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Carotid body denervation improves autonomic and cardiac function and attenuates disordered breathing in congestive heart failure

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Key points

- A strong correlation between disordered breathing patterns, elevated sympathetic nerve activity and enhanced chemoreflex sensitivity exists in patients with heart failure.
- Evidence indicates that disordered breathing patterns and increased sympathetic nerve activity increases arrhythmia incidence in patients with heart failure.
- Enhanced coupling between sympathetic and respiratory neural drive underlies elevated sympathetic nerve activity in an animal model of sleep apnoea.
- We investigated the impact of carotid body chemoreceptor denervation on sympathetic nerve activity, disordered breathing and sympatho-respiratory coupling in an animal model of heart failure.
- Renal sympathetic nerve activity, apnoea/hypopnoea incidence, variability measures of tidal volume and respiratory rate and arrhythmia incidence were quantified during resting breathing in heart failure animals with and without carotid body ablation.
- Our results indicate that carotid body chemoreceptor denervation reduces sympathetic nerve activity, disordered breathing patterns, arrhythmia incidence and sympatho-respiratory coupling in experimental heart failure.
- These findings suggest that device-oriented ablation of carotid body chemoreceptors is a viable treatment option for reduction of sympathetic nerve activity, disordered breathing patterns and arrhythmia incidence in heart failure.

Abstract In congestive heart failure (CHF), carotid body (CB) chemoreceptor activity is enhanced and is associated with oscillatory (Cheyne–Stokes) breathing patterns, increased sympathetic nerve activity (SNA) and increased arrhythmia incidence. We hypothesized that denervation of the CB (CBD) chemoreceptors would reduce SNA, reduce apnoea and arrhythmia incidence and improve ventricular function in pacing-induced CHF rabbits. Resting breathing, renal SNA (RSNA) and arrhythmia incidence were measured in three groups of animals: (1) sham CHF/sham–CBD (sham–sham); (2) CHF/sham–CBD (CHF–sham); and (3) CHF/CBD (CHF–CBD). Chemoreflex sensitivity was measured as the RSNA and minute ventilatory ($\dot{V}_E$) responses to hypoxia and hypercapnia. Respiratory pattern was measured by plethysmography and quantified by an apnoea–hypopnoea index, respiratory rate variability index and the coefficient of variation of tidal volume. Sympatho-respiratory coupling (SRC) was assessed using power spectral analysis and the magnitude of the peak coherence function between tidal volume and RSNA frequency spectra. Arrhythmia incidence and low frequency/high frequency ratio of heart rate variability were assessed using ECG and blood pressure waveforms, respectively. RSNA and $\dot{V}_E$ responses to hypoxia were augmented in CHF–sham and abolished in CHF–CBD animals. Resting RSNA was greater in CHF–sham compared to sham–sham animals ($43 \pm 5\%$ max *vs.* $23 \pm 2\%$ max,

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$P < 0.05$), and this increase was not found in CHF–CBD animals (25 ± 1% max, $P < 0.05$ *vs.* CHF–sham). Low frequency/high frequency heart rate variability ratio was similarly increased in CHF and reduced by CBD ($P < 0.05$). Respiratory rate variability index, coefficient of variation of tidal volume and apnoea–hypopnoea index were increased in CHF–sham animals and reduced in CHF–CBD animals ($P < 0.05$). SRC (peak coherence) was increased in CHF–sham animals (sham–sham 0.49 ± 0.05; CHF–sham 0.79 ± 0.06), and was attenuated in CHF–CBD animals (0.59 ± 0.05) ($P < 0.05$ for all comparisons). Arrhythmia incidence was increased in CHF–sham and reduced in CHF–CBD animals (213 ± 58 events h^{-1} CHF, 108 ± 48 events h^{-1} CHF–CBD, $P < 0.05$). Furthermore, ventricular systolic (3.8 ± 0.7 *vs.* 6.3 ± 0.5 ml, $P < 0.05$) and diastolic (6.3 ± 1.0 *vs.* 9.1 ± 0.5 ml, $P < 0.05$) volumes were reduced, and ejection fraction preserved (41 ± 5% *vs.* 54 ± 2% reduction from pre-pace, $P < 0.05$) in CHF–CBD compared to CHF–sham rabbits. Similar patterns of changes were observed longitudinally within the CHF–CBD group before and after CBD. In conclusion, CBD is effective in reducing RSNA, SRC and arrhythmia incidence, while improving breathing stability and cardiac function in pacing-induced CHF rabbits.

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Abbreviations AHI, apnoea–hypopnoea index; CHF, congestive heart failure; CB, carotid body; CBD, carotid body denervation; CV of V_t, coefficient of variation of tidal volume; HRV, heart rate variability; HF, high frequency of HRV; iSNA, integrated renal sympathetic nerve activity; LF, low frequency of HRV; RRVI, respiratory rate variability index; RSNA, renal sympathetic nerve activity; SNA, sympathetic nerve activity; SRC, sympatho-respiratory coupling

Introduction

Clinical evidence indicates a positive correlation between chemosensitivity, neurohumoral activation, oscillatory (Cheyne–Stokes) breathing and arrhythmia incidence (Giannoni *et al.* 2008). The importance of these relationships is underscored by the fact that mortality risk is significantly higher in patients with congestive heart failure (CHF) with high chemosensitivity relative to comparable patients with CHF with normal chemosensitivity (Ponikowski *et al.* 2001; Giannoni *et al.* 2008). While the causes of higher mortality associated with high chemosensitivity in CHF are incompletely understood, it is known that increased sympathetic nerve activity (SNA) (Meredith *et al.* 1991) and oscillatory breathing (Leung *et al.* 2004) are associated with a higher incidence of arrhythmias, and that arrhythmia incidence is independently associated with mortality risk in patients with CHF (Meinertz *et al.* 1985).

Multiple lines of evidence suggest that increased SNA in CHF can be attributed to the presence of oscillatory breathing (Naughton *et al.* 1995; van de Borne *et al.* 1998) as well as alterations in cardiovascular reflexes and central neural control of SNA (Zucker *et al.* 2004). Previous work from our lab has shown that both the afferent and efferent arms of the carotid body (CB) chemoreflex are enhanced in an animal model of CHF (Sun *et al.* 1999a,*b*), and that normalization of CB chemoreflex function using pharmacological or genetic techniques results in reductions in resting SNA (Li *et al.* 2005, 2006; Ding *et al.* 2009). Despite the observed correlations between chemoreflex sensitivity, SNA and oscillatory breathing (Giannoni *et al.* 2008), no studies have definitively determined the role of the CB chemoreflex in the aetiology of disordered breathing patterns and autonomic function in CHF. The primary purpose of this study was to assess the viability of therapeutic denervation of the CB chemoreceptors for the reduction of SNA and disordered breathing patterns in CHF, and to determine the subsequent effects on arrhythmia incidence and cardiac function.

Recent studies suggest that enhanced coupling between sympathetic and respiratory neural drive (sympatho-respiratory coupling, SRC) may contribute to sympathetic overactivity in cardiovascular diseases (Zoccal *et al.* 2008; Molkov *et al.* 2011; Costa-Silva *et al.* 2012). In an animal model that mimics the episodic hypoxia associated with sleep apnoea, enhanced sensitivity of the CB chemoreflex (Peng *et al.* 2003) contributes to enhanced ventilatory and sympathetic responses to hypoxia (Peng *et al.* 2006; Marcus *et al.* 2010) as well as alterations in resting sympathetic outflow (Marcus *et al.* 2010). Furthermore, the CB chemoreceptors serve as the crucial link in this model between enhanced sympathetic activity and the development of increases in arterial pressure (Lesske *et al.* 1997; Peng *et al.* 2006). Other investigators have shown a link between CB chemoreceptors, SRC and hypertension in spontaneously hypertensive rats (Simms *et al.* 2009; Abdala *et al.* 2012). Based on these findings, a secondary

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aim of our study was to explore the nature of SRC in CHF, and to determine its dependence on the CB.

We hypothesized that selective CB denervation (CBD) applied after the development of CHF would attenuate resting renal SNA (RSNA), disordered breathing patterns and arrhythmia incidence. Furthermore, we hypothesized that SRC would be increased in CHF concomitant with enhanced CB chemoreceptor sensitivity, and that this enhanced coupling would be reduced after CBD. We measured RSNA, ventilation and the electrocardiogram (ECG) at rest and in response to hypoxia and hypercapnia to characterize the sensitivity of the CB chemoreflex and its contribution to resting RSNA, disordered breathing patterns, arrhythmia incidence and SRC in CHF. Left ventricular function was assessed by echocardiography.

Methods

Ethical approval

The experimental protocols were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the American Physiological Society's Guide for the Care and Use of Laboratory Animals.

Induction of congestive heart failure

Adult male New Zealand White rabbits weighing 3.5–4.0 kg were housed in individual cages under controlled temperature and humidity and a 12:12 h dark–light cycle, and fed standard rabbit chow with water available ad libitum.

CHF was induced by pacing and monitored by weekly echocardiograms as previously described (Sun *et al.* 1999b; Ding *et al.* 2011). Chronic pacing of the atrium or ventricle to produce heart failure has been used extensively and is considered a reliable disease model for replication and study of the mechanical, structural and neurohumoral alterations associated with dilated cardiomyopathy in humans (Houser *et al.* 2012). Our pacing protocol results in decreases in cardiac contractility and ejection fraction (EF), and increases in cardiac volume, resting heart rate and central venous pressure, in addition to increases in RSNA (Sun *et al.* 1999b).

Rabbits were anaesthetized with a cocktail of 5.8 mg kg^{-1} xylazine, 35 mg kg^{-1} ketamine and 0.01 mg kg^{-1} atropine given I.M., intubated and connected to a small animal anaesthesia respiration unit using 2.0–5.0% inhalation isoflurane with oxygen for the duration of the surgery. Using sterile technique, a pressure telemetry unit was implanted into a branch of the femoral artery and advanced into the iliac artery or into the abdominal aorta. A left thoracotomy was performed to implant a pin electrode based on the left ventricle for pacing. The leads of pacing electrodes were tunnelled subcutaneously and fixed on the back. Postoperatively, rabbits were given an antibiotic regimen consisting of 5 mg kg^{-1} Baytril (S.C.) repeated daily for 5 days. All experimental protocols began 10 days after surgery when the animals had fully recovered.

