

**Supplementary Information****CAROTID CHEMORECEPTOR ABLATION IMPROVES SURVIVAL IN HEART  
FAILURE: RESCUING AUTONOMIC CONTROL OF CARDIORESPIRATORY  
FUNCTION**

Rodrigo Del Rio Ph.D., Noah J. Marcus Ph.D. and Harold D. Schultz Ph.D.

## Expanded Methods

**Animals.** Seventy one (2 month old) male Sprague-Dawley rats, weighing between 430 and 560 g, were used in these experiments. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and meet NIH standards.

**Induction of heart failure.** Chronic heart failure (CHF) was produced by coronary artery ligation (CAL) as previously described (1,2). Briefly, rats were anesthetized (2% isoflurane/98%O<sub>2</sub>) and mechanically ventilated then a left thoracotomy was performed. The left anterior descending coronary artery was ligated near its branch point from the aorta with a 6-0 silk suture. Following these maneuvers the thorax was closed and the air within the thorax was evacuated. Sham-operated rats were prepared in the same manner but did not undergo CAL. All animals were allowed to resume spontaneous respiration and recover from anesthesia. Rats were then housed in a temperature and humidity control environment with ad libitum access to food and water. All experiments were performed at 16-17 weeks after CAL.

**Echocardiography.** Cardiac function and the degree of heart failure were determined by echocardiography (Vevo 770; Visualsonics, Inc.). Under isoflurane anesthesia, a two-dimensional, short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles. M-mode tracings were recorded through the anterior and posterior LV walls, and anterior and posterior wall thicknesses (end-diastolic and end-systolic) and LV internal dimensions were also measured. Rats with ejection fraction as determined by echocardiogram of less than 45%, were considered to be in CHF (1,2).

Echocardiography was performed before and after one week of CB denervation. In the studies in which early CB denervation was performed, echocardiographic measures were performed 2 weeks, 6 weeks and 16 weeks post CAL.

**Selective carotid body denervation** At 16 weeks (Protocol 1) or 2 weeks (Protocol 2) post-CAL surgery, the rats underwent carotid body denervation (CBD) or sham denervation surgery (3). Graphic timelines of the protocols are provided in Supplemental Figure 1. Rats were anesthetized (2% isoflurane/98%O<sub>2</sub>) and using sterile techniques, the carotid sinus regions were exposed and the CB region visually identified. The CBs were cryogenically destroyed using a fine tipped forceps cooled in liquid nitrogen. We found that this surgical approach allows the elimination of the CB chemosensory but not the carotid baroreceptor afferents (Supplemental Figure 2 and 3). No effects of CBD on water consumption or daily food intake was found between Sham and CHF rats that undergo CBD (Supplemental Figure 4). Sham-sham and CHF-sham groups were subjected to a similar surgical exposure, but the CBs were not destroyed.

**Radiotelemetric monitoring of arterial blood pressure and heart rate.** After 14 weeks of sham or CAL surgery, rats were anesthetized (2% isoflurane) and a blood pressure (BP) radio-telemetry probe (Physiotel TA11PA-C40, Data Science International, USA) was inserted into the left femoral artery and advanced to place the pressure-sensing catheter tip in the aorta. After 14 days of recovery, continuous changes in BP and heart rate were measured in the conscious freely moving state before and after selective CBD.

**Evaluation of heart rate and blood pressure variability.** Heart rate variability (HRV) and the low frequency component of the systolic blood pressure variability (SBPV) were used as indirect measures of autonomic balance as previously described (4). Briefly, BP recordings were obtained at 2 kHz over a 60 min period during the resting breathing test. Heart rate (HR) was derived from the beat to beat interval. HRV was analyzed using the HRV extension for LabChart 7 software (AD Instruments) over a 10-min recording characterized by the absence of movement-induced artifacts. Power spectral analysis in the frequency domain was performed to calculate the HRV. Fast Fourier transform (FFT) algorithm was applied to the inter-pulse signal using the followings cut-offs frequencies: low frequency (LF) at 0.04-0.6 Hz and high frequency (HF) at 0.6-2.4 Hz. Changes in the LF of SBPV (LFSBPV) reflect the level of sympathetic vasoconstrictor activity (5). Accordingly, the power of the LFSBPV was calculated following FFT using 0.2-0.6 Hz as cut-off frequency (6).

