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Cardiac sympathetic afferent reflex control of cardiac function in normal and chronic heart failure states

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Key points

- Cardiac sympathetic afferents are considered to be essential pathways for transmission of cardiac nociception to the central nervous system during myocardial ischaemia. However, a potential contribution of the CSAR control of cardiac dysfunction in both normal and chronic heart failure (CHF) states remains unknown.
- We found that activation of the CSAR evokes little increase in cardiac contractility with an exaggerated peripheral vasoconstriction in the CHF state.
- CSAR inhibition by epicardial lidocaine decreased cardiac contractility to a greater extent in CHF rats than sham rats. Furthermore, we also found that epicardial lidocaine paradoxically decreased left ventricular end-diastolic pressure (LVEDP) and left ventricular end-diastolic volume (preload) in CHF rats, which was not observed in sham rats.
- Chronic ablation of the CSAR by epicardial application of the afferent neurotoxin, RTX, selectively lowered diastolic blood pressure CHF rats.
- The observation suggests that CSAR has a differential effect on cardiac function in normal and CHF states. CSAR activation in normal state causes significant increase in cardiac contractility and cardiac output.

Abstract The enhanced 'cardiac sympathetic afferent reflex' (CSAR) critically contributes to the exaggerated global sympathetic tone in chronic heart failure (CHF). However, a potential contribution of the cardio-cardiac reflex control of cardiac function in both normal and CHF states remains unknown. In this study, we evaluated the effects of direct activation or inhibition of the CSAR on cardiac function by pressure–volume (*P–V*) loop analysis in ∼12-week sham-operated and myocardial infarcted (MI) rats. In sham rats, acute CSAR activation by epicardial application of bradykinin (BK) increased heart rate (HR), left ventricular systolic pressure (LVSP), the maximum first derivative of left ventricular pressure (dp/dt_{max}), and the slope of the end-systolic *P–V* relationship (ESPVR), suggesting that acute CSAR activation in the normal state enhances myocardial contractility. CSAR activation also decreased left ventricular (LV) systolic and diastolic volumes with little effect on LV end-diastolic pressure (LVEDP) or the end-diastolic *P–V* relationship (EDPVR) in sham rats. Compared to sham, CHF rats exhibit a reduced increase in the slope of the ESPVR and dp/dt_{max} in response to BK, indicating a poor contractile response to CSAR activation. Interestingly, BK application in CHF rats increased cardiac systolic and diastolic volumes and further increased the elevated LVEDP, neither of which was seen in sham rats. Following CSAR inhibition by epicardial lidocaine, blood pressure, HR, LVSP, dp/dt, LVEDP and ESPVR decreased in CHF rats whereas lidocaine had little effect in sham rats, indicating that the CSAR is tonically active in CHF and contributes to cardiac dysfunction. Furthermore, we found that epicardial lidocaine paradoxically decreased LV end-diastolic volume

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(preload) in CHF rats, which was not observed in sham rats. The decreased preload by lidocaine in CHF rats may be due to a reduction in peripheral vascular resistance since epicardial lidocaine significantly lowered peripheral (renal) sympathetic nerve activity in CHF rats but not in sham rats. Furthermore, chronic ablation of CSAR by epicardial application of a selective afferent neurotoxin, resiniferatoxin, selectively lowered diastolic blood pressure both at daytime and night-time with less effect on systolic blood pressure in CHF rats. Our data suggest that there is an imbalance between cardiac and peripheral responses to CSAR in CHF animals compared to sham-operated controls.

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Abbreviations AP, arterial pressure; BK, bradykinin; CHF, chronic heart failure; CO, cardiac output; CSAR, cardiac sympathetic afferent reflex; dp/dt, first derivative of left ventricular pressure; dp/dt_{max}, maximum first derivative of left ventricular pressure; dp/dt_{min}, minimum first derivative of left ventricular pressure; EDPVR, end-diastolic pressure–volume relationship; EDV, end-diastolic volume; EF, ejection fraction; ESPVR, end-systolic pressure–volume relationship; HR, heart rate; LV, left ventricular; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; MI, myocardial infarction; PRSW, preload recruitable stroke work; *P–V* loop, pressure–volume loop; RSNA, renal sympathetic nerve activity; RTX, resiniferatoxin; SV, stroke volume; SW, stroke work; TRPV1, transient receptor potential cation channel subfamily V member 1.

Introduction

Chronic heart failure (CHF) is a major health care concern in the industrialized world. In the United States over 5 million people suffer from this disease with over 660,000 new diagnoses each year (Lloyd-Jones *et al.* 2009). Exaggerated sympatho-excitation, a hallmark of CHF, is a critical factor in the development and progression of the CHF state (Cohn *et al.* 1984; Watson *et al.* 2006; Barretto *et al.* 2009; Floras, 2009; May *et al.* 2013). This sympathetic dysfunction takes the form of persistent and adverse activation of sympathetic outflow to the heart, kidneys and other organs, and causes chronic effects that contribute to disease progression. However, the neural mechanisms underlying the exaggerated sympatho-excitation in CHF are not fully understood. Several abnormal cardiovascular reflexes have been reported to be involved in the excessive sympatho-excitation at rest and during exercise in CHF, including a blunted baroreflex, an augmented cardiac sympathetic afferent reflex (CSAR), an enhanced chemoreflex and an elevated exercise pressor reflex (Wang *et al.* 1991, 1999, 2010*a*,*b*, 2014; Wang & Zucker, 1996; Sun *et al.* 1999; Zucker *et al.* 2004; Gao *et al.* 2005; Del Rio *et al.* 2013).

For many years our laboratory has studied the role of the CSAR, a sympatho-excitatory reflex originating in the heart, in mediating the exaggerated sympathetic tone in CHF (Wang & Zucker, 1996; Wang *et al.* 1999, 2007, 2008). Early work from our laboratory (Wang & Zucker, 1996; Gao *et al.* 2005) demonstrated that the CSAR is tonically activated and contributes to the elevated sympathetic tone in CHF. We also reported that the sites at which the CSAR is sensitized reside at both the afferent endings (Wang *et al.* 1999) and in the central nervous system (Ma *et al.* 1997). In a recent study (Wang *et al.* 2014), we further demonstrated that chronic and selective cardiac sympathetic afferent denervation by epicardial application of resiniferatoxin (RTX), an ultrapotent analogue of capsaicin capable of inducing rapid degeneration of TRPV1-expressing afferent neurons and fibres (Szallasi & Blumberg, 1989; Szolcsanyi *et al.* 1991; Zahner *et al.* 2003), markedly reduced left ventricular end-diastolic pressure (LVEDP) and increased cardiac contractile reserve in post-myocardial infarcted rats. Furthermore, these effects were associated with decreased cardiac and renal sympathetic efferent nerve activities (Wang *et al.* 2014), indicating a chronic detrimental effect of the CSAR on cardiac remodelling and dysfunction in CHF. However, the underlying mechanisms that elucidate how the CSAR-evoked sympatho-excitation affects cardiac function in both normal and CHF states remain unclear. Therefore, the primary goal of the current study was to evaluate the acute effects of direct activation or inhibition of the CSAR on cardiac function using pressure–volume (*P–V*) loop analysis (a technique that has become a gold standard function), in ∼12-week sham-operated and rats subjected to myocardial infarction (MI). To further explain the cardiac function data observed from the *P–V* loop experiments, both electrophysiological recording (i.e. renal sympathetic nerve recording) in sham and MI rats and conscious blood pressure measurements in chronic CSAR-ablated and vehicle-treated MI rats were performed in order to (1) determine if there is tonic activation of the CSAR in the control of sympathetic outflow in

the post-MI rats, and (2) elucidate a potential effect of the CSAR-evoked haemodynamic response (i.e. vasoconstriction) on cardiac function in the CHF state.

Methods

Experiments were performed on male sham Sprague–Dawley rats weighing ∼350–500 g and male CHF rats weighing 410–520 g at the time of the acute experiment. These experiments were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and were carried out under the guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Model of CHF and chronic cardiac sympathetic afferent desensitization

Heart failure was produced by coronary artery ligation as previously described (Wang *et al.* 2010*b*, 2012). Briefly, each rat was ventilated at a rate of 60 breaths min^{-1} with 3% isoflurane in oxygen during the surgical procedure. A left thoracotomy was performed through the fifth intercostal space, the pericardium was opened, the heart was exteriorized, and the left anterior descending coronary artery was ligated. Sham-operated rats were prepared in the same manner but did not undergo coronary artery ligation.