The rapid ventricular pacing model of inducing CHF was carried out using a pacemaker of our design. We initially began pacing at a rate of 340 bpm, which was held for 7 days, and then the rate was gradually increased to 380 bpm, with an increment of 20 bpm each week. Each rabbit was checked daily to insure chronic pacing. Rabbits were paced continually for 3–4 weeks. The progression of CHF was monitored by weekly echocardiograms (Siemens/Acuson Sequoia 512C with a 4 MHz probe; Siemens Medical Solutions USA, Inc., Malvern, PA, USA) before CBD, and at 3 day intervals after CBD, with the pacemaker turned off for at least 30 min before the recordings were started. CHF was characterized by >35% reduction in EF and fractional shortening (FS).

Selective denervation of carotid body chemoreceptors and implantation of renal nerve electrodes

After CHF induction (3–4 weeks of pacing), the rabbits underwent CBD or sham denervation surgery, using the method described by Verna *et al.* (1975). Anaesthesia was induced with an anaesthetic cocktail (5.8 mg kg^{-1} xylazine, 35 mg kg^{-1} ketamine and 0.01 mg kg^{-1} atropine given I.M.) and maintained with inhalation of isoflurane (see 'thoracotomy' in 'Induction of congestive heart failure'). Using sterile techniques, the carotid sinus regions were exposed and the CB visually identified. The CBs were cryogenically destroyed using a fine-tipped forceps cooled in liquid nitrogen. Sham–sham and CHF–sham groups were subjected to a similar surgical exposure, but the CBs were not destroyed.

In all groups, a pair of electrodes was implanted on the left renal sympathetic nerves during the same surgical procedure. Using sterile techniques, an incision was made along the costal margin on the left side. Muscles were retracted and the kidney was approached in the retroperitoneal space. The renal artery and nerve were identified. Using glass hooks, the renal nerve was gently separated from the artery and a pair of Teflon coated silver wire electrodes were secured to the nerve using a fast-setting silicone gel (Wacker Sil-Gel). A ground wire was sutured to a nearby muscle before the incision was closed. The electrode wires were tunnelled beneath the skin to exit on the upper back. After surgery, all animals were placed on an antibiotic regimen consisting of 5 mg kg^{-1} Baytril (S.C.) for 5 days. Rabbits were allowed to recover for 24 h postsurgery before cardiac pacing resumed in the CHF–sham and CHF–CBD groups. All animals were

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allowed to recover for 3 days postsurgery before any experiments were performed.

Experimental groups

Animals were randomly assigned to one of three experimental groups: (1) sham–sham: underwent operation to implant pacing electrodes but were not paced and underwent sham–CBD surgery; (2) CHF–sham: pacing protocol with sham–CBD surgery; (3) CHF–CBD: pacing protocol with CBD surgery. All baseline and experimental measurements were obtained from paced animals with the pacemaker turned off for at least 30 min.

Chemoreflex evaluation with renal sympathetic nerve activity and minute ventilation

To determine changes in CB chemoreflex sensitivity with CHF induction and to assess the efficacy of CBD, reflex changes in $\dot{V}_E$ and RSNA were measured in response to hypoxia. Tidal volume (V_t) and respiratory rate (RR) were calculated from the pressure changes in the chamber (MP45 differential pressure transducer and amplifier; Validyne Engineering Corp., Northridge, CA, USA) connected to a PowerLab (Model 8SP) analysis system (ADInstruments Inc., Colorado Springs, CO, USA) as described previously (Sun *et al.* 1999b). Ventilatory responses to hypoxia and hypercapnia were determined longitudinally in the same animal through the course of the study (before and after CHF induction or sham pacing, and after CBD or sham–CBD surgery). An experimental timeline is provided in the online supplement (Fig. S1).

RSNA responses to hypoxia were measured along with ventilation in these groups, 3–9 days after renal electrode implant concomitant with CBD or sham–CBD surgery (see timeline Fig. S1). RSNA was recorded using a Grass P511 differential amplifier (Grass Technologies/Astro-Med Inc., West Warwick, RI, USA) and a storage oscilloscope. The RSNA was filtered at a bandwidth of 100 Hz–3 kHz. The nerve signal was also fed to an audio amplifier and loudspeaker. The nerve signal was rectified and integrated (iSNA), and both raw and integrated signals were recorded. All analog signals were captured and digitized by the PowerLab data acquisition system.

RSNA and $\dot{V}_E$ responses to chemoreceptor stimulation were measured in the conscious resting state. CB chemoreceptors were stimulated preferentially by allowing the rabbits to breathe graded mixtures of hypoxic gas under poikilocapnic conditions. Different concentrations of O_2 with balance of N_2 were delivered into the chamber in the following sequence: 21% O_2 (normoxia), 15% O_2 (mild hypoxia) and 10% O_2 (moderate hypoxia). To assess CO_2 sensitivity, the rabbits breathed different concentrations of normoxic gas with CO_2 added (5% and 7% CO_2 with balance O_2 and N_2). Linear regression was applied to determine the slope of the ventilatory response to CO_2, which was used to quantify this response. Each stimulation was held for 2.5 min until a steady response was achieved. Ten minutes of recovery time at 21% O_2 was allowed between stimuli to ensure that all variables returned to baseline levels.

Evaluation of resting variables

For all measurements, pacemakers were turned off 30 min before the beginning of experiments. Rabbits were placed in a Plexiglas chamber (volume 11 litres) with exit ports for ECG wires and renal nerve electrodes, and the chamber was sealed, except for an inlet and outlet port that allowed a continuous flow of air through the chamber. Measurements of resting blood pressure (BP), ECG, ventilation and RSNA (when available) were recorded over 2 h. Resting ventilation, BP, ECG were obtained longitudinally from each animal over the course of the study (Fig. S1). Arterial BP was measured via a radio-telemetry transmitter (Data Sciences International, Saint Paul, MN, USA).

Apnoea–hypopnoea index and respiratory variability

Resting breathing pattern was determined by plethysmography. To quantify respiratory disturbances associated with oscillatory breathing behaviour we calculated an apnoea–hypopnoea index (AHI), RR variability index (RRVI) and the coefficient of variation (CV) of tidal volume (V_t). The CV of V_t was calculated as the V_t standard deviation/V_t mean taken over the course of 20 min of resting breathing. The AHI was the tally of the number of apnoeas and hypopnoeas occurring per hour. An event was tallied if there was a 50% or greater reduction in V_t for three or more respiratory cycles. Apnoeas occurring immediately after a sigh were excluded from the analysis. Oscillations in RR (RRVI) were tallied when there was a change in ventilatory rate greater than 3 standard deviations from the mean (taken over the course of 1 h). All events for AHI or RRVI were identified visually, tallied and expressed as the number of events per hour (AHI) or the number of oscillations per hour (RRVI).

Power spectrum and coherence analysis

Respiration (V_t), BP and iSNA data were collected at 10 kHz at rest while the rabbits were in the plethysmograph. Auto- and cross-spectral estimates were computed in 5 min artefact-free recordings using the Welch's overlapped segment averaging method. A fast Fourier transform (FFT) algorithm was applied to each variable. The FFT size (n) was equal to 4,194,304 points and a hamming window of $n/16$ was used to account for spectral leakage. Power spectral resolution was 0.04 Hz. The auto-spectrum

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for each variable of each animal was normalized by expressing the power relative to the total spectral power. With oscillations in respiration or BP taken as the input signal and iSNA as the output signal, coherence analysis was performed over a range of 0.1 Hz centred at the frequency of the maximum respiratory or BP spectral peak. Estimation of the squared coherence function is a mathematic bivariate spectral method that ranges between 0 (no relation) and 1 (strong relation). High squared coherence values (>0.5) between variations in respiration, BP and SNA reflect a positive interaction between these signals. Transfer function gain and phase were also calculated at the respiratory spectral peak. Both transfer function gain and phase are considered reliable only at those frequencies at which input and output variables were coherent (>0.5).

Arrhythmia incidence

ECG data taken during the resting baseline periods was evaluated for the incidence of arrhythmias. Premature beats or delayed beats (beat to beat R-R interval greater than 3 standard deviations from the mean) or beats with ectopic foci were visually identified and recorded to determine an index of events per hour. All events meeting the stated criteria were tallied and combined to derive a single index. Measurements of arrhythmia incidence were taken longitudinally at the pre-pace, CHF and CHF–CBD time points.

Evaluation of heart rate and systolic blood pressure variability

Heart rate was derived from dP/dt BP signal recorded at 10 kHz sampling rate from the radiotelemetry transmitter (Data Sciences International), and calculated on a beat-to-beat basis by using the Powerlab system and LabChart software (ADInstruments). Heart rate variability (HRV) was analysed by using the HRV extension for LabChart 7 as previously described (Pliquett *et al.* 2003). The same BP used to calculate HRV were used for low frequency component of systolic BP variability analysis in the same analytical manner as for HRV analysis (Egi *et al.* 2007). These methods are described in more detail in the online supplement.

Baroreflex sensitivity

Spontaneous cardiac baroreflex sensitivity was assessed by FFT of the R-R interval and the systolic BP in the low frequency domain (Parati *et al.* 2000). Spontaneous RSNA baroreflex sensitivity was determined by relating spontaneous changes in RSNA and diastolic BP (Sundlof & Wallin, 1978). These methods are described in more detail in the online supplement.

Data analysis

All parameters were recorded with the Powerlab data acquisition and analysis system. RSNA was expressed as the percentage of maximal nerve activity evoked by eliciting the oropharyngeal reflex with a puff of cigarette smoke as previously described (Sun *et al.* 1999b; Li *et al.* 2006; Ding *et al.* 2011), determined after completing each experiment. Resting and chemoreflex-evoked respiratory responses (minute ventilation, $\dot{V}_E$) were calculated as the product of tidal volume (V_t) and RR ($\dot{V}_E = V_t \times$ RR). Chemoreflex function curves were analysed by plotting data points averaged over 10 s for RSNA and $\dot{V}_E$ against the corresponding F_{iO_2}.