**Baroreflex sensitivity.** The spontaneous baroreflex function was assessed by means of spectral calculation. Briefly, the squared root of the ratio of HRV and SBP powers in the LF domain was calculated since the coupling between the R-R and SBP in the high frequencies are not of baroreflex origin (7). Beat-to-beat interval and SBP was obtained using radiotelemetry (Data Science International, USA) and data was calculated over a 10-min period at rest.

**Evaluation of respiratory variability and ventilatory chemoreflex function.** Tidal volume ( $V_t$ ), respiratory frequency (RR), and minute ventilation ( $V_E$ :  $V_t \times RR$ ) were determined by unrestrained whole body plethysmography as previously described (2). Tidal volume was measured by temporarily (15-30 s) sealing the air ports and

measuring the pressure changes in the sealed chamber using a Validyne (MP-45) differential pressure transducer and amplifier connected to a PowerLab System (AD Instruments). Chamber pressure fluctuations were proportional to tidal volume. Resting breathing was recorded for 2 h while the rats breathed room air. Peripheral chemoreceptors were stimulated preferentially by allowing the rats to breathe hypoxic (10% O<sub>2</sub>/balance N<sub>2</sub>) gas for 2-5 min under isocapnic conditions. Because hypoxic stimulation of ventilation induces hyperventilatory hypocapnia, 2–3% CO<sub>2</sub> was added to the hypoxic mixture to maintain relatively constant P<sub>a</sub>CO<sub>2</sub> during hyperventilation. Normoxic hypercapnia (7% CO<sub>2</sub>) gas challenges were given for 2-5 min. All recordings were made at an ambient temperature of 25 ± 2 °C. Respiratory stability was calculated during resting breathing recordings by Poincare plots and analysis of SD1 and SD2 of inter-breath intervals variability (2,8) over 500 consecutive breaths. Apnea episodes (cessation of breathing ≥ 3 breaths), hypopneas (reductions ≥50% in V<sub>t</sub>) and sigh frequency (single breath ≥50% increase in V<sub>t</sub>) were averaged during resting breathing. During chemoreflex testing, respiratory variables (RR and V<sub>t</sub>) were averaged for at least 20 consecutive breaths over a period of 4 min of inspired hypoxic and hypercapnic challenges.

**Western blotting.** Micropunches of the rostral ventrolateral medulla (RVLM) were obtained as previously described (9). Briefly, after euthanizing the rats, brains were removed and quickly frozen on dry ice. Coronal sections (100 µm) were cut through the medulla at the level of the RVLM using a cryostat. According to the technique of Palkovits and Brownstein (10), RVLM nuclei were bilaterally punched using a diethylpyrocarbonate (DEPC)-treated blunt 18-gauge needle attached to a syringe.

Tissue was lysed in RIPA buffer containing 1% complete protease inhibitor cocktail (Sigma, USA) and proteins concentration determined using a BCA protein assay kit (ThermoScientific, USA). Equal amount of protein was loaded into 10% polyacrylamide gel for electrophoretic separation. Gels containing the proteins were then transferred to PVDF membrane (Immobilon-P, Millipore, USA) at 4°C. The membranes were first incubated with blocking buffer (Odyssey Blocking Buffer, Li-Cor, USA) and then incubated overnight at 4°C with a rabbit polyclonal anti- Fra-1 antibody (1:100, Santa Cruz Biotechnology, USA) followed by 1h incubation with a goat anti-rabbit secondary antibody (1:10000, IRDye 800CW, Li-Cor, USA). Membranes were then developed using the Odyssey infrared fluorescence imaging systems (Li-Cor, USA). Following stripping of the membranes (NewBlot™, Li-Cor, USA), protein loading was evaluated by probing all membranes with a mouse anti- $\beta$ -actin antibody (1:2500, Sigma, USA). Analysis of the specific bands optical densities were performed using the Odyssey Infrared Fluorescence Imaging Software (Li-Cor, USA). The relative amount of protein of interest was calculated as the ratio of intensity of the band relative to the intensity of  $\beta$ -actin. No difference in the level of expression of  $\beta$ -actin was found between groups.