In a subgroup of MI rats, cardiac sympathetic afferent desensitization was produced by epicardial application of resiniferatoxin (RTX, 50 μg ml^{-1}) as previously described (Wang *et al.* 2014). Briefly, RTX (1 mg; Sigma-Aldrich, St Louis, MO, USA) was dissolved in 2 ml of ethanol and mixed with 18 ml of Tween in isotonic saline (2 ml of Tween 80 (Sigma-Aldrich) dissolved in 16 ml of isotonic saline). RTX (50 μg ml^{-1}) was painted twice on the entire surface of the left and right ventricles with a small brush just prior to coronary ligation. Vehicle (2 ml of ethanol mixed with 18 ml of Tween in isotonic saline) was painted on the ventricles as a control. The dose of RTX was determined by our previous study (Wang *et al.* 2014) in which we found nearly complete ablation of TRPV1-expressing cardiac nerve endings on the surface of the rat heart. The same dose of RTX also abolished functional activation of CSAR by epicardial application of exogenous bradykinin (10 μg ml^{-1}) (Wang *et al.* 2014). Following coronary artery ligation and RTX or vehicle application, the thorax was closed with continuous 4-0 Dexon II suture (Ethicon, Somerville, NJ, USA) and the skin was closed with 3-0 polypropylene suture (Ethicon) and evacuated. Betadine was applied to the wound and the rats were allowed to recover from the anaesthesia. For post-procedure pain management, buprenorphine (0.05 mg kg^{-1}) was injected subcutaneously after surgery and twice daily for 2 days.

Cardiac function in all experimental animals was measured 6 weeks after MI by echocardiography (VEVO 770, Visual Sonics, Inc., Toronto, Ontario, Canada) as previously described (Wang *et al.* 2010*a*,*b*, 2012). In all acute terminal experiments, except for pressure volume (*P–V*) loop analysis, a Millar catheter (SPR 524; size, 3.5-Fr; Millar Instruments, Houston, TX, USA) was advanced through the right carotid artery into the left ventricle to determine left ventricular (LV) end-diastolic pressure (LVEDP), LV systolic pressure (LVSP), the maximum first derivative of left ventricular pressure (dp/dt_{max}) and the minimum first derivative of LV pressure (dp/dt_{min}). In *P–V* loop analysis experiments, cardiac function was measured by using a Millar *P–V* loop System (Millar Instruments). At the end of these acute experiments, the rats were killed with a rapid I.V. injection of saturated potassium chloride. The hearts and lungs were removed, and the ratio of the infarct area to total LV area was measured.

General surgical preparation and recording renal sympathetic nerve activity

In acute terminal experiments that were performed ∼12 weeks post-MI, rats were anaesthetized with urethane (800 mg kg^{-1} I.P.) and α-chloralose (40 mg kg^{-1} I.P.). The trachea was cannulated, and the rats were ventilated artificially with room air supplemented with 100% oxygen. A catheter (SPR 524; size, 3.5-Fr; Millar Instruments) was advanced through the right common carotid artery into the left ventricle to determine basal cardiac functional parameters such as LVEDP, LVSP, dp/dt_{max} and dp/dt_{min}. The transducer was then pulled back into the aorta and left in place to record arterial pressure (AP) and mean arterial pressure (MAP). Heart rate was derived from the AP pulse using Chart 7.1 software and a PowerLab model 16S (ADInstruments, Colorado Springs, CO, USA) data acquisition system. The right jugular vein was cannulated for intravenous injections. To selectively activate the CSAR in the following experiments, the bilateral vagal nerves were cut after jugular vein cannulation. Body temperature was maintained between 37 and 38°C by a heating pad.

Renal sympathetic nerve activity (RSNA) was recorded as previously described (Gao *et al.* 2005; Wang *et al.* 2010*b*). Generally, the left kidney, renal artery and nerves were exposed through a left retroperitoneal flank incision. Sympathetic nerves running on or beside the renal artery were identified. The renal nerve was cut distally to avoid recording afferent activity. The renal sympathetic nerves were placed on a pair of platinum–iridium recording electrodes and then were covered with a fast-setting silicone (Kwik-Sil; World Precision Instruments, Sarasota, FL, USA). Nerve activity was amplified (×10,000) and filtered (bandwidth: 100–3000 Hz) using a Grass P55C preamplifier (Grass - AstroMed, Inc., West Warwick RI, USA). The nerve signal was monitored on an oscilloscope

Table 1. Haemodynamic and morphological data in sham, CHF, CHF + vehicle and CHF + RTX rats

	Sham ($n = 21$)	CHF ($n = 18$)	CHF + vehicle ($n = 6$)	CHF + RTX ($n = 6$)
BW (g)	445 ± 10	460 ± 8	456 ± 12	439 ± 14
HW (mg)	1186 ± 27	2107 ± 60*	2214 ± 69*	1613 ± 75*
HW/BW (mg g^{-1})	2.7 ± 0.1	4.6 ± 0.1*	4.9 ± 0.2*	3.7 ± 0.2*
WLW/BW (mg g^{-1})	4.4 ± 0.1	9.0 ± 0.3*	9.4 ± 0.6*	5.4 ± 0.3*
LVEDP (mmHg)	3.2 ± 0.4	19.8 ± 1.0*	21.5 ± 2.1*	10.6 ± 1.5*
EF (%)	71.5 ± 0.7	39.6 ± 1.8*	37.8 ± 1.8*	40.9 ± 1.6*
FS (%)	41.9 ± 0.5	20.0 ± 0.9*	18.9 ± 1.1*	20.5 ± 1.0*
Infarct size (%)	0	40.8 ± 1.2*	42.4 ± 1.6*	39.3 ± 1.6*

Values are means ± SEM. BW, body weight; EF, ejection fraction; FS, fractional shortening; HW, heart weight; LVEDP, left ventricle end-diastolic pressure; WLW, wet lung weight. *$P < 0.05$ *vs.* CHF.

and was displayed on a computer where it was rectified, integrated, sampled (1 kHz), and converted to a digital signal by the PowerLab data acquisition system.

At the end of the experiment, the rat was killed with a rapid I.V. injection of saturated potassium chloride. The maximum nerve activity (Max) occurred 1–2 min after the rat was killed. Background noise levels for sympathetic nerve activity were recorded 15–20 min after the rat was killed. Using the unit conversion of the Powerlab Chart (ADInstruments) system, baseline nerve activity was taken as a percentage of Max after the noise level was subtracted. The change in RSNA in response to bradykinin (BK) or lidocaine was also normalized as % Max, which allowed us to compare the RSNA response between sham and CHF rats (Wang *et al.* 2010*b*).

Measurement of left ventricular performance

LV function was assessed using a Millar *P–V* loop System (Millar Instruments) as previously described (Wang *et al.* 2014). Briefly, a microtip *P–V* catheter (SPR-838) was inserted into the right carotid artery and advanced into the left ventricle. A polyethylene catheter was inserted into the left jugular vein for fluid administration. After stabilization for 10–20 min, LV *P–V* signals were recorded continuously at a sampling rate of 1000 Hz using an MPVS-300 conductance system (Millar Instruments) coupled to a PowerLab 8/30 (ADInstruments); 50 μl of 20% saline was injected intravenously so as to establish a parallel conductance volume from the shift of *P–V* relations, and this was used for correction of the cardiac mass volume. LV *P–V* loops were also captured by transiently compressing the inferior vena cava. LV parameters were computed using cardiac *P–V* analysis software (PVAN3.2, Millar Instruments). Volume calibrations were performed with the Millar volume calibration cuvette, which consisted of a 1 cm-deep cylindrical block with cylindrical holes of known diameters ranging from 2 to 11 mm filled with fresh heparinized whole rat blood. The linear volume–conductance regression of the absolute volume in each cylinder *versus* the raw signal acquired by the conductance catheter was used for the volume calibration using PVAN 3.2 (Millar, Houston, TX, USA). In addition to the haemodynamic parameters described above, the system calculated the time constant of isovolumetric relaxation (τ), ejection fraction (EF), end-diastolic volume (EDV), cardiac output (CO), stroke volume (SV), stroke work (SW), preload recruitable stroke work (PRSW), end systolic pressure volume relationship (ESPVR), and the end diastolic pressure volume relationship (EDPVR).

Activation and inhibition of the CSAR

Epicardial application of bradykinin or lidocaine has been demonstrated to effectively stimulate or inhibit cardiac sympathetic afferents respectively (Zahner *et al.* 2003; Gao *et al.* 2005) in vagotomized rats. The chest was opened through the fourth intercostal space. The pericardium was removed to expose the left ventricle. A square of filter paper (3 mm × 3 mm) saturated with BK (10 μg ml^{-1}) was applied to the anterior surface of the left ventricle to stimulate regional cardiac sympathetic afferents whereas two 3 mm × 3 mm filter papers (covering both basal and apex regions) saturated with 2% lidocaine was simultaneously applied to the anterior surface of the left ventricle to maximally inhibit ventricular cardiac sympathetic afferents in vagotomized rats. Haemodynamic and neural parameters were continuously recorded. After the peak response, the epicardium was rinsed three times with 10 ml of warm normal saline (38°C). The time interval between BK and lidocaine was at least 20 min to allow the AP, HR and RSNA to return to, and stabilize at, their control levels.