Because it was not possible to maintain viable renal nerve electrodes through the course of pacing, they were implanted after the development of CHF, and RSNA was measured at 3 day intervals after CBD or sham–CBD surgery. Owing to drop-off of viable recordings after 3 days, data for time points at 6 and 9 days post-CB surgery were averaged if there were measurements at both 6 and 9 days. The differences in resting and chemoreflex-evoked RSNA between groups were determined using one-way ANOVA. Other variables were measured longitudinally through the course of pacing and CBD. Thus, chemoreflex $\dot{V}_E$ responses, measures of resting breathing pattern, echocardiographic parameters and arrhythmia incidence in the experimental groups were analysed by two-way ANOVA for repeated measures. The Student–Newman–Keuls procedure was used as the multiple comparison test to determine the significance between groups. All data are expressed as means ± S.E.M. Statistical significance was accepted when $P < 0.05$. An experimental timeline is provided in the online supplement (Fig. S1).

Results

Effects of congestive heart failure and carotid body denervation on chemoreflex sensitivity

Changes in the sensitivity of the CB chemoreflex with CHF and the efficacy of CBD are illustrated in Fig. 1. Activation of RSNA in response to hypoxia (F_{iO_2} 0.10) was markedly and significantly enhanced in CHF–sham animals compared to sham–sham (+16 ± 2% max sham–sham *vs.* +36 ± 8% max CHF–sham) and was abolished in CHF–CBD animals (+1 ± 1% max, Fig. 1). The hypoxic ventilatory response (F_{iO_2} 0.10) was significantly enhanced in CHF–sham animals compared to sham–sham (+155 ± 30 ml min^{-1} sham–sham *vs.* +264 ± 42 ml min^{-1} CHF–sham) and was nearly abolished in CHF–CBD (+25 ± 29 ml min^{-1}) (Fig. 1). Similar changes in the hypoxic ventilatory response were observed longitudinally in CHF–CBD animals (+182 ± 37 ml min^{-1} pre-pace, +279 ± 46 ml min^{-1} CHF, +25 ± 28 ml min^{-1} CHF–CBD). The ventilatory response to hypercapnia was

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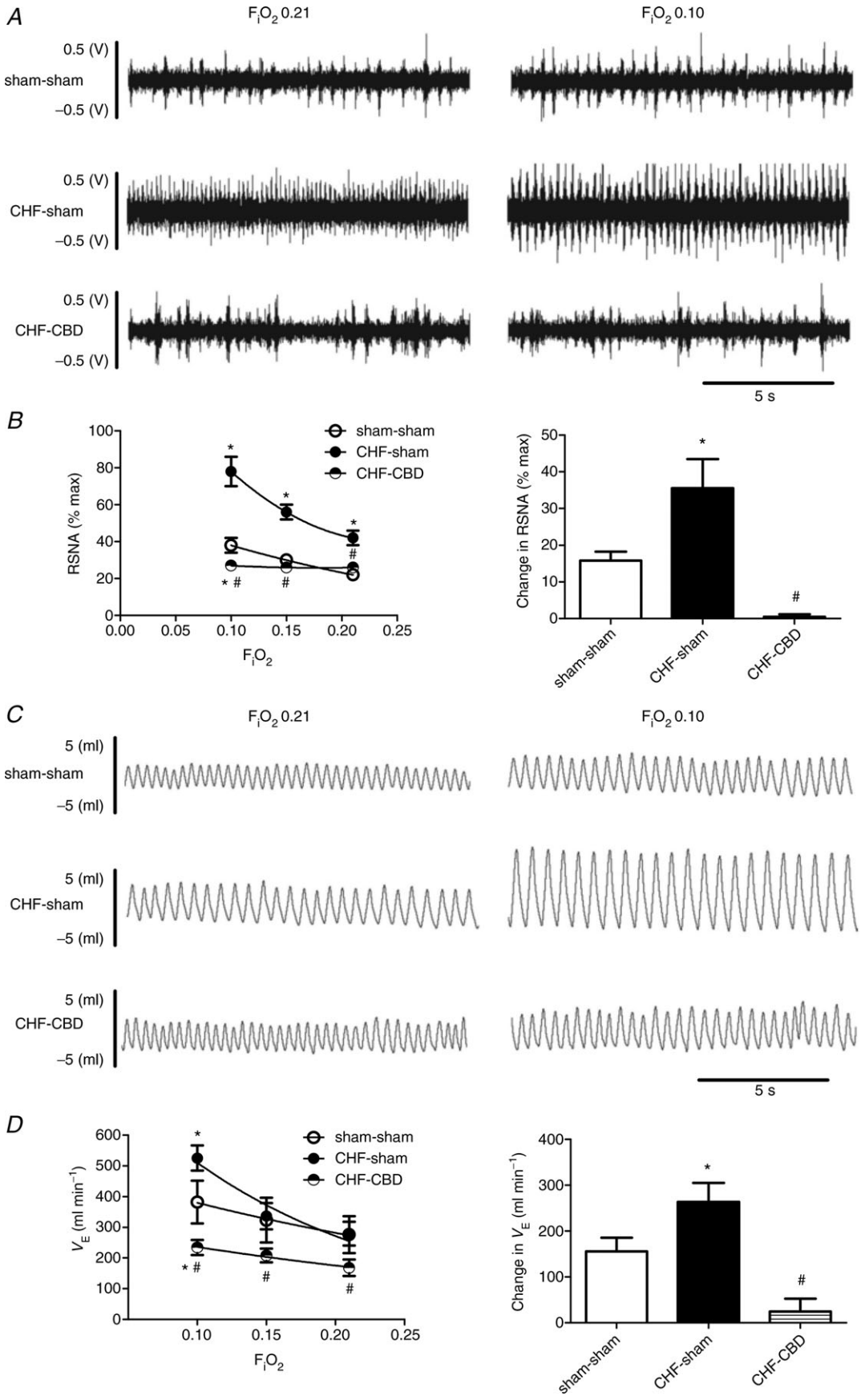

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enhanced in CHF, and was returned to normal after CBD (Fig. S2A and S2B) but not sham–CBD surgery. The ventilatory response to hypercapnia remained unchanged in sham–sham animals: CO_2 response slope [pre-pace sham 24 ± 4; sham–sham (day 3) 26 ± 4 ml min^{-1} % F_{iCO_2}; $P = 0.6677$] and resting $\dot{V}_E$ [pre-pace sham 289 ± 68; sham–sham (day 3) 233 ± 64 ml min^{-1}; $P = 0.4760$].

Effects of congestive heart failure and carotid body denervation on resting renal sympathetic nerve activity and $\dot{V}_E$

We were able to maintain RSNA recordings in eight CHF animals for 3 days post-CBD and in five of these animals for 6–9 days. Resting RSNA in CHF–sham rabbits was nearly double that compared to the sham–sham group at 3 days post-CB surgery and remained elevated through the 6–9 day interval (Fig. 2*B*). RSNA was significantly lower in CHF–CBD animals compared to the CHF–sham group at 3 day postsurgery (25 ± 1% max CHF–CBD; Fig. 2*B*), as well as at 6–9 days postsurgery (28 ± 2% max CHF–CBD, Fig. 2*B*), with resting RSNA levels equivalent to that of sham animals. Thus, CBD normalized resting RSNA in CHF rabbits.

Resting $\dot{V}_E$ was not different between groups at the start of the experimental protocol (sham–sham 276 ± 61 ml min^{-1}, CHF–sham 218 ± 41 ml min^{-1}, CHF–CBD 288 ± 24, $P = 0.49$; Tables S3 and S4). In both paced groups there was a trend for $\dot{V}_E$ to increase in CHF; however, the differences were not statistically significant. At the 3 day post-CB surgery time point, $\dot{V}_E$ was not different between CHF–sham (246 ± 38 ml min^{-1}) and sham–sham (277 ± 45 ml min^{-1}) animals (Tables S3 and S4). $\dot{V}_E$ was significantly lower in CHF–CBD animals (150 ± 17 ml min^{-1}, $P < 0.05$) compared to the CHF–sham and sham–sham groups. A similar trend was seen longitudinally in resting $\dot{V}_E$ in CHF–CBD animals (Tables S3 and S4).

Effect of congestive heart failure and carotid body denervation on apnoea–hypopnoea index, respiratory rate variability index and coefficient of variation of tidal volume

AHI significantly increased with the development of CHF at 3–4 weeks pacing (Fig. 3*A* and *B*), and AHI continued to increase with continued pacing after sham–CBD surgery [pre-pace 11 ± 1; CHF 20 ± 1; CHF–sham (day 3) 21 ± 1; CHF–sham (days 6–9) 23 ± 3 events h^{-1}]. However, in the group of animals that underwent CBD after being paced to CHF, AHI was significantly reduced subsequent to denervation [pre-pace 8 ± 2; CHF 16 ± 2; CHF–CBD (day 3) 5 ± 1; CHF–CBD (days 6–9) 8 ± 2 events h^{-1}]. A similar pattern was observed in the other measures of breathing pattern variability (Fig. 3*C* and *D*). Both the RRVI [pre-pace 9 ± 2; CHF 34 ± 4; CHF–sham (day 3) 32 ± 9; CHF–sham (days 6–9) 27 ± 5 RR oscillations h^{-1}] and the CV of V_t [pre-pace 0.23 ± 0.03; CHF 0.39 ± 0.04; CHF–sham (day 3) 0.41 ± 0.05; CHF–sham (days 6–9) 0.34 ± 0.03] increased with CHF and remained elevated above pre-pace levels after sham–CBD surgery. In the animals that underwent CBD after CHF, both the RRVI [pre-pace 9 ± 3; CHF 29 ± 3; CHF–CBD (day 3) 12 ± 4; CHF–CBD (days 6–9) 10 ± 2 oscillations h^{-1}] and the CV of V_t [pre-pace 0.23 ± 0.02; CHF 0.38 ± 0.04; CHF–CBD (day 3) 0.23 ± 0.02; CHF–CBD (days 6–9) 0.20 ± 0.03] were reduced.