**Immunohistochemistry.** Localization of Fra-1 immunoreactivity in RVLM catecholaminergic pre-sympathetic neurons was assessed by immunofluorescence. Anesthetized rats were perfused intracardially with PBS (pH 7.4) for 20 min, followed by buffered paraformaldehyde (PFA 4%, Sigma) for 20 min. Brains were harvested from the rats and postfixed by immersion in buffered-PFA 4% for 24 h at 4°C followed by three 15 min washes in PBS, sucrose gradient (5%, 10%, 20% in PBS), and then embedded in OCT. Cryostat coronal sections (15  $\mu$ m) of the brain were obtained and

mounted on Superfrost Plus slides (Thermo Fisher Scientific). Sections were blocked/permeabilized in 0.5% Triton X-100, 2% fish skin gelatin (Sigma-Aldrich), 1%BSA in PBS for 1 h at RT (11). Sections were incubated overnight at 4°C with a mixture of a rabbit anti- Fra-1 polyclonal antibody (a marker for neuronal activation 1:100 in the same blocking media, Santa Cruz Biotechnology, USA) and a mouse anti-TH monoclonal antibody (a marker for catecholaminergic cells 1:250 in the same blocking media, Millipore). Then sections were washed with PBS and incubated for 1 h with a mixture of Alexa-Fluor 488 goat anti-mouse IgG (1:200, Molecular Probes) and Alexa-Fluor 546 goat anti-rabbit IgG (1:200, Molecular Probes). Finally, sections were mounted in DAPI-containing media (Vectashield, Vector Laboratory) and visualized using a confocal laser microscope (Leica).

**Mortality studies.** In a subset of animals we assessed the effects of early CBD (eCBD) on survival rate during CHF. For this, 2 weeks after CAL surgery, cardiac function was assessed by echocardiography. Heart failure rats (EF<45%) were randomly assigned to CHF+eCBD or CHF+sham surgery. No differences in the initial EFs were found between CHF+eCBD and CHF+sham groups. Survival rates were then followed through the remaining 14 weeks of the protocol. After 14 weeks post-CAL surgery, a radiotelemetry unit was implanted to assessed heart rate and calculation of arrhythmia incidence. At the end of the 16 week post-CAL, rats were euthanized for infarct size and cardiac remodeling measurements.

**Cardiac remodeling.** The effects of early CBD (second week after infarct) on cardiac remodeling was assessed after 16 weeks post-CAL surgery. Excised hearts from anaesthetized rats were fixed in 4% buffered-PFA, embedded in paraffin and sectioned

(5  $\mu$ m). Short-axis ventricular slices were stained with Masson's trichrome stain for the analysis of infarct size. Collagen content was determined by quantitative morphometry of Sirius red-stained sections. Sections were deparaffinized, treated with 0.1% Sirius red (Sigma) solution for 60 min, rinsed with acidified water twice, dehydrated and then permanently mounted. Multiple digital images were acquired from both the noninfarcted LV free wall and the interventricular septum (IVS). Images were analyzed offline using ImageJ software (NIH, <http://rsb.info.nih.gov/ij/>). Collagen content was determined from the following equation: collagen content (%) [Sirius red-positive stained area/total tissue area] x 100.

**Arrhythmia score.** Heart rate time series were derived from arterial blood pressure waveforms (12) obtained by radiotelemetry during two hours while the rats were resting. Irregular heartbeats were visually inspected. Arrhythmic episodes were defined as premature or delayed beats with changes greater than 3 standard deviations from the mean beat to beat interval duration. All events meeting the stated criteria were identified and recorded to determine an index of events per hour.

**Statistics.** Data were expressed as means  $\pm$  SEM. Differences among 3 or more groups were assessed with one or two-way ANOVA tests, followed by Newman-Keuls or Bonferroni posthoc comparisons. The Student's t-test was employed to compare the differences between two groups. The log rank test was used to compare survival rates after eCBD in CHF.  $P < 0.05$  was considered statistically significant.