Telemetry recording of blood pressure in rats

To record 24-hour conscious blood pressure in ∼12-week post-MI rats treated with vehicle or RTX, the rats were instrumented with blood pressure monitoring

radiotelemeter devices (model no. TA11PA-C40, Data Sciences International, St Paul, MN, USA) ~10 weeks after the ligation or sham surgery. Briefly, rats were anaesthetized using 2%–3% isoflurane in oxygen using a nose cone. The surgical site (i.e. the femoral area) was cleared of hair and prepared with betadine, and the femoral artery was exposed. After a small incision in the femoral artery, the telemetry unit catheter was inserted and retrogradely placed into the abdominal aorta for measurement of systolic and diastolic blood pressure and HR. The telemetry unit was then secured to the abdominal muscle wall and buried under the skin in the femoral area. The skin was closed and rats were given antibiotics and analgesics. Following 2 weeks of recovery,

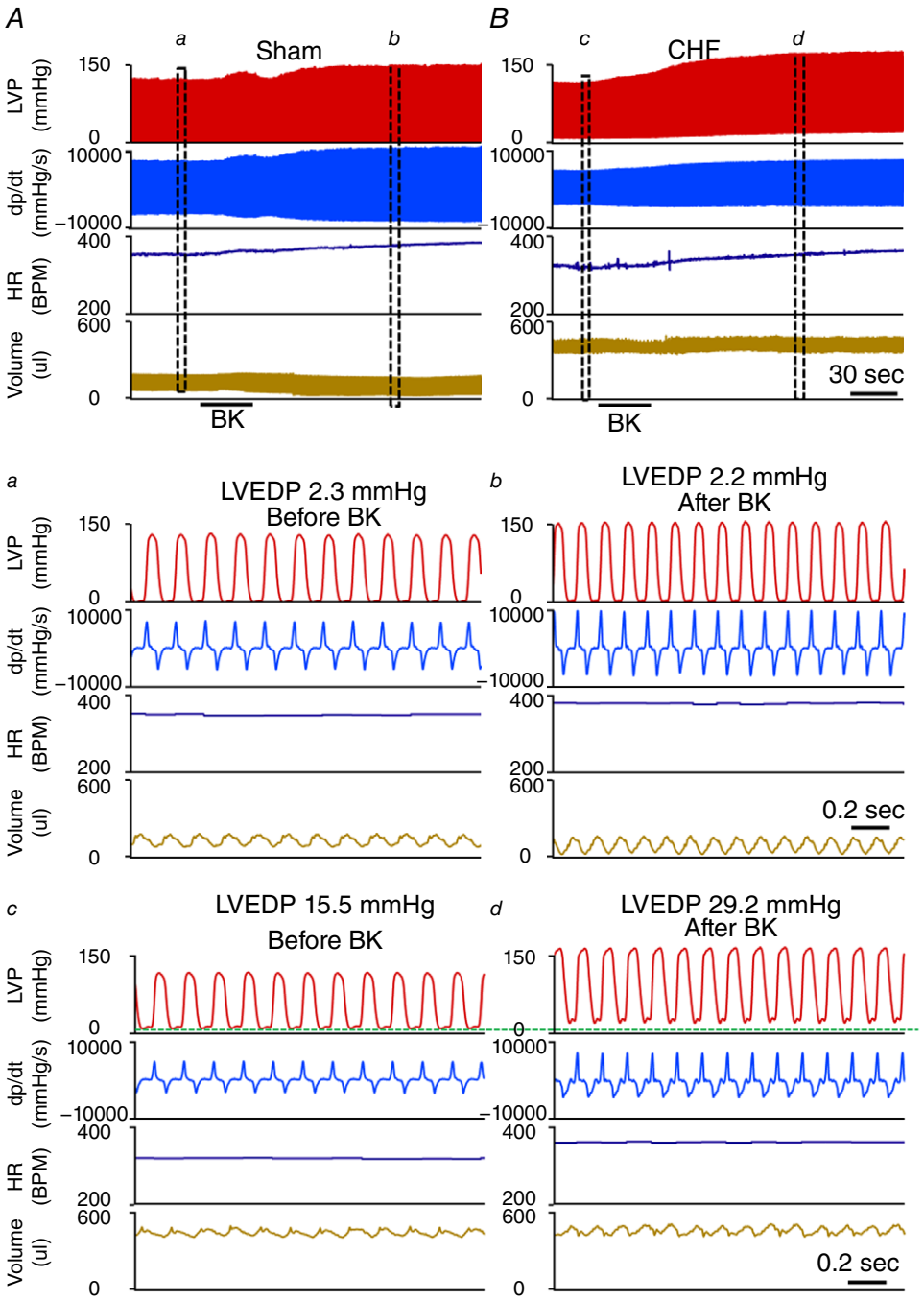

Figure 1. Representative tracings showing cardiac functional changes in response to epicardial application of bradykinin (BK, 10 μg ml^{-1}) in a sham (*A*) or CHF (*B*) rat
a–d, 2 s recordings before and after BK from *A* and *B* as shown. Note that LVEDP was dramatically increased in the CHF rat after epicardial application of BK whereas it was not affected by BK in the sham rat.

Table 2. Summary data for *P–V* loop experiments before and after epicardial BK

	Sham ($n = 6$)		CHF ($n = 10$)	
Parameter	Before BK	After BK	Before BK	After BK
EF (%)	67.2 ± 3.3	75.7 ± 2.6*	20.0 ± 3.8†	18.1 ± 2.4†
SW (mmHg μl)	13454 ± 1423	16598 ± 1603*	4132 ± 496†	5060 ± 786†
SV (μl)	147 ± 14.4	159.6 ± 14.6*	68.7 ± 8.4†	74.0 ± 10.1†
CO (ml min^{-1})	49.7 ± 4.5	56.7 ± 4.9*	22.6 ± 3.1†	26.4 ± 3.9†
Afterload				
LVESP (mmHg)	129.4 ± 1.9	143.0 ± 3.0*	105.8 ± 3.6†	134.7 ± 2.2*
E_a (mmHg μl^{-1})	1.10 ± 0.10	0.95 ± 0.07*	1.96 ± 0.38†	2.26 ± 0.48†
Preload				
LVEDP, mmHg	3.2 ± 1.0	3.4 ± 1.0	14.7 ± 1.5†	21.4 ± 2.6†*
LVEDV (μl)	218 ± 17	210 ± 17	427.0 ± 50.5†	444.6 ± 50.5†
Contractility				
dp/dt_{max} (mmHg s^{-1})	8213 ± 248	9704 ± 533*	5298 ± 344†	6531 ± 326†
ESPVR	2.8 ± 0.2	4.1 ± 0.2*	1.3 ± 0.1†	1.8 ± 0.2†*
PSRW (mW μl^{-1})	98.8 ± 2.3	133.8 ± 4.3*	61.4 ± 1.9†	77.8 ± 7.3†*
Lusitropy				
dp/dt_{min} (mmHg s^{-1})	−7002 ± 288	−7511 ± 341*	−3605 ± 206†	−3875 ± 357†
τ_G (ms)	11.7 ± 0.8	10.3 ± 0.6*	16.2 ± 1.5†	19.4 ± 2.3*†
EDPVR	0.003 ± 0.0005	0.003 ± 0.0005	0.015 ± 0.001†	0.015 ± 0.001†

Values are means ± SEM. CO, cardiac output; dp/dt_{max}, maximal slope of systolic pressure increment; dp/dt_{min}, maximal slope of diastolic pressure increment; E_a, arterial elastance; EDPVR, end diastolic pressure volume relationship; ESPVR, end systolic pressure volume relationship; LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; PSRW, preload-recruitable stroke work; SV, stroke volume; SW, stroke work; τ_G, time constant of isovolumetric relaxation. *$P < 0.05$ *vs.* before BK. †$P < 0.05$ *vs.* sham + vehicle. ESPVR, EDPVR and PSRW data are from $n = 9$ rats, whereas $n = 10$ for all other cardiac function parameters in CHF rats.

24-hour blood pressure and HR were measured in sham, CHF + vehicle and CHF + RTX rats.

Statistics. All data are expressed as the mean ± standard error of the mean (SEM). Differences between groups were determined by a two-way ANOVA followed by Tukey's *post hoc* test. Changes in cardiac function before and after epicardial application of BK or lidocaine were determined by Student's paired *t* test. A *P* value < 0.05 was considered statistically significant.