Effects of congestive heart failure and carotid body denervation on renal sympathetic nerve activity/respiratory coherence

SRC was evident in rabbits with CHF. The bursting of RSNA showed low correlation with the respiratory cycles at rest in sham–sham rabbits 3 days after sham–CBD surgery (Figs. 4 and 5*A*). In CHF–sham rabbits, the synchronicity of the RSNA bursting with respiratory cycles was markedly increased over the same period. Moreover, concomitant oscillations in RSNA and ventilation typically occurred in the CHF state (Figs. 4 and 5*A*). The magnitude of coherence between respiration and RSNA increased 1.6-fold in CHF–sham rabbits compared to the values obtained in sham–sham rabbits (Fig. 5*C*). Denervation of the CB during CHF significantly reduced SRC coupling in CHF–CBD rabbits (Figs. 4 and 5*A–C*; $P < 0.05$). Accordingly, CHF–CBD rabbits exhibited RSNA/respiratory coherence values comparable to those obtained from sham–sham rabbits (0.48 ± 0.05 *vs.* 0.59 ± 0.04, sham–sham *vs.* CHF–CBD, respectively) at 3 days post-CB surgery that persisted over the remaining 6–9 day period (Fig. 5*C*). CHF–sham rabbits displayed a negative transfer function phase angle (−22.4 ± 34.9°)

Figure 1. Effect of CBD on RSNA and $\dot{V}_E$ responses to hypoxia
Representative RSNA (*A*) and plethysmographic (*C*) recordings taken at 3 days post-CB surgery showing the response to poikilocapnic hypoxia exposure (0.10 F_{iO_2}) in sham–sham, CHF–sham, and CHF–CBD animals. (*B* and *D*) Mean data illustrating RSNA and minute ventilation ($\dot{V}_E$) at each level of F_{iO_2} (left), and the change in RSNA and $\dot{V}_E$ from 0.21 F_{iO_2} (baseline) to 0.10 F_{iO_2} (hypoxia) (right). RSNA is expressed as percentage of the maximum response to cigarette smoke exposure at each level of F_{iO_2}. $n = 6$ sham–sham, $n = 7$ CHF–sham, $n = 8$, CHF–CBD. *$P < 0.05$ *vs.* sham–sham, #$P < 0.05$ *vs.* CHF–sham. CBD, denervation of the carotid body; CHF, congestive heart failure; RSNA, renal sympathetic nerve activity.

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supporting the notion that respiratory oscillations in RSNA were in phase with the peak to late inspiratory (V_t) cycle. A high coherence was found between RSNA bursting and BP cycles in all conditions and was not impacted by CBD (Fig. 5*D*).

Effects of congestive heart failure and carotid body denervation on heart rate and systolic blood pressure variability and baroreflex sensitivity

Time domain analysis revealed that the standard deviation of the normal R-R interval was significantly decreased from pre-pace with the development of CHF, and CBD significantly reversed this effect (Fig. S3A and S3B). From power spectral analysis, the total spectral power of HRV was reduced from pre-pace with the development of CHF, and CBD reversed this effect (Fig. S3C). The LF/HF ratio (Fig. S3D) was increased in CHF, and CBD reversed this effect (see Supplementary Tables S5 and S6 for power densities). The increase in LF/HF ratio during CHF was associated with a significant reduction in the HF component (Tables S5 and S6).

Furthermore, analysis of the R-R interval, RSNA and BP variability indicated that spontaneous cardiac baroreflex sensitivity and renal sympathetic baroreflex sensitivity (Fig. S4A and S4B, Tables S5 and S6) were reduced in CHF and were restored to normal after CBD.

Finally, we evaluated the low frequency component of systolic BP variability (Fig. S4C, Tables S5 and S6). In accordance with resting RSNA, the low frequency component of systolic BP variability was significantly increased from pre-pace with the development of CHF and was significantly reduced after CBD (182 ± 38% of pre-pace CHF, 104 ± 18% of pre-pace CHF–CBD day 3).

None of the time domain or power spectral parameters changed significantly in sham–sham animals over the same time course (Tables S5 and S6).

Effects of congestive heart failure and carotid body denervation on arrhythmia incidence

As shown in Fig. 6, and indicated by the abrupt deflections in the heart rate tracings (Fig. 6*A*), there was a marked increase in the arrhythmia incidence with the development of CHF (34 ± 10 events h^{-1} pre-pace *vs.* 213 ± 58 events h^{-1} CHF, Fig. 6*B*) before CB surgery. After CBD, arrhythmia incidence was significantly reduced in this same group of animals [108 ± 48 events h^{-1} (day 3), Fig. 6*A* and *B*], and remained significantly reduced for 6–9 days after CBD. In CHF–sham animals, arrhythmia incidence remained elevated through the course of pacing (Fig. 6*B*). Arrhythmia incidence was not altered through the course of the protocol in sham–sham animals [30 ± 19 *vs.* 18 ± 9 events h^{-1} (day 3), and 24 ± 12 events h^{-1} (days 6–9)]. The vast majority of arrhythmias we observed were premature ventricular contractions with a compensatory pause; however, we also noted isolated sinus pauses as well as sustained bradyarrhythmias in one or two cases. CBD reduced arrhythmias with no selective effect on type.

Effects of congestive heart failure and carotid body denervation on cardiac function

After 3–4 weeks of pacing and in the resting state with the pacer turned off, EF and FS were reduced from pre-pace levels by ∼39% and 46%, respectively, concomitant with increased left ventricular systolic (LVs Vol) and left ventricular diastolic (LVd Vol) dimensions in both CHF groups

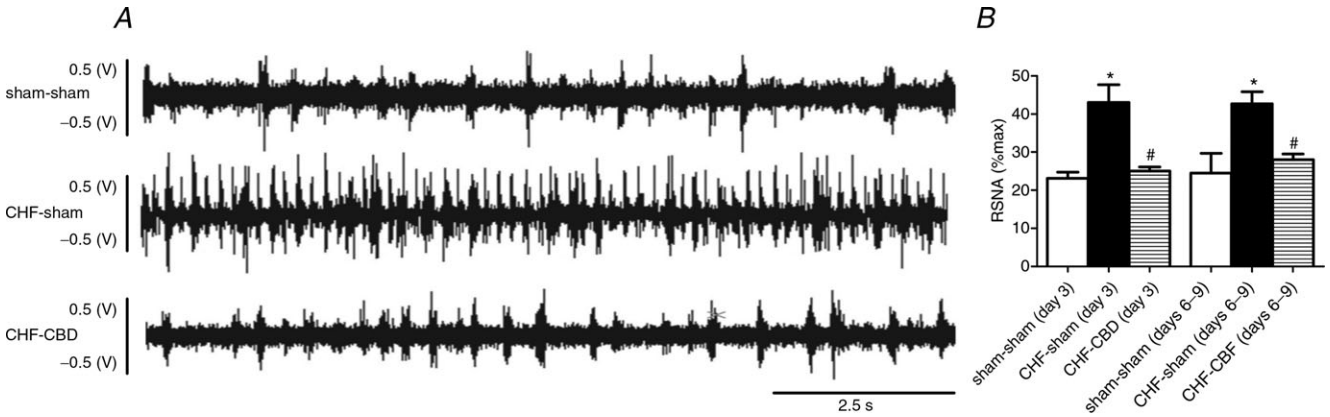

Figure 2. Effect of CBD on resting RSNA in CHF
A, representative RSNA recordings taken at rest at 3 days post-CB surgery in sham–sham (upper trace), CHF–sham (middle trace) and CHF–CBD (lower trace) animals with pacer off. *B*, resting RSNA was significantly increased in CHF–sham animals and was significantly reduced with CBD at 3 days and 6–9 days post-CB surgery. $^*P < 0.05$ *vs.* sham–sham, $\#P < 0.05$ *vs.* CHF–sham. CBD, denervation of the carotid body; CHF, congestive heart failure; RSNA, renal sympathetic nerve activity.

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before CBD or sham–CBD surgery (Table 1 and Fig. 7). Cardiac function was not altered through the course of the study in sham–sham animals (Table 1). Cross-sectional comparison of the CHF groups to the sham group showed similar differences in cardiac function to those observed longitudinally before and during pacing within the CHF groups (Tables 1 and 2).

In the CHF groups, the pacemaker was turned off for a period of 24 h during the CBD/sham–CBD surgery and recovery and then reinstated. There were no changes in any of the cardiac function parameters over the initial 3 days following CBD or sham–CBD surgery (Table 1); however, cardiac function progressively worsened in CHF–sham animals at 6–9 days post-CB surgery. EF was reduced an additional 20% (54.1 ± 2.0% reduction from pre-pace) and FS an additional 16% (61.1 ± 1.6% reduction from pre-pace) from the paced state just before CB surgery (Fig. 7*A* and Table 2) in this group. Furthermore, LVd Vol increased an additional 8% (61.1 ± 6.75% increase from pre-pace levels) and LVs Vol increased an additional 63% (239.4 ± 32.5% increase from pre-pace levels) over the same period (Fig. 7*A*).