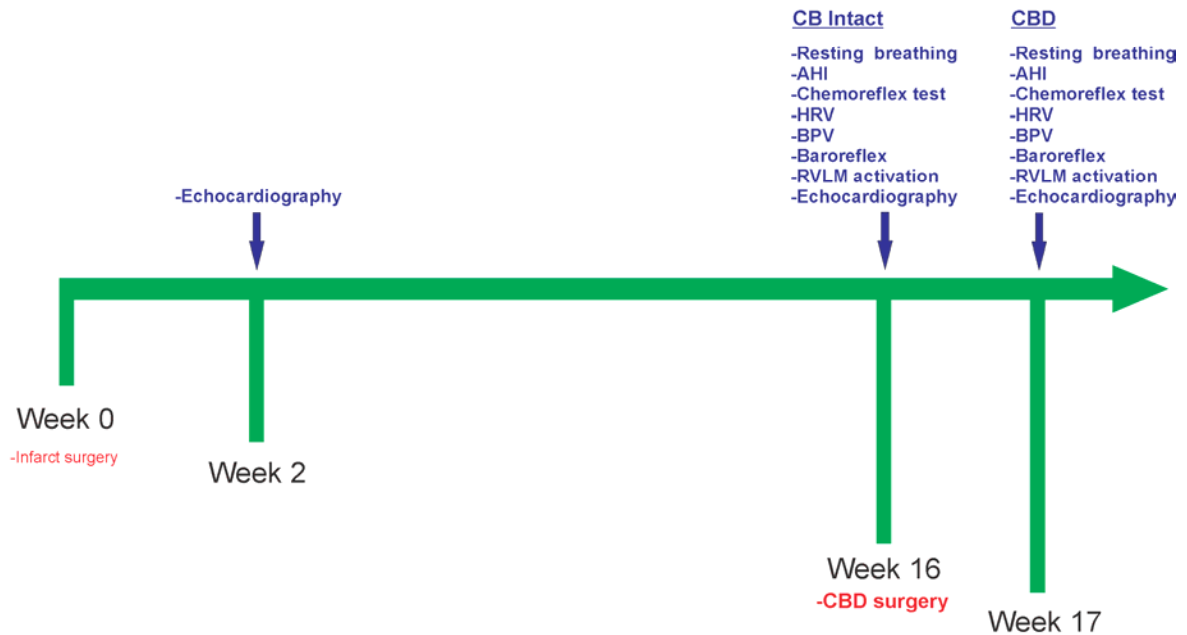
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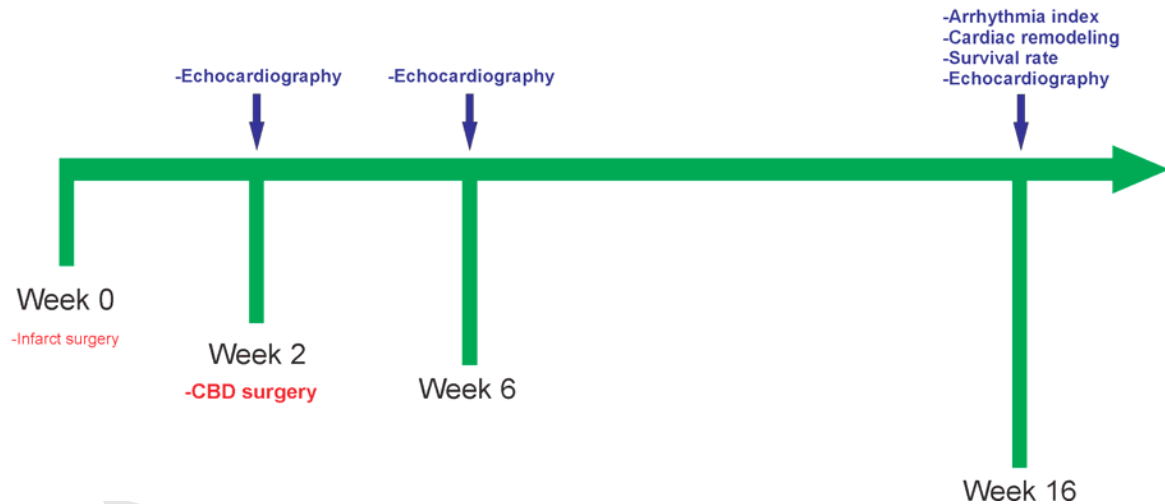
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## Supplemental Figures