Results

Evaluation of body weight, organ weight and baseline haemodynamics

Echocardiographic and haemodynamic measurements in all groups are summarized in Table 1. MI rats exhibited reduced ejection fraction and fractional shortening compared with sham rats, indicating decreased cardiac systolic function. Haemodynamic data collected at the time of the terminal experiments (~12 weeks) further demonstrated that there was a significant increase in LVEDP in CHF rats compared to sham rats. The heart weight and lung weight to body weight ratio were significantly higher in CHF rats than in sham-operated rats, suggesting cardiac hypertrophy and substantial pulmonary congestion in the CHF state. Compared to CHF rats or CHF + vehicle rats, CHF + RTX rats exhibited a significant decrease in LVEDP, heart weight and lung weight to body weight ratio. There was no significant difference in infarct size between CHF, CHF + vehicle and CHF + RTX rats. These data were consistent with our previous finding demonstrating that cardiac sympathetic afferent ablation by RTX at the time of MI has a cardio-protective effect (Wang *et al.* 2014).

Effects of acute CSAR activation by epicardial application of BK on cardiac function in sham and CHF rats

Although it is well established that the CSAR evokes sympatho-excitation, its direct effects on cardiac function in normal and CHF states remain largely unknown. In the present study, acute CSAR activation in sham rats by epicardial application of BK increased HR, LVSP, first derivative of left ventricular pressure (dp/dt), and the slope of the ESPVR (Fig. 1 and Table 2). In addition, this intervention decreased LV systolic and diastolic volumes with little effect on LVEDP or the EDPVR in the sham rats (Table 2), suggesting that acute CSAR activation in the normal state enhances myocardial contractility.

Compared to sham, CHF rats exhibited less increase in the slope of the ESPVR and dp/dt_{max} in response to acute CSAR activation (Fig. 2*A*–*C*, Table 2), indicating a poor contractile response to CSAR activation. Interestingly, in CHF rats epicardial application of BK (1) increased cardiac systolic and diastolic volumes (Fig. 1 and Table 2) and (2) further increased the elevated LVEDP (Fig. 2*D*), neither of which were seen in sham rats. In addition, the elevated LVEDP was further increased as was cardiac systolic and diastolic volumes (Table 2), predisposing to a syndrome of acute fulminating heart failure. The above phenomena may be expected if the sensitivity of the CSAR was augmented and evoked a sympathetically mediated peripheral vasoconstriction thus increasing afterload in the failing heart. This notion is consistent with previous data from our laboratory showing a greater pressor response to epicardial application of capsaicin in dogs with pacing-induced CHF (Wang & Zucker, 1996).

Effects of acute CSAR inhibition by epicardial application of lidocaine on cardiac function in sham and CHF rats

Acute epicardial application of lidocaine, which blocks superficial cardiac sympathetic afferents, decreased the HR, LVSP, dp/dt_{max} and ESPVR in CHF rats whereas it had little effect in sham rats (Table 3), indicating that the CSAR is tonically active in CHF and contributes to cardiac dysfunction. Interestingly, we observed that epicardial application of lidocaine paradoxically decreased

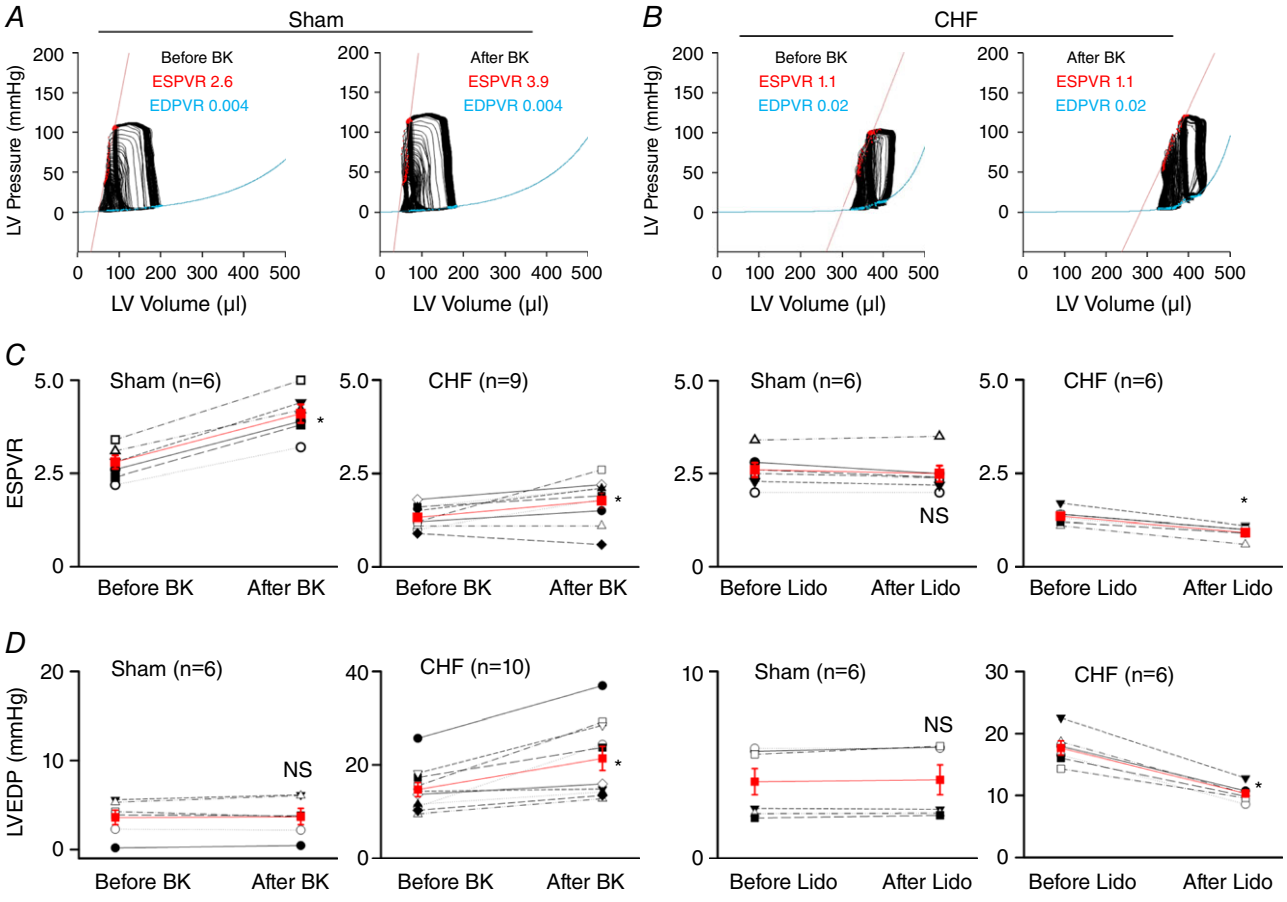

Figure 2. Representative tracings and summary data showing that there were differential changes in systolic (end-systolic pressure–volume relationship, ESPVR) function and left ventricle end-diastolic pressure (LVEDP) in responses to epicardial application of bradykinin (BK, 10 μg ml^{-1}) or 2% lidocaine (Lido) in sham and CHF rats

A and *B*, original recordings of steady state *P*–*V* loops obtained with a Millar *P*–*V* conductance catheter system before and after epicardial application of BK in sham and CHF rats. Red line represents ESPVR and the blue curved line demonstrates the diastolic (end-diastolic pressure–volume relationship, EDPVR) function, both of which are independent of systemic vascular resistance. *C*, individual (black and white symbols) and average (red symbols) data showing changes in ESPVR in response to epicardial application of either BK or lido in sham and CHF rats. *D*, individual (black and white symbols) and average (red symbols) data showing changes in LVEDP in response to epicardial application of either BK or lido in sham and CHF rats. Data are expressed as means ± SEM. $^*P < 0.05$ *vs*. sham rats. NS, not significant.

Table 3. Summary data for *P–V* loop experiments before and after epicardial lidocaine (Lido)

	Sham ($n = 6$)		CHF ($n = 6$)	
Parameter	Before Lido	After Lido	Before Lido	After Lido
EF (%)	64.4 ± 1.1	61.1 ± 0.8*	19.9 ± 2.4†	18.4 ± 2.5†
SW (mmHg μl)	12247 ± 867	11527 ± 810	4251 ± 610†	3525 ± 689†
SV (μl)	119.4 ± 7.0	115.5 ± 6.8	74.7 ± 10.0†	65.4 ± 8.3†
CO (ml min^{-1})	43.3 ± 2.2	39.4 ± 2.0*	25.9 ± 2.9†	19.3 ± 2.2†*
Afterload				
LVESP (mmHg)	133.0 ± 2.5	128.1 ± 3.3*	113.6 ± 2.7†	88.7 ± 3.7*
E_a (mmHg μl^{-1})	1.21 ± 0.03	1.27 ± 0.03	1.83 ± 0.22†	1.64 ± 0.16†*
Preload				
LVEDP, mmHg	4.1 ± 0.7	4.2 ± 0.8	17.7 ± 1.1†	10.3 ± 0.6†*
LVEDV (μl)	186 ± 12	189 ± 12	383.0 ± 43.4†	367.3 ± 40.0†*
Contractility				
dp/dt_{max} (mmHg s^{-1})	8552 ± 224	7694 ± 302*	6003 ± 278†	4141 ± 413†*
ESPVR	2.6 ± 0.2	2.5 ± 0.2	1.4 ± 0.1†	0.9 ± 0.1†*
PSRW (mW μl^{-1})	98.0 ± 1.7	94.0 ± 2.6	62.7 ± 3.6†	51.0 ± 3.6†*
Lusitropy				
dp/dt_{min} (mmHg s^{-1})	−7031 ± 276	−6553 ± 320*	−3785 ± 205†	−3088 ± 255†
τ_G (ms)	10.6 ± 0.7	10.9 ± 0.7*	16.8 ± 1.6†	15.8 ± 1.0†
EDPVR	0.003 ± 0.0004	0.003 ± 0.0004	0.015 ± 0.001†	0.015 ± 0.001†