In striking contrast to the CHF–sham animals at 6–9 days post-CB surgery, CHF–CBD animals exhibited a

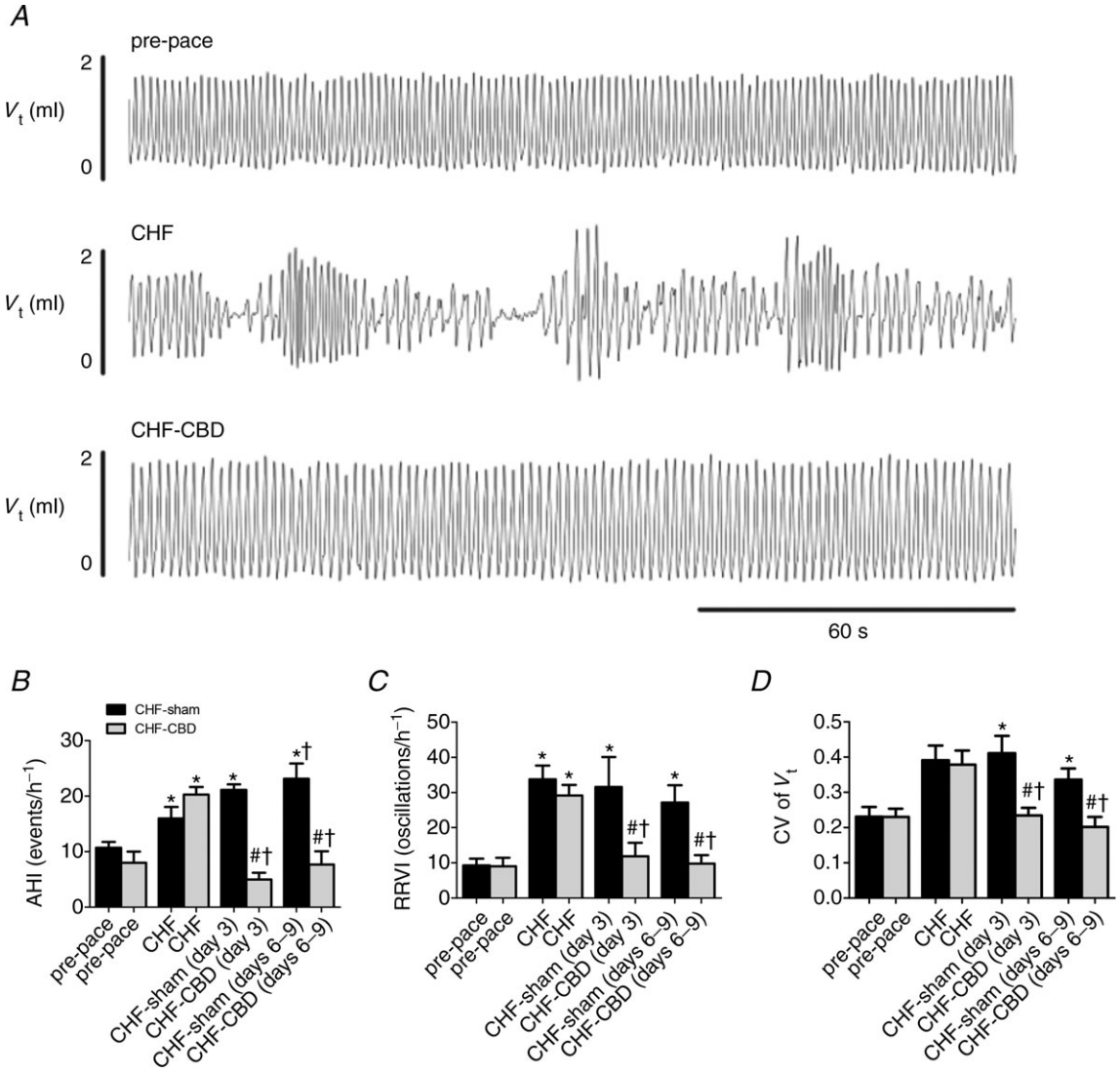

Figure 3. Effect of CBD on breathing patterns in CHF
A, representative tidal volume (V_t) traces from one animal illustrating changes in resting breathing patterns with the development of CHF, and the effect of CBD. *B–D*, mean data for measures of breathing pattern (AHI, RRVI and CV of V_t). Apnoea/hypopnoea incidence, respiratory rate variability and tidal volume variability all increased in CHF, and were attenuated after CBD. $n = 7$ CHF–sham, $n = 8$ CHF–CBD. *$P < 0.05$ *vs.* pre-pace. #$P < 0.05$ *vs.* CHF–sham. †$P < 0.05$ *vs.* respective CHF condition before CB surgery. AHI, apnoea–hypopnoea index; CBD, denervation of the carotid body; CHF, congestive heart failure; CV, coefficient of variation; RRVI, respiratory rate variability index.

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marked reversal of LVs Vol and LVd Vol to decrease (144% and 60% respectively) relative to the 3 day post-CB surgery time point (Fig. 7*B*). These improvements in cardiac volumes were accompanied by complete abrogation of a decline in EF and FS in CHF–CBD animals as compared to the CHF–sham group over the same period (Fig. 7*B* and Table 2).

Discussion

Our results confirm a role for the CB chemoreflex in the aetiology of autonomic imbalance and abnormal breathing patterns associated with CHF. Ablation of the CB chemoreceptors was shown to be an effective means of reducing both tonic and hypoxia-evoked RSNA in CHF. Furthermore, CBD nearly abolished ventilatory responses to hypoxia, decreased CO_2 sensitivity and resting ventilation, and decreased all indices of oscillatory breathing. The attenuation of AHI and measures of oscillatory breathing after CBD underscore the importance of CB for the occurrence of disordered breathing patterns in CHF. CB ablation also normalized SRC in CHF. The increased coherence between RSNA bursting and respiration in CHF rabbits is indicative of enhanced coupling of a sympathetic and

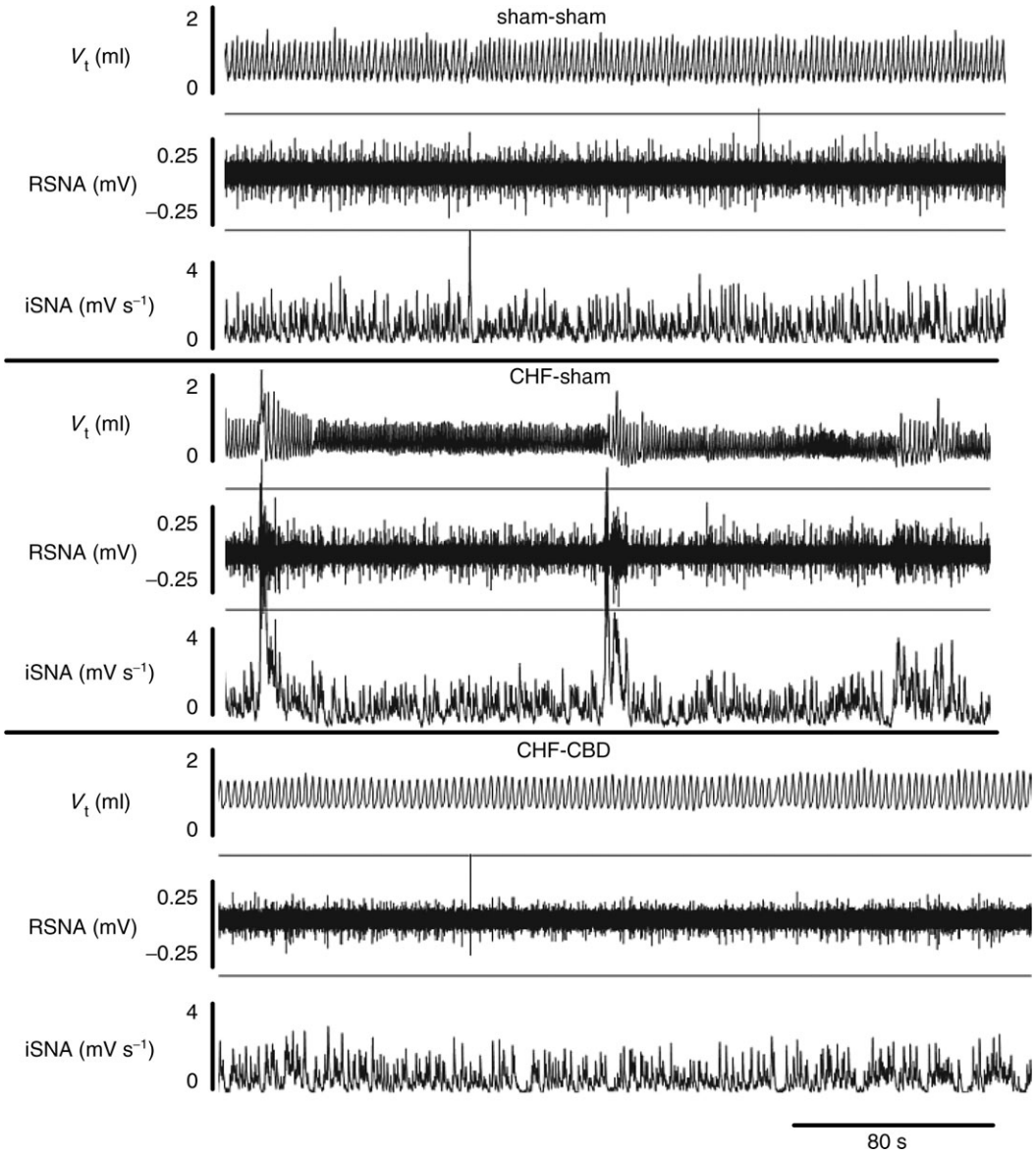

Figure 4. Effect of CBD on oscillations in respiration and RSNA
Representative recordings of V_t, RSNA and iSNA in sham–sham, CHF–sham and CHF–CBD animals. Qualitatively these tracings illustrate the concomitant oscillations in respiration and RSNA that occur in CHF, and the effect of CBD to normalize these oscillations. CBD, denervation of the carotid body; CHF, congestive heart failure; iSNA, integrated renal sympathetic nerve activity; RSNA, renal sympathetic nerve activity.

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respiratory neural drive. CBD reduced this coherence suggesting that the CB chemoreflex plays a role in enhancing SRC in CHF. Finally, CBD in established CHF improved cardiac function as evidenced by decreased arrhythmia incidence, decreased ventricular volumes and abrogation of the decline in EF. The reductions in AHI after CBD in CHF animals correlated with improvements in resting RSNA and indicators of cardiac autonomic balance, and these changes were in turn followed by improvements in cardiac function. The temporal sequence of the autonomic changes underscores a relationship between AHI and resting RSNA, and suggests that improving breathing stability may contribute to reducing sympathetic drive and lead to the improvements in arrhythmia incidence and cardiac function we observed after CBD. Cumulatively, these results support the notion that the CB contributes importantly to the cardiac, autonomic, respiratory and haemodynamic maladaptations that occur in the development of heart failure.