## A

*Protocol 1: CBD*

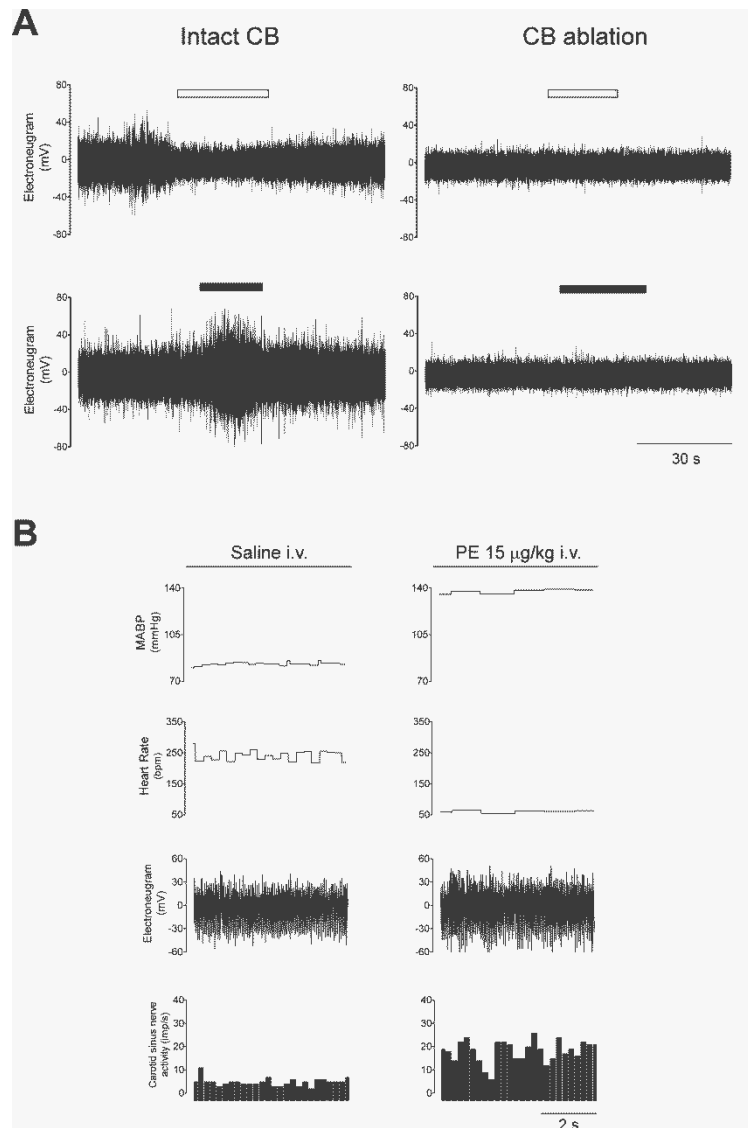
## B

*Protocol 2: eCBD*

**Supplemental Figure 1.** Timelines of the selective carotid body denervation (CBD) interventions in CHF. **(A)** Ablation of the CB was performed after 16 weeks of induction of heart failure (Protocol 1). **(B)** Early CBD was performed at the second week after the infarct was induced (Protocol 2). AHI, apnea and hypopnea index; BPV, blood pressure