Values are mean ± SEM. CO, cardiac output; dp/dt_{max}, maximal slope of systolic pressure increment; dp/dt_{min}, maximal slope of diastolic pressure increment; E_a, arterial elastance; EDPVR, end diastolic pressure volume relationship; ESPVR, end systolic pressure volume relationship; LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; PSRW, preload recruitable stroke work; SV, stroke volume; SW, stroke work; τ_G, time constant of isovolumetric relaxation. *$P < 0.05$ *vs.* before lidocaine †$P < 0.05$ *vs.* sham + vehicle.

LV end-diastolic volumes (LVEDV, Table 3) and the elevated LVEDP (Figs 2 and 3), both of which were never observed in sham rats.

Effects of acute CSAR activation or inhibition on blood pressure and RSNA in sham and CHF rats

As shown in Fig. 4, compared to sham rats, CHF rats exhibited exaggerated MAP, HR and RSNA responses to epicardial application of BK. On the other hand, epicardial application of lidocaine slightly decreased MAP and HR in anaesthetized sham rats. However, RSNA was not affected by lidocaine, indicating that there was no tonic CSAR control of RSNA in sham rats. In contrast, epicardial application of lidocaine decreased blood pressure, HR and RSNA in CHF rats to a greater extent than in sham rats, confirming that tonic CSAR control of sympatho-excitation exists in the CHF state.

Effects of chronic CSAR ablation by epicardial application of RTX on blood pressure in conscious sham and CHF rats

Based on the acute *P–V* loop observations, we speculated that CSAR control of sympatho-excitation results in an overwhelming vasoconstriction (i.e. arteries and veins) and poor or little increase in cardiac output in CHF rats, which could shift blood volume from the periphery to the chest and eventually increase cardiac systolic and diastolic volumes, resulting in increases in LVEDP in CHF rats. If this hypothesis is correct, we would expect that chronic ablation of the CSAR would reduce peripheral sympatho-excitation-induced vasoconstriction and decrease blood pressure (especially diastolic pressure) in CHF rats. As shown by the 24-h telemetry recording data (Fig. 5), we observed significant decreases in SBP and MAP but not DBP at many of the daytime time points and almost all of the night-time time points in CHF rats compared to sham rats. After averaging the 12-h daytime and 12-h night-time BP data (Fig. 6), we found that daytime averaged SBP and MAP was slightly decreased but did not reach statistical differences in CHF rats compared to sham rats. However, night-time averaged SBP was significantly lower in CHF rats compared to sham rats. Both average daytime and night-time DBP were similar in sham and CHF rats. These data indicate that a moderate decrease in blood pressure in CHF rats is likely to be due to a decrease in cardiac output. Compared to CHF + vehicle rats, CHF + RTX rats exhibited a selective decrease in DBP at both daytime and night-time whereas SBP was not different (Figs 5 and 6) in either group.

Discussion

The primary goal of this study was to investigate the acute effects of CSAR activation and inhibition on cardiac

function in sham and CHF rats. Here, we provide the first evidence, to our knowledge, showing that there is an imbalance between cardiac and peripheral responses to CSAR in CHF animals compared to sham-operated controls. First, we found that acute CSAR activation by epicardial application of BK in sham rats significantly enhanced cardiac systolic function and decreased LV systolic and diastolic volumes with little effects on diastolic function parameters (i.e. LVEDP or the EDPVR). In contrast, acute CSAR activation in CHF rats caused very little increase in myocardial contractility (ESPVR and dp/dt_{max}) but further aggravated the elevated LVEDP as

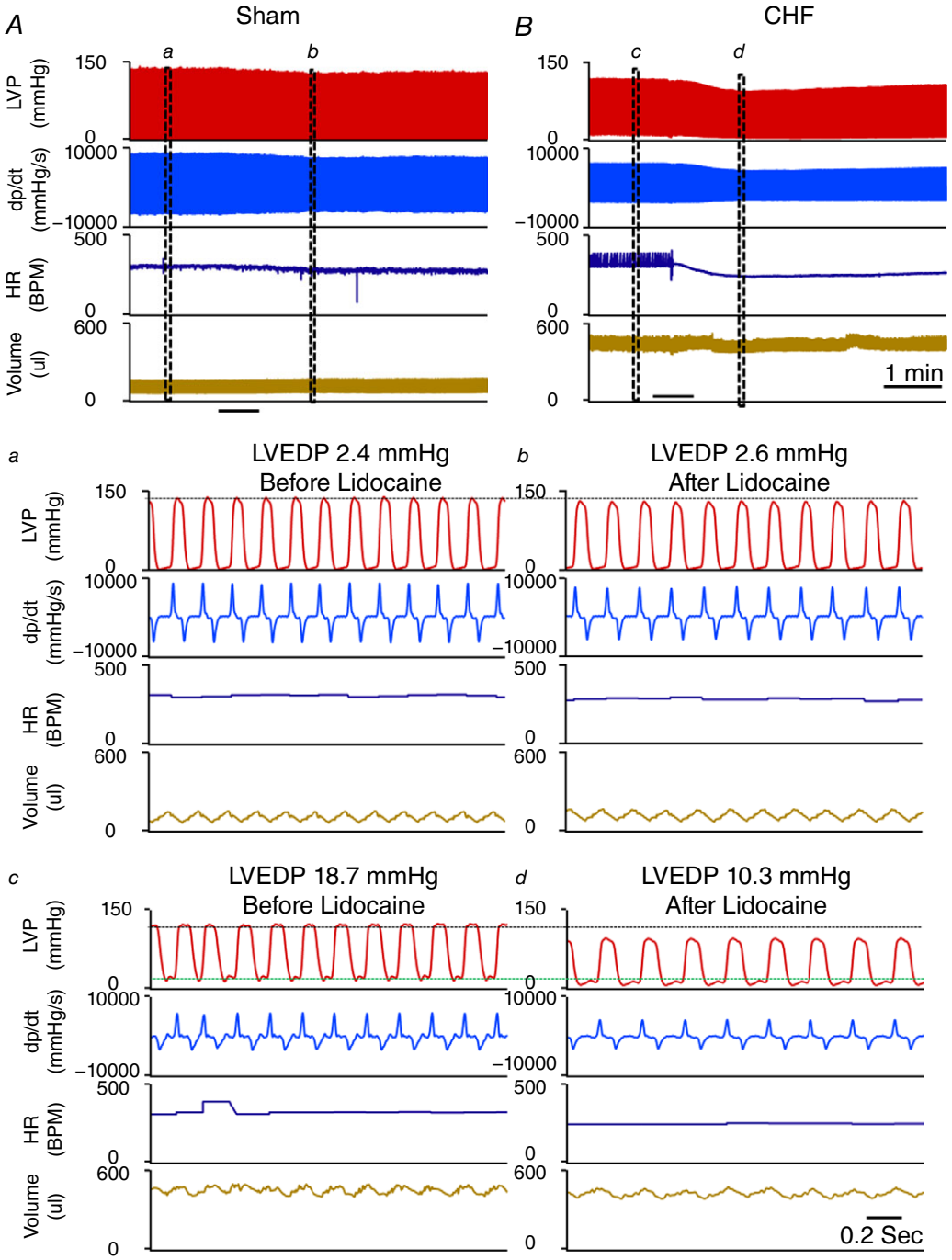

Figure 3. Representative tracing showing cardiac functional changes in response to epicardial application of 2% lidocaine in a sham (*A*) or CHF (*B*) rat
a–d, 2 s recordings before and after lidocaine from *A* and *B* as shown. Note that LVEDP was dramatically decreased in the CHF rat after epicardial application of lidocaine whereas it was less affected by lidocaine in the sham rat.