Carotid body chemoreceptors and control of renal sympathetic nerve activity in congestive heart failure

Alterations in multiple cardiovascular reflexes that influence neural control of cardiovascular function have been identified in CHF (Zucker *et al.* 2004). Previous studies from our laboratory have clearly shown that the CB

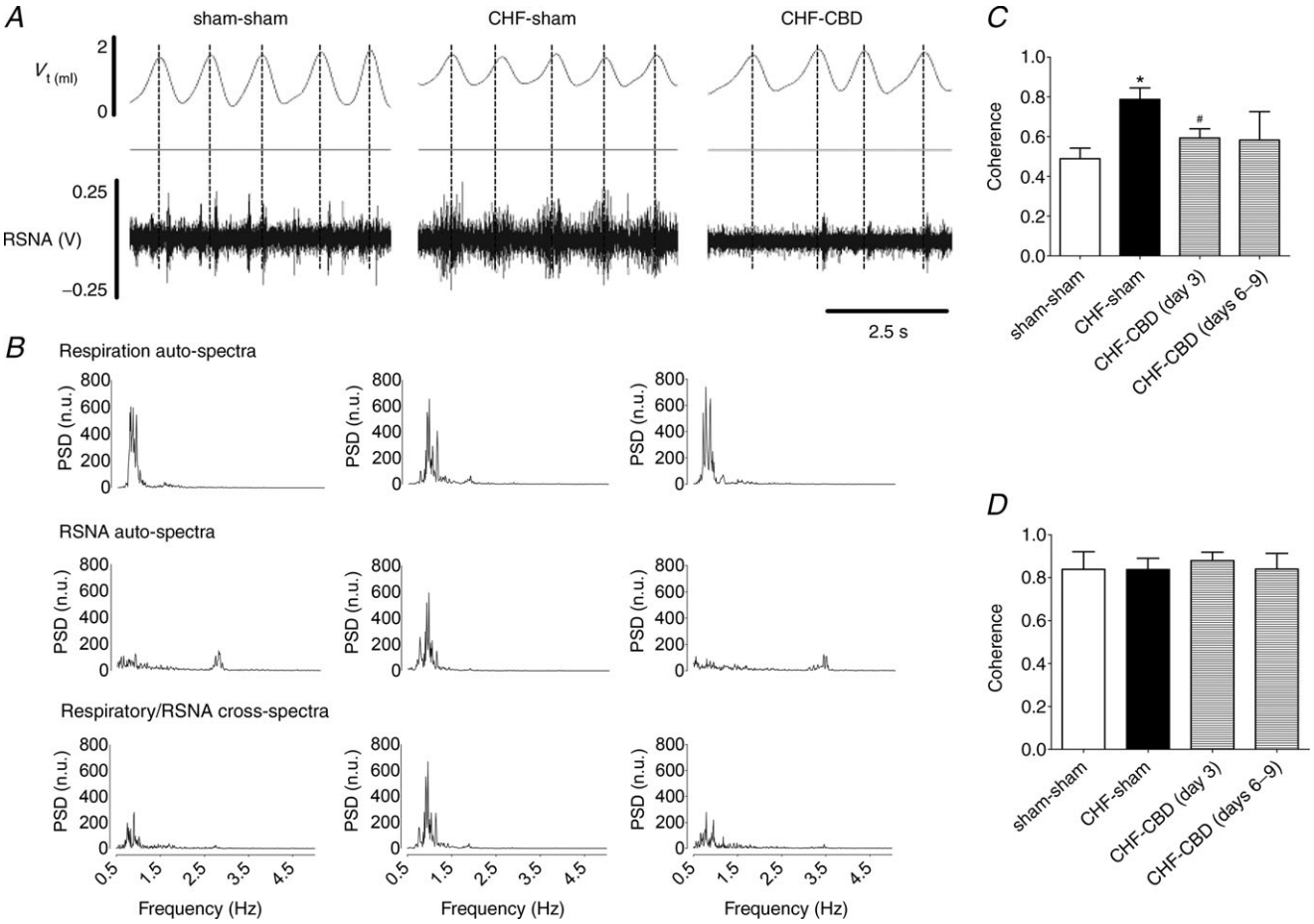

Figure 5. Effect of CBD on sympatho-respiratory coupling in CHF
A, representative recordings of resting V_t and RSNA in sham–sham (left trace), CHF–sham (middle trace) and CHF–CBD (right trace) animals. The dotted lines indicate one respiratory cycle and are placed to illustrate the relationship between respiration and RSNA. Qualitatively, these tracings show increased SRC in CHF that is reversed after CBD. *B*, power spectral densities for respiration (V_t), RSNA and the cross spectra of these two signals. *C*, magnitude of coherence at the peak respiratory frequency (a quantitative measure of SRC) was significant in CHF, and was reduced in CHF–CBD animals. *D*, the magnitude of arterial pressure and RSNA spectral coherence was significant; however, their coherence was unaffected by CHF or CBD. $n = 5$ sham–sham, $n = 5$ CHF–sham, $n = 6$ CHF–CBD at 3 days post-CB surgery, $n = 4$ CHF–CBD at 6–9 days post-CB surgery. $^*P < 0.05$ *vs.* sham–sham. $\#P < 0.05$ *vs.* CHF–sham. CBD, denervation of the carotid body; CHF, congestive heart failure; PSD, power spectral density; RSNA, renal sympathetic nerve activity.

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chemoreflex plays an important role in modulating sympathetic outflow in CHF (Sun *et al.* 1999a,*b*; Li *et al.* 2005, 2006; Ding *et al.* 2009, 2010, 2011). Our current findings that resting RSNA is lower in CHF animals with denervation of the CB further underscores the relationship between CB afferents and tonic control of efferent sympathetic outflow in CHF.

This study demonstrates that CBD lowers and normalizes tonic RSNA, eliminates hypoxic renal sympathetic activation in CHF and improves-inhibitory RSNA baroreflex sensitivity. Importantly, these specific CB chemoreflex-mediated influences on RSNA suggest a linkage between the CB chemoreflex and sympathetically mediated renal pathology, such as sodium retention, renin release and reductions in renal blood flow and may, in part, explain the cardio-renal syndrome of fluid retention, diuretic resistance and episodic decompensations in CHF (Fallick *et al.* 2011). Considering that sleep-disordered breathing and the attendant intermittent periods of hypoxia with CB activation are relatively common in patients with CHF (Rao *et al.* 2006) our results lend even greater significance to the role of the carotid chemoreceptors in development of cardio-renal syndrome in high chemosensitivity patients who experience episodic hypoxia.

Chemoreflex/baroreflex interaction in congestive heart failure

In addition to alterations in chemoreflex sensitivity and autonomic control of the heart, there is ample evidence that indicates the arterial baroreflex is altered in CHF as well (Eckberg *et al.* 1971; Chen *et al.* 1991, 1992). Recent evidence suggests an inverse relationship between chemoreflex and baroreflex sensitivity in patients with CHF (Despas *et al.* 2012). We found that that CBD improves spontaneous cardiac baroreflex and renal sympathetic baroreflex sensitivity in CHF. However, additional studies will be needed to determine the effect of CBD on the maximal gain of the baroreflex, as the spontaneous baroreflex sensitivity indices used in the present study only assess baroreflex sensitivity over the operational range of resting BP.

Enhanced chemoreflex sensitivity and disordered breathing patterns in congestive heart failure

CB chemoreceptor-mediated responses to hypoxia and hypercapnia are often augmented in patients with CHF (Wilcox *et al.* 1993; Javaheri, 1999; Giannoni *et al.* 2008).