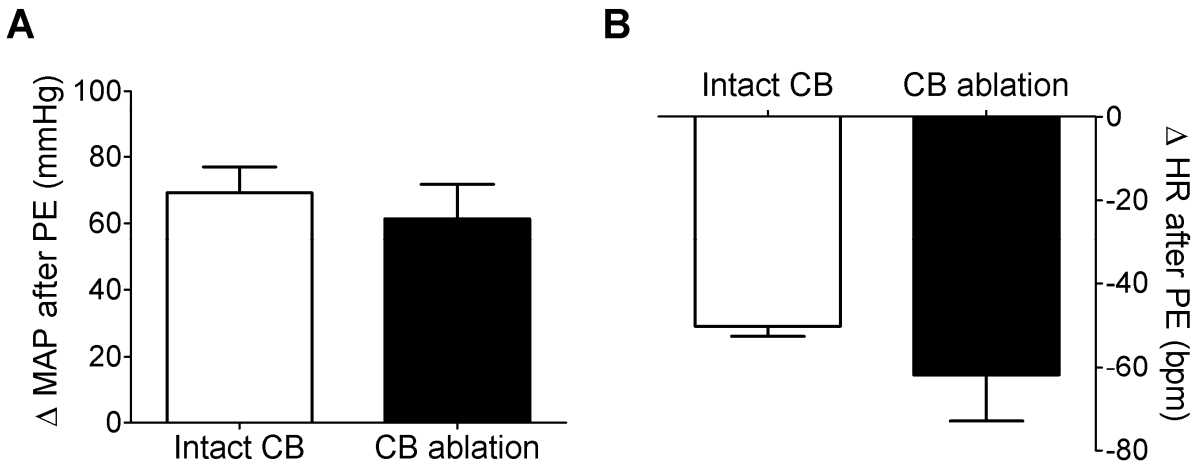
variability; CBD, carotid body denervation; HRV, heart rate variability; RVLM, rostral ventrolateral medulla.

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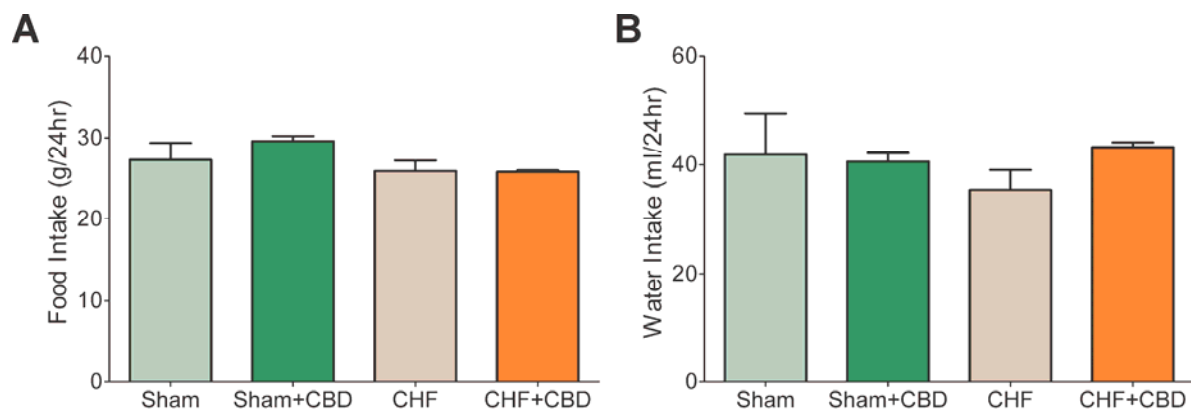


**Supplemental Figure 2.** Efficacy of selective ablation of the CB chemosensory afferents. **(A)** Afferent discharge from the carotid sinus nerve recorded before and after carotid body denervation (CBD). Open bars depict period of exposure to hyperoxia ( $F_{iO_2} \sim 100\%$ ) and the filled bars to hypoxia ( $F_{iO_2} \sim 5\%$ ). Note that CBD completely eliminated both hyperoxic (inhibition) and hypoxic (excitation) chemosensory responses. **(B)** Carotid sinus baroreceptor activity and baroreflex responses remained intact after

CBD. Left panel displays mean arterial blood pressure (MABP), heart rate (HR) and afferent carotid sinus nerve discharge during iv infusion of saline solution. Right panel illustrates the baroreflex bradycardia in response elevated arterial pressure due to iv injection of phenylephrine (PE 15 $\mu$ g/kg). Note that carotid sinus nerve activity increased during PE infusion.