well as cardiac systolic and diastolic volumes, neither of which was seen in sham rats. On the other hand, we also observed that CSAR inhibition by epicardial lidocaine decreased cardiac systolic function to a greater extent in CHF rats than sham rats, indicating that the CSAR is tonically active in CHF and contributes to cardiac dysfunction. Furthermore, we also found that epicardial lidocaine paradoxically decreased LVEDP and LV end-diastolic volume (preload) in CHF rats, which was not observed in sham rats. We postulate that the decreased LVEDP and preload induced by lidocaine in CHF rats may be due to a reduction in peripheral vascular resistance since we found that epicardial lidocaine lowered peripheral (renal) sympathetic nerve activity in CHF rats but not in sham rats. To support this notion, we provide evidence that chronic ablation of the CSAR by epicardial application of a selective afferent neurotoxin, RTX, selectively lowered diastolic blood pressure during both daytime and night-time with less effect on systolic blood pressure in CHF rats. Our data suggest that CSAR activation fails to significantly improve cardiac performance in the established CHF state. Rather, the elevated LVEDP induced by CSAR activation in CHF is likely to contribute to acute fulminating heart failure,

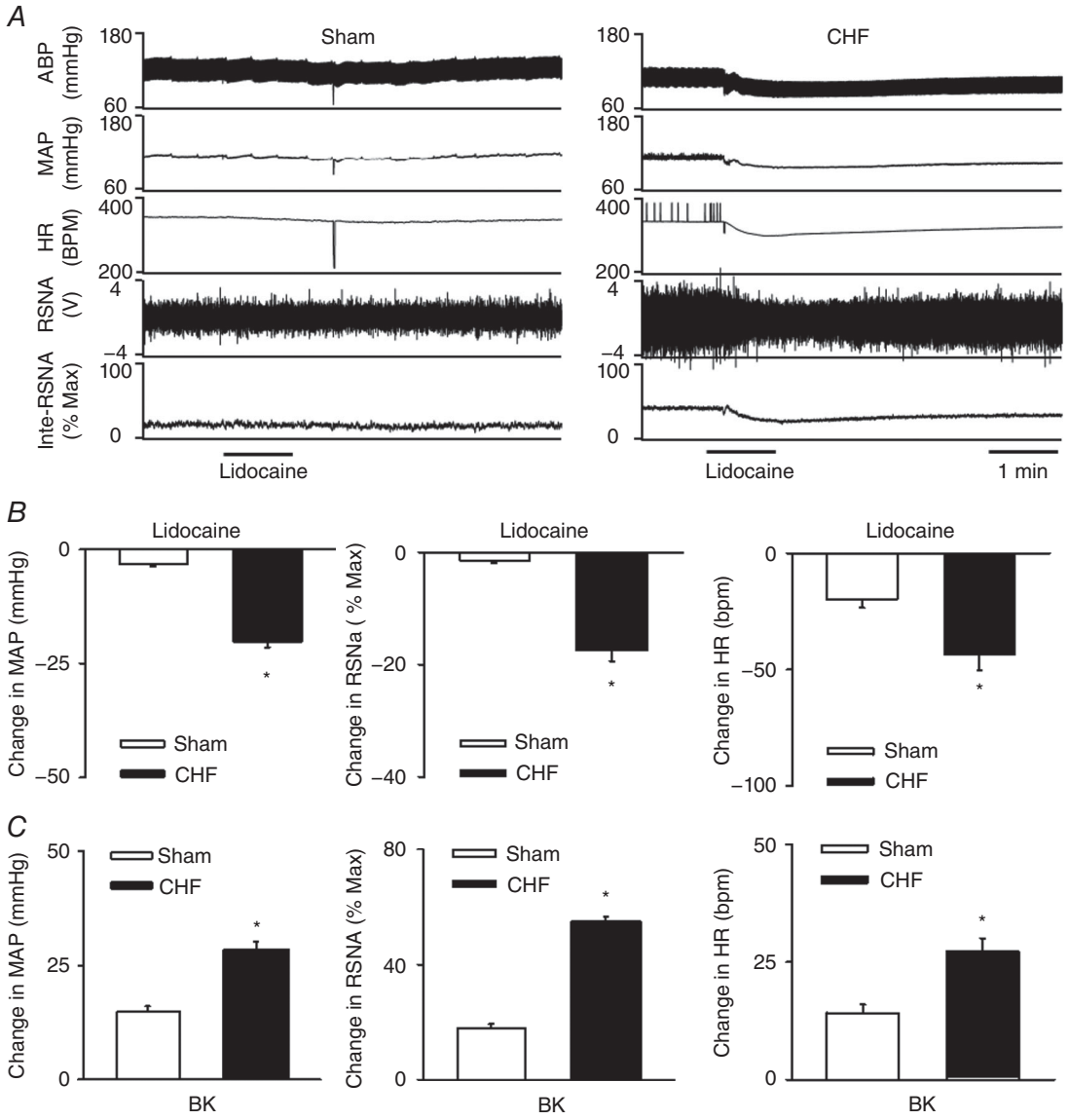

Figure 4. Representative tracings (*A*) and summary data (*B* and *C*) showing that acute epicardial application of either bradykinin (BK) or 2% lidocaine increased or decreased, respectively, mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) to a greater extent in the CHF rats compared to sham rats ($n = 8$ in each group)
Data are expressed as means ± SEM. $^*P < 0.05$ *vs*. sham.

which can be ameliorated by CSAR inhibition through a reduction in peripheral vascular resistance.

Cardiac sympathetic afferents are considered to be essential pathways for transmission of cardiac nociception to the central nervous system during myocardial ischaemia. Stimulation of the CSAR has been demonstrated to increase sympathetic outflow, MAP and HR (Malliani & Lombardi, 1986; Zucker & Wang, 1991; Wang & Zucker, 1996; Ma *et al.* 1997; Wang *et al.* 1999; Gao *et al.* 2005). Studies from this laboratory have demonstrated that the discharge of cardiac sympathetic afferents is increased and CSAR-evoked responses of BP, HR and sympathetic nerve activity are exaggerated in CHF animals (Wang & Zucker, 1996; Wang *et al.* 1999, 2007, 2008, 2009; Wang & Ma, 2000). We previously reported that an enhanced CSAR contributes to increased global sympathetic outflow and impaired baroreflex function in the CHF state (Gao *et al.* 2005). In a more recent study (Wang *et al.* 2014), we reported that chronic ablation of the CSAR by epicardial application of a selective afferent neurotoxin (RTX) not only reduced the exaggerated sympatho-excitation but also improved cardiac dysfunction and cardiac fibrosis in the post-MI rats. These data suggested that the CSAR chronically contributes to cardiac dysfunction and cardiac remodelling in the development of CHF. However, it is not fully understood how the CSAR plays a critical role in modulating cardiac function in both normal and CHF states. On the one hand, CSAR-evoked sympatho-excitation could directly target the heart and potentially cause positive chronotropic and inotropic effects. On the other hand, CSAR control of sympathetic outflow could potentially target the peripheral circulation and cause vasoconstriction of both the arterial and venous sides, which would increase both afterload and preload, respectively, and thus haemodynamically alter cardiac function. An increased afterload via CSAR-evoked arterial vasoconstriction would increase impedance to ejection and therefore decrease cardiac output and increase cardiac volumes, whereas venous vasoconstriction by CSAR activation would increase venous return and also increase cardiac chamber volume (preload). Therefore, the net effect of the CSAR on cardiac function will most likely be the sum of both direct effects on the heart and indirect peripheral circulatory effects. However, it is unclear how the central and peripheral circulatory systems are integrated by the CSAR to alter cardiac function in both normal and CHF conditions.

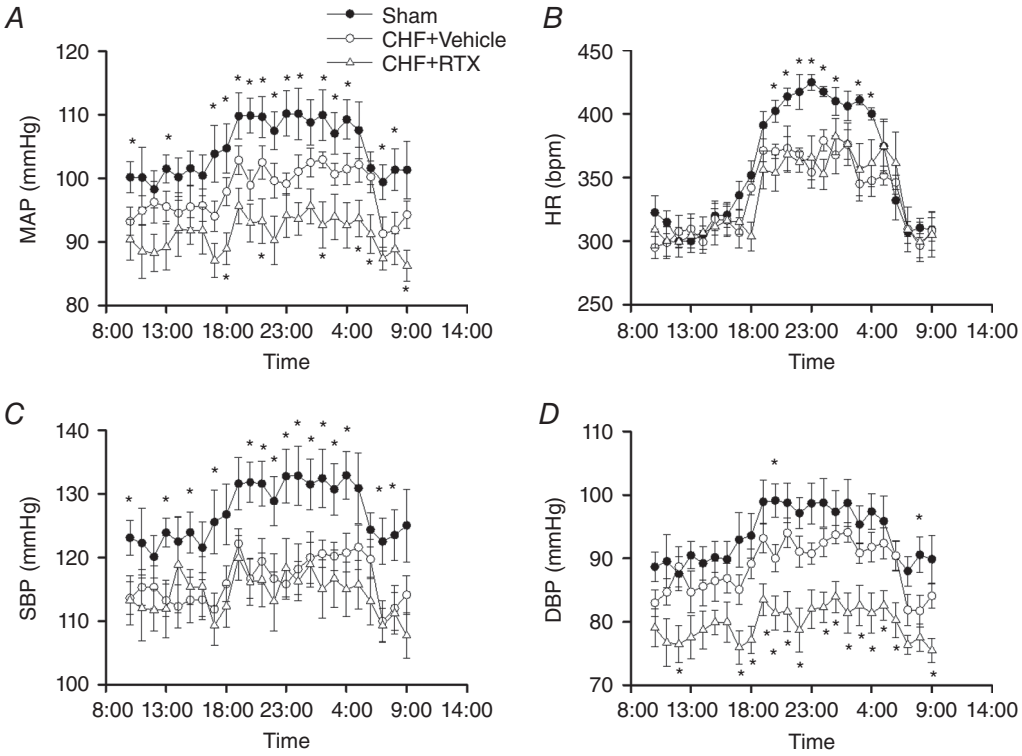

Figure 5. Conscious 24-h telemetry blood pressure data showing the effects of chronic cardiac sympathetic afferent denervation by epicardial application of RTX (50 μg ml^{-1}) on mean arterial pressure (MAP, *A*), heart rate (HR, *B*), systolic blood pressure (SBP, *C*) and diastolic blood pressure (DBP, *D*) in CHF rats 12 weeks post-MI

