern generator[4,35] and to the RVLM,[31,36] indicating they have the ability to regulate both pulmonary ventilation and autonomic function.[4] Activation of RTN chemosensitive neurons induces cardiorespiratory coupling and generation of active expiration, both of which are associated with heightened sympathetic nerve activity.[31,32] A recent study from our laboratory found that episodic stimulation of central chemoreceptors in high output HF results in entrainment of respiratory and sympathetic activity.[28] In the present study, we found that central chemoreflex gain, active expiration, and cardiorespiratory coupling in high output HF are abolished by attenuating ERS with TUDCA treatment. Therefore, it is plausible that ERS in the RTN also contributes to increased central chemoreflex gain. Identification of the molecular mechanisms responsible for enhanced central chemoreflex gain in high output HF is outside of the scope of the present study; however, the fact that TUDCA treatment reduced central chemoreflex gain and normalized resting breathing patterns suggests that ERS also plays a role in altering RTN neuron

function in this model of high output HF. With that said, it has been shown that RVLM neurons can regulate/modulate breathing.[37] Therefore, it is plausible that RVLM neurons projecting to the RTN contribute at least in part to changes in central chemoreflex gain in high output HF. Indeed, we recently reported that ablation of RVLM-C1 neurons markedly improved breathing irregularity in rats with high output HF.[9] Whether these effects are associated with direct changes in RTN neuron function or with changes in RVLM neuron function that is transmitted to the RTN remains to be determined. Regardless, TUDCA effectively reduced central chemoreflex drive and reduced the incidence of breathing disorders in the setting of high output HF. The precise contribution of the RVLM, RTN, or other brain regions to these beneficial effects requires additional investigation.

Limitations

Experimental volume overload HF results in deterioration of passive properties of the heart (EDPVR and LVEDP) without reductions in ejection fraction. While these changes are present in human HF (eg, aortic regurgitation, HFpEF) further validation of the volume overload HF model is required if it is to be used as a model of preserved ejection fraction HF. However, the presence of sympathoexcitation, chemoreflex potentiation, and breathing disorders in volume overload HF rats make this a useful model for understanding these pathophysiological changes in HF independent of changes in ejection fraction. In this study, we show that attenuation of central ERS with intracerebroventricular. TUDCA in volume overload HF rats improves cardiorespiratory function, suggesting that targeting central ERS may be beneficial in the treatment of high output HF. At this point, whether our results can be translated to human HF requires further study.

Due to the nature of TUDCA administration (continuous infusion into the lateral cerebral ventricle for 4 weeks), we cannot rule out the possibility that TUDCA may have affected other RVLM projecting brain regions that experience ERS which, in turn, could potentially contribute to changes in ERS gene expression in the RVLM. Additionally, we showed upregulation of ERS genes in HF rats which may not perfectly reflect changes in protein expression. However, we found that BiP protein expression (a main chaperone of ERS signaling) matches gene expression data showing a significant increase in HF rats and a marked reduction after TUDCA treatment (See Figure S1). Whether other changes in gene expression related to ERS in high output HF also lead to a significant changes in protein expression remains to be determined. Finally, the data from this study and any inference that can be drawn from it is limited to male sex. Future studies of volume overload HF should be conducted in females as well.

Perspectives

Brainstem catecholaminergic neurons are a nodal point for sympathetic and respiratory dysfunction in high output HF. The molecular/cellular mechanisms underlying these changes in sympathetic/respiratory regulation in high output HF are relatively unknown, thus limiting development of therapeutics which could target the source of dysfunction. This study indicates that inhibition of central ERS is a potentially promising avenue for treatment of cardiorespiratory dysfunction in high output HF.

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Disclosures

None.

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