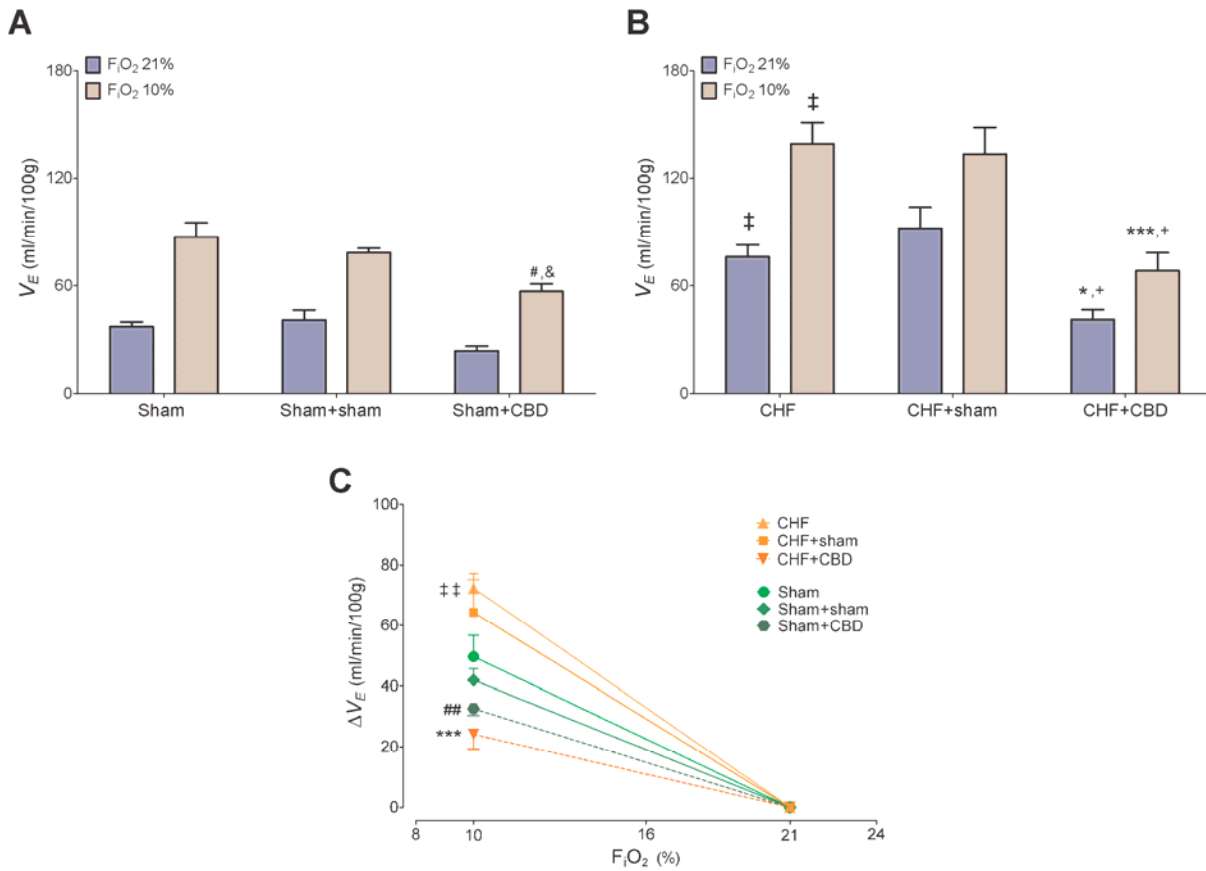


**Supplemental Figure 3.** Selective ablation of the CB maintained normal baroreflex function. **(A)** Changes in mean arterial pressure (MAP) after PE. Note that before and after denervation PE elicits a similar rise in MAP. **(B)** Baroreflex-mediated changes in heart rate (HR) following PE. Ablation of the CB did not modify the acute baroreflex response to increase blood pressure.



**Supplemental Figure 4.** Effects of CBD on food and water consumption in Sham and CHF rats. CBD did not affect average daily food intake (**A**) or water consumption (**B**) in either group. Sham / Sham+CBD, n=6; CHF / CHF+CBD, n=8.