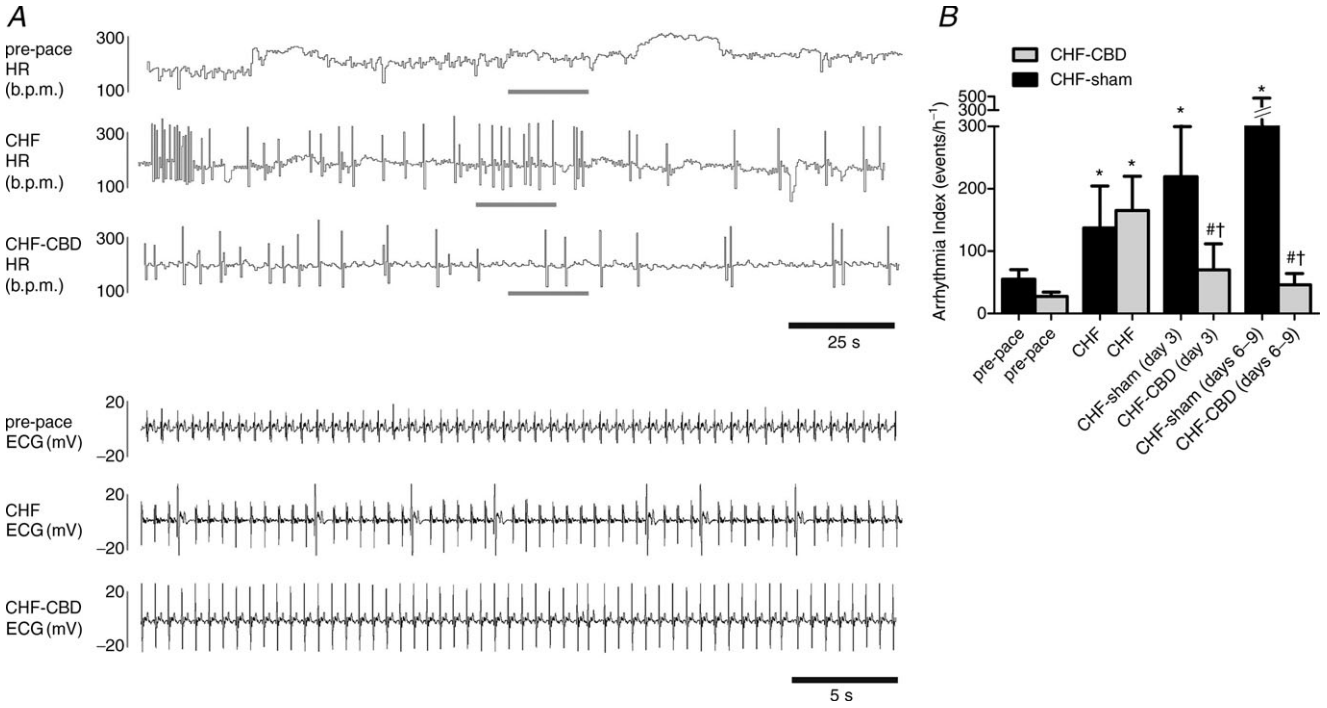

Figure 6. Effect of CBD on arrhythmia incidence in CHF
A, top, representative tracings of resting heart rate in one animal in the pre-pace, CHF and CHF–CBD states; bottom, an expanded scale of the ECG tracings taken during the time periods indicated by the grey lines in (*A*), illustrating the arrhythmic episodes indicated by the deflections in the heart rate tracing shown in (*A*). *B*, the arrhythmia incidence was increased in CHF and this effect was attenuated by CBD. Reductions in arrhythmia incidence were maintained in the subset of CHF–CBD animals in which RSNA recordings persisted for 6–9 days post-CBD. $n = 7$–8 rabbits in each CHF group at 3 days post-CB surgery, and $n = 5$ rabbits in each group at 6–9 days post-CB surgery. $^*P < 0.05$ *vs*. pre-pace, $\#P < 0.05$ *vs*. CHF–sham. $\dagger P < 0.05$ *vs*. respective CHF condition before CB surgery. CBD, denervation of the carotid body; CHF, congestive heart failure; HR, heart rate.

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Table 1. Echocardiographic measurements and body weight (day 3 post-CB surgery)

	Pre-pace	CHF	Postsurgery (day 3)
Sham–sham			
Body weight (kg)	3.8 ± 0 .2		3.9 ± 0.2
LVd Vol (ml)	6.0 ± 1.0		6.1 ± 0.3
LVs Vol (ml)	2.2 ± 0.5		1.9 ± 0.3
Ejection fraction	65.2 ± 2.9		68.3 ± 3.0
% change from pre-pace			1.8 ± 2.4
Cardiac output (ml min^{-1})	233.5 ± 23.4		271.0 ± 23.4
Fractional shortening	32.8 ± 2.0		35.3 ± 2.2
CHF–sham			
Body weight (kg)	3.5 ± 0.2	3.7 ± 0.2	3.6 ± 0.2
LVd Vol (ml)	5.7 ± 0.5	8.7 ± 1.0*	8.6 ± 0.4*
LVs Vol (ml)	2.0 ± 0.3	5.1 ± 0.6*	4.8 ± 0.3*
Ejection fraction	65.9 ± 2.5	40.7 ± 1.5*	36.8 ± 1.9*†
% change from pre-pace		–38.2 ± 1.2*	–44.1 ± 2.3*†
Cardiac output (ml min^{-1})	285.9 ± 44.4	221.2 ± 22.2	192.0 ± 6.2*†
Fractional shortening	33.3 ± 1.9	18.1 ± 0.1*	16.2 ± 1.0*†
CHF–CBD			
Body weight (kg)	3.6 ± 0.3	3.9 ± 0.2	3.6 ± 0.2
LVd Vol (ml)	6.0 ± 0.6	8.13 ± 1.0	10.0 ± 0.7*
LVs Vol (ml)	2.2 ± 0.4	5.0 ± 0.8*	6.3 ± 0.5*
Ejection fraction	66.7 ± 2.6	40.3 ± 3.7*	39.3 ± 1.6*†
% change from pre-pace		–40.1 ± 4.4*	–39.5 ± 2.5*†
Cardiac output (ml min^{-1})	226.0 ± 33.2	202.3 ± 19.1	203.2 ± 10.2†
Fractional shortening	32.7 ± 2.7	18.08 ± 2.0*	17.4 ± 0.8*†

CBD, denervation of the carotid body; CHF, congestive heart failure; LVd Vol, left ventricular diastolic volume; LVs Vol, left ventricular systolic volume. $n = 6$ sham–sham, $n = 7$ CHF–sham, $n = 8$ CHF–CBD. *$P < 0.05$ compared to pre-pace, †$P < 0.05$ compared to sham–sham (day 3).

Table 2. Echocardiographic measurements and body weight (days 6–9 post-CB surgery)

	Pre-pace	CHF	Postsurgery (day 3)	Postsurgery (days 6–9)
CHF–sham				
Body weight (kg)	3.7 ± 0.2	3.8 ± 0.2	3.6 ± 0.1	3.6 ± 0.2
Ejection fraction	66.4 ± 3.4	41.5 ± 2.1*	43.3 ± 1.3*	30.6 ± 2.5*†‡
% change from pre-pace		–37 ± 1.0*	–34 ± 5.0*	–54 ± 2.0*†‡
Cardiac output (ml min^{-1})	306.6 ± 58.8	231.3 ± 29.6	222.9 ± 11.8	228.9 ± 14.5
Fractional shortening	33.9 ± 2.6	18.6 ± 1.1*	19.6 ± 0.7*	13.2 ± 1.2*†‡
CHF–CBD				
Body weight (kg)	3.8 ± 0.2	3.9 ± 0.2	3.5 ± 0.2	3.4 ± 0.2
Ejection fraction	60.2 ± 2.9	34.3 ± 3.2*	30.8 ± 2.1*	33.5 ± 1.8*
% change from pre-pace		–41 ± 6*	–47 ± 4*	–41 ± 5*
Cardiac output (ml min^{-1})	304.2 ± 40.7	222.8 ± 21.7*	199.3 ± 32.7*	195.5 ± 18.4*
Fractional shortening	35.3 ± 1.8	18.2 ± 2.4*	16.0 ± 1.0*	17.7 ± 1.1*

CBD, denervation of the carotid body; CHF, congestive heart failure. $n = 5$ CHF–sham, $n = 5$ CHF–CBD. The ventricular volumes for this time point are shown in Fig. 7. *$P < 0.05$ compared to pre-pace, †$P < 0.05$ compared to CHF, ‡$P < 0.05$ compared to 3 days post-CB surgery time point.

A recent study in patients with CHF showed that those without augmented chemosensitivity did not exhibit oscillatory breathing, and that the incidence of oscillatory breathing progressively increased with enhancement of the CB chemoreflex (Giannoni *et al.* 2008). In other studies of patients with CHF, deactivation of CB chemoreceptors reduced or abolished cyclical breathing patterns and the incidence of central apnoeas (Ponikowski *et al.* 1999; Fontana *et al.* 2011). These findings suggest an important relationship between disordered cyclical breathing patterns and changes in CB chemoreflex sensitivity. In concordance with these findings, we have shown that CBD

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decreases AHI and measures of respiratory variability in CHF, indicating that the CB chemoreceptors contribute to the disordered breathing patterns associated with CHF.

Sympatho-respiratory coupling in congestive heart failure

Alterations in the relationship between central control of respiratory and sympathetic neural drive may underlie our observations of altered respiratory patterns and increased sympathetic outflow in CHF. Recent evidence indicates that SRC is increased in animal models of disease states associated with enhanced CB sensitivity (Zoccal *et al.* 2008; Simms *et al.* 2009; Kc *et al.* 2010; Kline, 2010; Toney *et al.* 2010; Silva & Schreihofer, 2011; Costa-Silva *et al.* 2012). We show for the first time in this study that SRC is enhanced in CHF concomitant with increases in CB chemoreflex sensitivity, and that ablation of CB chemoreceptors in turn reduces SRC. These results indicate that CB chemoreceptors contribute to SRC in CHF. Oscillatory breathing patterns in CHF have the potential to drive increased tonic sympathetic outflow as a result of increased SRC, but episodic desaturations associated with cyclical breathing patterns also result in surges in SNA (van de Borne *et al.* 1998), which may contribute to morbidity and mortality in patients with CHF.

Autonomic imbalance, arrhythmia incidence and disordered breathing patterns in congestive heart failure

Multiple lines of evidence indicate that oscillatory breathing patterns in patients with CHF contribute to autonomic imbalance (Naughton *et al.* 1995; van de Borne