Sham rats served as controls. Data are expressed as means ± SEM. n = 6–7 in each group. *P < 0.05 *vs.* CHF + vehicle.

To address the questions above, we used *P–V* loop analysis (the gold standard technique for cardiac functional analysis) to evaluate the effects of the CSAR on cardiac parameters in both normal and CHF states. Our data suggest that in the normal state (sham rats), activation of the CSAR causes a significant increase in cardiac contractility and moderate increases in peripheral sympatho-excitation and vasoconstriction, which results in increased cardiac output with only a moderate pressor response associated with decreased diastolic and systolic volumes. We consider these haemodynamic responses to CSAR activation in the normal state as protective because they assist the heart to overcome emergent challenges such as ischaemia or cardiac injury. Interestingly, we found that CHF rats exhibited a very different CSAR activation pattern compared to sham rats. Our data suggest that acute CSAR activation in CHF rats caused very little increase in cardiac contractility (ESPVR) whereas it resulted in exaggerated peripheral sympatho-excitatory (RSNA) and vasoconstriction/pressor responses. Consequently, CSAR

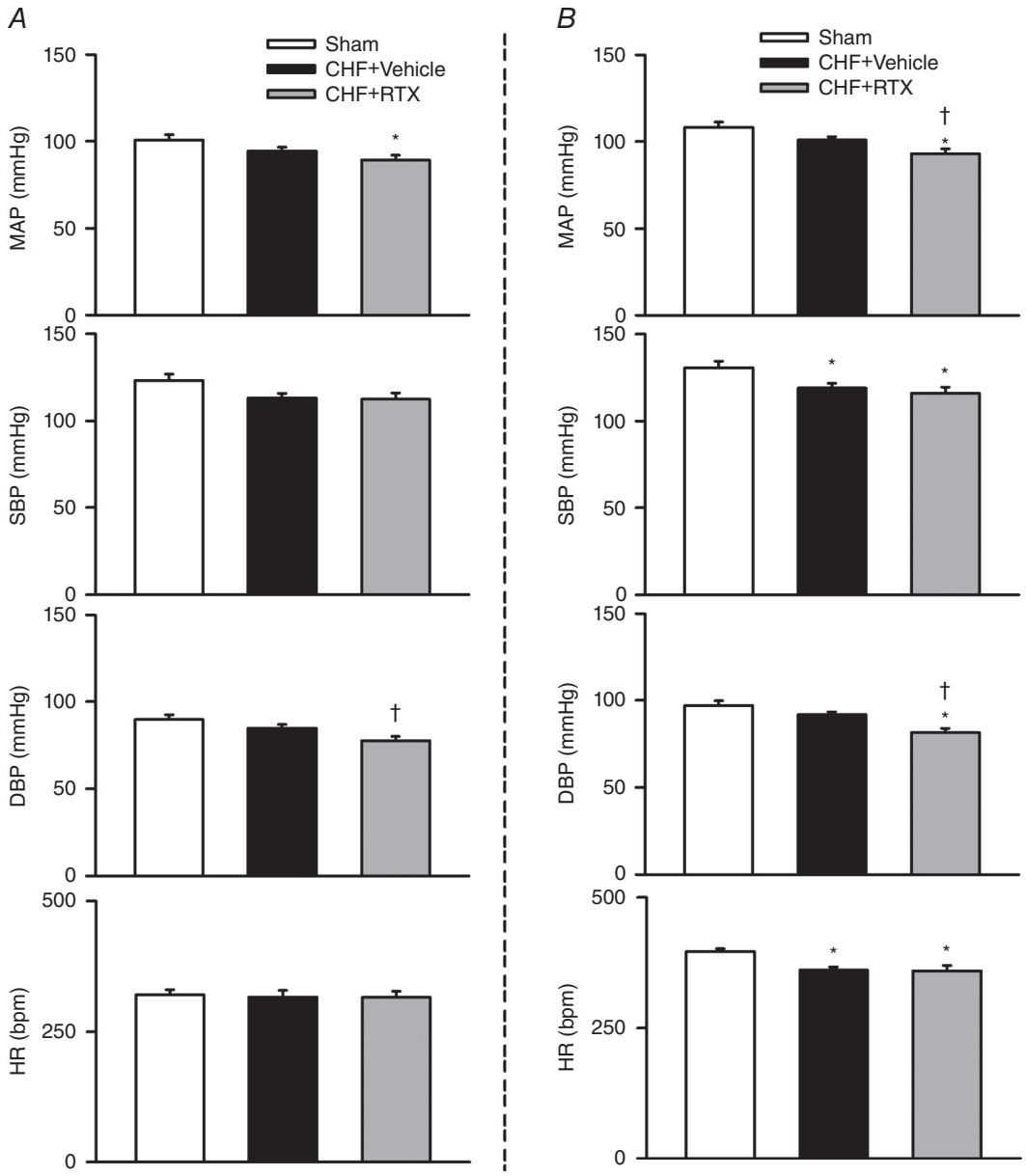

Figure 6. Conscious daytime (*A*) and night-time (*B*) telemetry blood pressure data showing the effects of chronic cardiac sympathetic afferent denervation by epicardial application of RTX (50 μg ml^{-1}) on mean arterial pressure (MAP), heart rate (HR), systolic arterial pressure (SBP) and diastolic blood pressure (DBP) in CHF rats 12 weeks post-myocardial infarction
Data are expressed as means ± SEM. $n = 6$–7 in each group. *$P < 0.05$ *vs*. sham. †$P < 0.05$ *vs*. CHF + vehicle.

activation in CHF rats caused very little increase in cardiac output with an exaggerated vasoconstriction/pressor response, which may cause an acute volume shift from the peripheral to central circulation (i.e. the chest) as evidenced by increased cardiac systolic and diastolic volumes and LVEDP. This acutely elevated LVEDP evoked by CSAR activation in the CHF state would be detrimental because it could cause acute pulmonary oedema and increase the risk of acute LV failure.

To determine if there is a tonic regulation of the CSAR on cardiac function in both normal and CHF states, we also investigated the acute effect of epicardial application of lidocaine on cardiac parameters in sham and CHF rats. We found very little effect of lidocaine on cardiac contractility (ESPVR and PSRW) in sham rats, indicating that the CSAR is not tonically involved in the regulation of cardiac function in the normal state. We did observe a moderate decrease in HR (~20 bpm) in response to lidocaine in sham rats, which may be due to a non-specific inhibition of lidocaine on cardiac conduction rather than inhibition of the CSAR because the CSAR reflex control of RSNA was not affected by lidocaine. Since dp/dt and cardiac output are heart rate dependent, both were slightly decreased by lidocaine in sham rats. Compared to sham rats, we observed a greater reduction in contractility (ESPVR and PSRW) by lidocaine in CHF rats, suggesting that the enhanced CSAR in CHF exerted a tonic control of cardiac function in the CHF state.

Another important finding is that CSAR inhibition by lidocaine significantly decreased both LVESP and LVEDP in CHF rats with little effect on sham rats. Since cardiac contractility was reduced by lidocaine-induced CSAR inhibition in CHF rats, it should have resulted in increased LV volume and LVEDP after lidocaine treatment. However, in fact, we saw the opposite effect that both LVEDV and LVEDP were decreased after lidocaine. A possible explanation for the decreased LVEDP in this condition is that lidocaine-induced CSAR inhibition in CHF rats reduced sympathetic outflow to peripheral vascular beds and caused a potent vasodilation, which decreased both arterial resistance (afterload) and venous return, resulting in decreased LVEDV and LVEDP. To support this hypothesis, we provided additional evidence showing that acute CSAR inhibition by lidocaine significantly attenuated the exaggerated RSNA in CHF rats. Furthermore, our radiotelemetry data demonstrated that chronic CSAR ablation by epicardial application of RTX selectively reduced diastolic blood pressure (a marker for peripheral vascular resistance) in conscious CHF rats during both the daytime and night-time. These additional electrophysiological and haemodynamic data strongly suggest that both acute and chronic CSAR inhibition by lidocaine and RTX, respectively, in CHF can attenuate the exaggerated sympathetic outflow to peripheral vascular beds and improve peripheral vascular compliance. This mechanism also provides additional explanation for our previous data (Wang *et al.* 2014) where we showed that chronic CSAR ablation reduced LVEDP in the post-MI state.

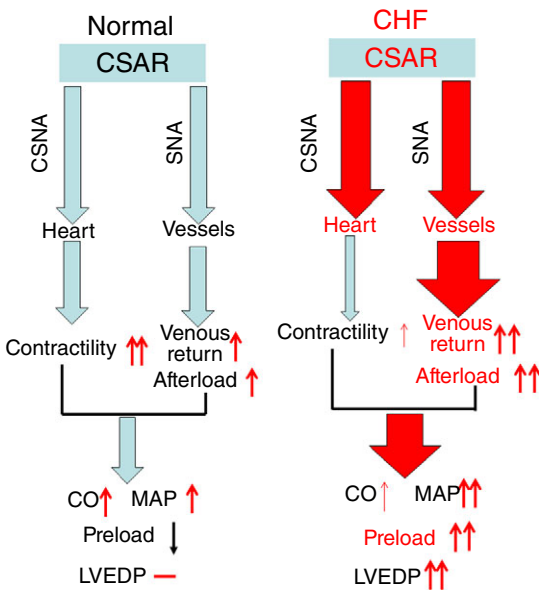

Figure 7. A schematic diagram describing how CSAR regulates cardiac function in the normal and CHF states
Activation of the CSAR causes a potent increase in cardiac contractility with a moderate increase in peripheral vasoconstriction in the normal state, which results in increased cardiac output with decreased cardiac diastolic and systolic volumes. However, activation of the CSAR causes very little increase in cardiac contractility with an exaggerated peripheral vasoconstriction in the CHF state, which causes a small increase in cardiac output associated with increased cardiac systolic and diastolic volumes and LVEDP.

Perspective

CHF is one of the leading causes of death in the USA. In spite of the fact that the use of β-adrenergic blocking agents, Angiotensin Converting Enzyme inhibitors, and angiotensin II receptor blockers have been highly effective in slowing the progression of the disease and reducing mortality, there remains an extremely high mortality and morbidity rate for patients diagnosed with CHF (Kiel & Deedwania, 2015; Bonsu *et al.* 2016; Nabeebaccus *et al.* 2016; Naegele *et al.* 2016). In a recent study, we used RTX to ablate the CSAR as an intervention to improve cardiac function and remodelling that could potentially be useful in patients following an MI and perhaps in CHF (Wang *et al.* 2014). We believe that it is possible to deliver RTX to the surface of the heart using percutaneous epicardial application and thereby selectively ablate cardiac sympathetic afferents in CHF patients. Based on our previous (Wang *et al.* 2014) and current studies, we propose that RTX-induced cardiac sympathetic afferent

denervation acts in a cardio-protective manner primarily through inhibition of cardiac sympathetic efferent nerve activity through a β-adrenergic mechanism (Wang *et al.* 2014) or through inhibition of peripheral sympathetic outflow to vascular beds that affects vascular compliance in the CHF state. These protective mechanisms following RTX administration mimic, to some degree, current first line therapy for CHF. Importantly, many current pharmacological therapies for CHF target a single downstream cascade related to cardiac dysfunction, whereas an upstream therapeutic target (e.g. CSAR-driving sympatho-excitation) may affect the remodelling process and contribute to the development of further cardiac dysfunction. Previous work in this area has largely ignored this intriguing target. We postulate that the concept of CSAR ablation may offer a new therapeutic target in the treatment of CHF. The current study has further provided a mechanistic explanation for how the CSAR regulates cardiac function in both normal and CHF states. In particular, there is a significant clinical relevance of our findings that acute CSAR activation increases cardiac systolic and diastolic volumes (i.e. volume redistribution) as well as LVEDP in CHF rats, which might contribute to the genesis of acute decompensated heart failure at the late stage of CHF. We have identified the CSAR control of peripheral vasoconstriction as a major contributor to this phenomenon. We believe that CSAR-evoked potent peripheral sympatho-excitation/vasoconstriction in arteries and veins can cause volume redistribution from the peripheral circulation to the chest via increased afterload and venous return in the CHF state. Although we provide radiotelemetry and electrophysiological (RSNA) data to support the afterload hypothesis, we unfortunately have no direct evidence to support the venous return hypothesis due to lack of specific measurable parameters for venous return/venous vasoconstriction during CSAR activation in sham or CHF rats. Nevertheless, we argue that this mechanism might play a critical role in sympatho-excitation driving volume redistribution during CSAR activation in CHF because (1) the venous system contains approximately 70% of total blood volume (Gelman, 2008), and (2) the visceral veins contain large numbers of α-1 and -2 adrenergic receptors that are 5 times greater than in arteries (Birch *et al.* 2008; Fallick *et al.* 2011). Thus, further work needs to be done to document the CSAR control of venous return.

Limitations

There are several potential limitations in this study that should be discussed. First, most experiments were conducted under anaesthesia. Although the telemetry recording experiments in vehicle or RTX-treated MI rats were performed in the conscious state, all other acute experiments for evaluation of cardiac function were done under anaesthesia. We acknowledge that the impact of anaesthetics could potentially play a role in confounding the results. However, given the inherent difficulty in conducting selective activation of CSAR in conscious animals, anaesthetized preparations allow for the most selective and robust investigation into the CSAR reflex function. We also acknowledge a limitation that compared to RTX-mediated selective CSAR ablation in chronic experiments, epicardial application of lidocaine in the acute experiments might potentially affect other cardiac structures such as cardiac conducting system as well as cardiac sympathetic afferents. As a result, we even observed a moderate decrease in HR in response to lidocaine in sham rats, which is unlikely to be due to the CSAR inhibition because the CSAR control of RSNA was not affected by lidocaine. Therefore, we consider RSNA rather than BP and HR as a reliable index to evaluate the effect of lidocaine on the CSAR in the electrophysiological recording experiments. In the *P–V* loop experiments, we found very little effect of lidocaine on cardiac contractility (ESPVR and PRSW) in spite of HR reduction in sham rats whereas it had a greater reduction in ESPVR and PRSW in CHF rats. These data suggested that both ESPVR and PRSW may be more reliable parameters to evaluate the effect of lidocaine on the CSAR control of cardiac function. Finally, as we discussed above, any potential non-specific inhibition of cardiac function by lidocaine should have resulted in increased LV volume after lidocaine application in CHF rats. However, in fact we observed an opposite finding that LVEDV was decreased after lidocaine in CHF rats, which is cannot be explained by any lidocaine-evoked non-specific cardiac inhibition. A reasonable explanation is that lidocaine-induced CSAR inhibition reduced sympathetic outflow to peripheral vascular beds and caused a potent vasodilatation in both arteries and veins in CHF rats, which decreased both preload and afterload and caused the reduction in LVEDV. Therefore, although lidocaine is less specific than RTX for CSAR inhibition, it is still a useful tool to improve our understanding of the CSAR control of cardiovascular function in healthy and pathological states. In addition, it should be realized that MI-triggered remodelling may occur at various levels of the cardio-neural axis such as vagal and sympathetic afferents, and intrinsic and extrinsic cardiac ganglia. It is possible that RTX-mediated selective afferent ablation will affect multiple elements within the cardio-neural axis and eventually alter cardiac function in the CHF state. Further work needs to be done to verify this possibility.

Summary

The current study demonstrates, for the first time, to our knowledge, that there is an imbalance between cardiac and peripheral responses to CSAR in CHF animals compared

to sham-operated controls. In the normal state, CSAR activation causes a potent increase in cardiac contractility with a moderate increase in peripheral vasoconstriction, whereas it causes very little increase in cardiac contractility with an exaggerated peripheral vasoconstriction in the CHF state. The former results in increased cardiac output with decreased cardiac diastolic and systolic volumes, whereas the latter causes a small increase in cardiac output associated with increased cardiac systolic and diastolic volumes and LVEDP. Figure 7 provides a schematic model of our findings.

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Additional information

Competing interests

None declared.

Author contributions

H.-J.W.: Conception and design; financial Support; administrative support; provision of study materials or patients; collection and assembly of data; data analysis and interpretation; manuscript writing. G.R.: conception and design; data analysis and interpretation. I.Z.: conception and design; Data analysis and interpretation. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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