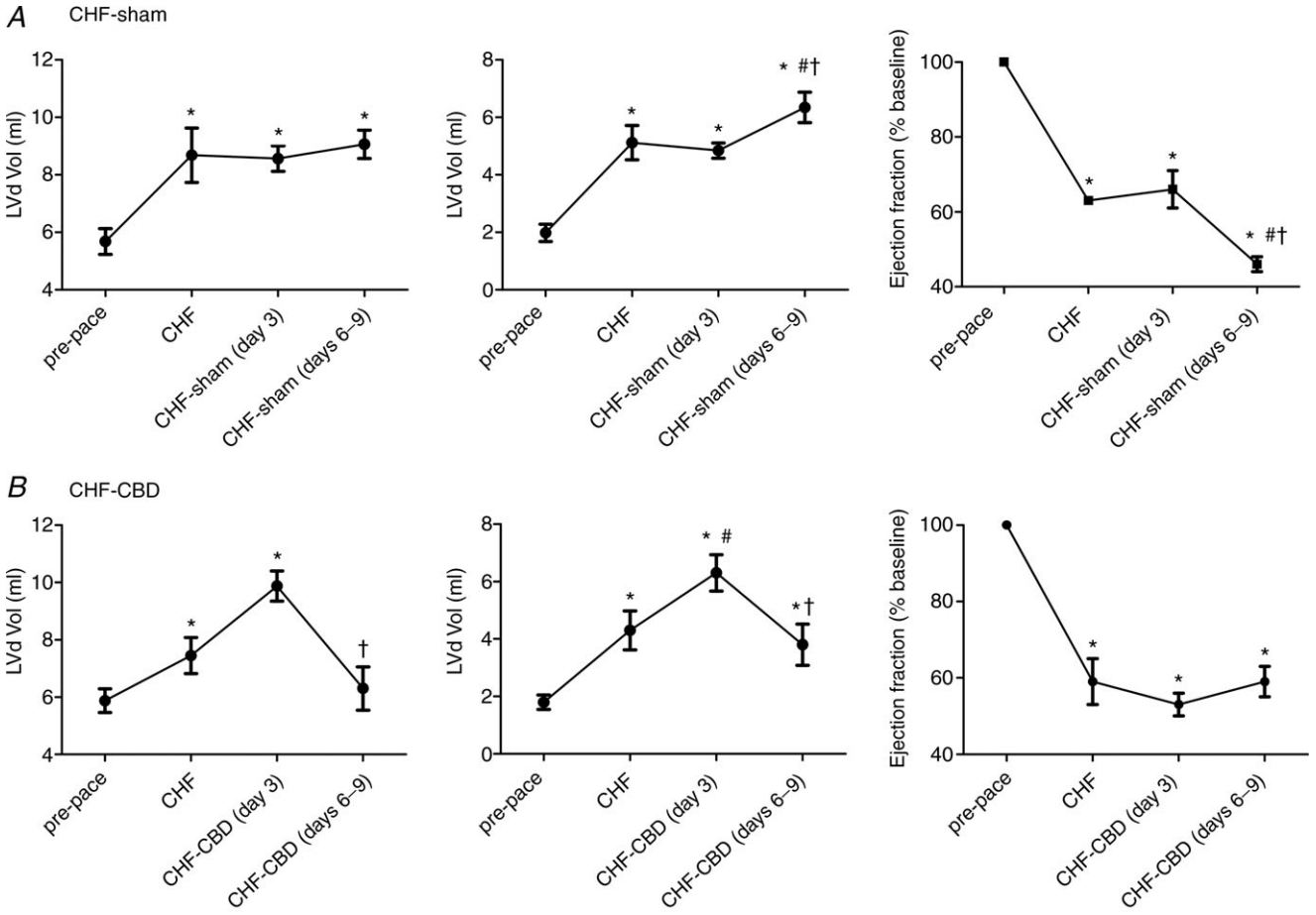

Figure 7. Effect of CBD on left ventricular volumes and ejection fraction
A, LVd Vol and LVs Vol were increased and ejection fraction was decreased in CHF–sham rabbits before CB surgery. After sham CB surgery with continued pacing, ventricular volumes continued to increase and ejection fraction decreased. *B*, ventricular volumes and ejection fraction were impaired in CHF–CBD rabbits before CBD to the same extent as in CHF–sham rabbits; however, ventricular volumes were significantly reduced after CBD with continued pacing for 6–9 days. CBD also prevented any further decline in ejection fraction 6–9 days after the surgery. Echocardiograms were obtained with the pacer turned off for 30–60 min. *$P < 0.05$ *vs.* pre-pace, #$P < 0.05$ *vs.* CHF, †$P < 0.05$ *vs.* day 3 post-CB surgery. CBD, denervation of the carotid body; CHF, congestive heart failure; LVd Vol, left ventricular diastolic volume; LVs Vol, left ventricular systolic volume.

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et al. 1998; Ryan *et al.* 2005; Yamada *et al.* 2011; Yoshihisa *et al.* 2012). The sympathetic activation resulting from oscillatory breathing may drive morbidity and mortality in patients with CHF in part by increasing the incidence of ventricular arrhythmias (Leung *et al.* 2006). Prevalence of ventricular arrhythmias has been shown to be significantly higher in patients with CHF with higher indices of sleep-disordered breathing (Yamada *et al.* 2013), and the frequency of ventricular arrhythmias in CHF patients is higher during periods of Cheyne–Stokes respiration with central sleep apnoea-than during spontaneous normal breathing (Leung *et al.* 2004). Finally, a reduction of apnoea incidence with either continuous positive airway pressure or adaptive servo-ventilation results in reductions in arrhythmia frequency and measures of sympathetic activation (Javaheri, 2000; Ryan *et al.* 2005; Yamada *et al.* 2011).

Our findings that decreased HRV and increased arrhythmia incidence in CHF were concomitantly reversed when the CB chemoreceptors were denervated expand on the previously observed correlation between enhanced chemoreflex sensitivity and arrhythmia incidence in a mouse model of CHF (Wang *et al.* 2012). Our data provide compelling evidence that improving cardiac autonomic balance can reduce the incidence of cardiac arrhythmias in CHF, and that CB chemoreceptors are a viable therapeutic target for attaining these goals. We demonstrate that CBD in CHF reduces direct and indirect indices of sympathetic outflow concurrent with reductions in AHI and arrhythmia incidence. The fact that these changes precede the improvements in cardiac function that we observed suggests that improvements in these parameters are attributable to CBD and not a secondary effect of improved cardiac function.

Carotid body denervation and cardiac function in congestive heart failure

Progressive decrements in EF and FS, as well as continued increases in ventricular volumes typically occur over the course of development of CHF by rapid pacing (Sun *et al.* 1999b). Our results show for the first time that CBD curbs the decreases in EF and FS and, perhaps more importantly, reverses the increases in systolic and diastolic ventricular volumes with chronic pacing. Studies have consistently shown that large increases in end systolic volume predict adverse cardiovascular outcomes in patients with left ventricular systolic dysfunction (White *et al.* 1987; Hamer *et al.* 1994; Yamaguchi *et al.* 1995), and that end systolic volume has better predictive value of long-term survival in patients with left ventricular dysfunction than EF or end diastolic volume (White *et al.* 1987). Furthermore, it should be clarified that the lower cardiac output observed in CHF–CBD relative to CHF–sham is probably a direct result of the reductions in cardiac volume that occurred after CBD, and is not indicative of a decrement in cardiac function.

Limitations

Recent studies of SRC have used phrenic and sympathetic neural signals to analyse the relationship between these two related drives in disease states. The chronic, conscious nature of our animal model and the need to limit interventions precludes the measurement of phrenic nerve activity and our ability to define more precisely neural coupling. In addition, we have used indirect measures of autonomic balance in the heart, and sympathetic vasomotor tone based on spectral analysis of heart rate and BP variability. As mentioned above, we are unable to simultaneously measure neural outflow to multiple organs/vascular beds in our conscious animal model. Nevertheless, the changes in heart rate and BP variability we observed are consistent with known autonomic disturbances in CHF, and demonstrate a marked effect of CBD to reverse and normalize these indices. Additional studies will be needed to verify more directly the effects of CBD on autonomic control of the heart and vasculature in CHF.

In our experiments, CBD resulted in a significant reduction in resting ventilation, which in turn would be expected to increase resting $PaCO_2$. The salutary effect of CBD on respiratory pattern in CHF may have been influenced by an increase in $PaCO_2$. However, we did not measure resting blood gases in these experiments, and therefore cannot state definitively the extent to which resting $PaCO_2$ was affected by CBD.

Translational perspective

Current trends in therapeutic treatment of hypertension have focused on modulation of the autonomic nervous system through manipulation of the baroreflex or efferent neural outflow to the kidneys (Sobotka *et al.* 2011). Recent evidence indicates that CBD lowers BP in hypertensive rats without adverse ventilatory effects (Abdala *et al.* 2012) and may provide an alternative therapeutic strategy for the treatment of hypertension (Paton *et al.* 2013). Surgical removal of the CB in patients has been extensively documented. Despite thousands of reported cases of CB resection in humans, there is little evidence of asymptomatic hypoxia or other adverse effects related to loss of the CB (Paton *et al.* 2013).

Chronic heart failure is a condition characterized by a 'dysautonomic' phenotype recognized to contribute to the progression and morbidity associated with the disease. Our findings of improved autonomic and cardiac function and reduced arrhythmia incidence after CBD combined with recent work from our lab showing that CBD improves survival in CHF (Del Rio *et al.* 2013) underscores the idea that the CB chemoreflex plays a central role in the

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autonomic imbalance that occurs in CHF, and that CBD is a potentially valuable therapeutic strategy. In support of this notion, a recent case report presented by Niewiński *et al.* (2013) showed that surgical removal of the CB from a patient with systolic heart failure significantly decreased sympathetic tone. In addition, as shown in the current study, CBD has the potential to improve the incidence of sleep-related breathing disorders, which are highly prevalent in patients with CHF with high chemosensitivity (Giannoni *et al.* 2008). Thus, our results showing the more extensive beneficial effects of ablation of the CB should be relevant and potentially transferrable to humans.

Conclusions

Our results indicate that selective denervation of CB improves autonomic, respiratory and cardiac function in a rapid ventricular pacing model of CHF. These findings suggest that CB-mediated disordered breathing plays an important role in the abnormalities of sympathetic outflow in CHF, which in turn has negative clinical implications for autonomic balance and cardiac function in this disease state.

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Additional information

Competing interests

This study was funded in part by a grant from Coridea Inc. NYC NY. The company is developing technology aimed at ablation of carotid body chemoreceptors for the treatment of autonomic dysfunction in cardiovascular and metabolic disease. N.M., R.D.R. and H.S. were consultants for Coridea during the period of funding.

Author contributions

All experiments were performed in the Department of Cellular and Integrative Physiology at the University of Nebraska Medical Center. N.J.M., R.D.R., X.X. and H.D.S. contributed to the conception and design of the experiments. N.J.M. contributed to collection, analysis and interpretation of data as well as drafting and revising the manuscript. R.D.R. contributed to analysis and interpretation of data, Matlab programming, and drafting and revising of the manuscript. E.P.S. contributed to analysis of data and Matlab programming. X.X. contributed to the collection and interpretation of preliminary data. H.D.S. supervised the study and contributed to the analysis and interpretation of data as well as revising the manuscript. All authors approved the manuscript.

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