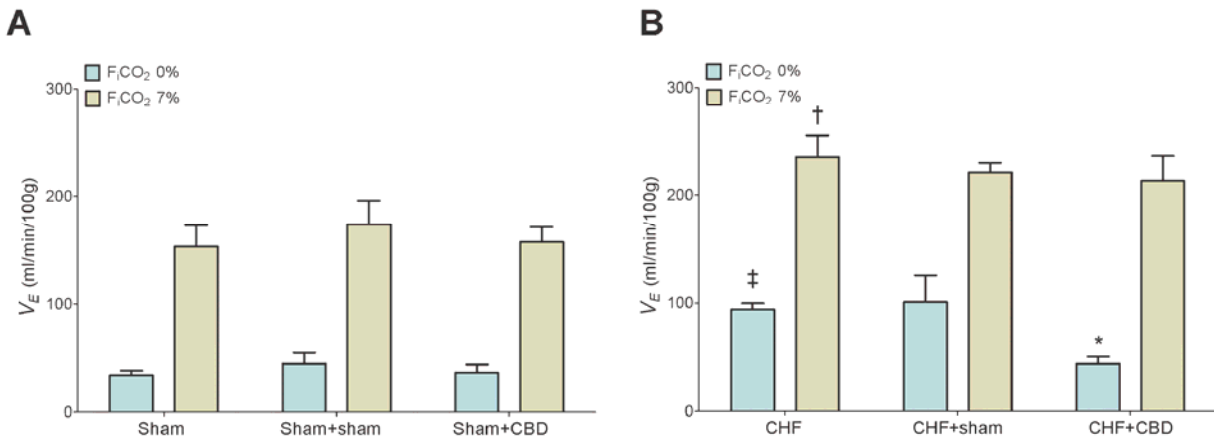




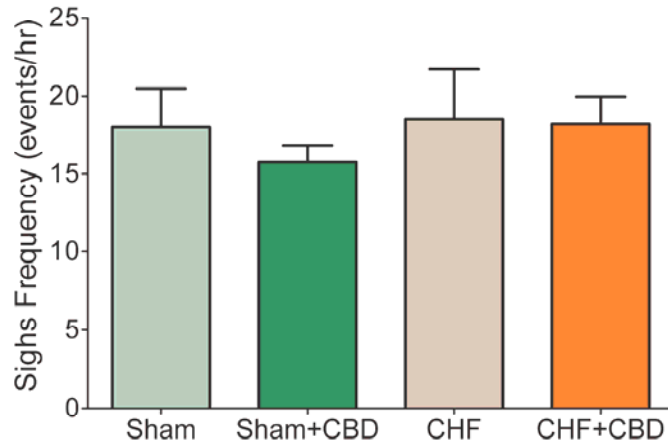
**Supplemental Figure 5.** Effects of sham (sham CBD) and CBD surgeries on the ventilatory chemoreflex response to hypoxia in Sham (Sham CHF) and CHF rats (Protocol 1). **(A)** Effects of sham and CBD surgeries on the ventilatory responses to hypoxia in Sham rats. CBD suppressed normoxic ( $F_{I}O_2 \sim 21\%$ ) and hypoxic ( $F_{I}O_2 \sim 10\%$ ) ventilation. **(B)** Effects of CBD on hypoxic ventilatory response in CHF rats. CHF increased both normoxic and hypoxic ventilation compared to Sham rats. CBD normalized normoxic and hypoxic ventilation in CHF rats. **(C)** CBD effects on the ventilatory response to acute hypoxia in CHF and sham rats expressed as the difference from the baseline values. † $P < .01$  Sham vs. CHF, †† $P < .001$  Sham vs. CHF; # $P < .05$  Sham vs. Sham+CBD, ## $P < .001$  Sham vs. Sham+CBD; & $P < .05$ , Sham+sham vs.

Sham+CBD; \* $P < .05$ , CHF vs. CHF+CBD, \*\*\* $P < .001$ , CHF vs. CHF+CBD; \* $P < .05$ , CHF+sham vs. CHF+CBD. Sham / Sham+CBD,  $n=6$ ; Sham+sham / CHF+sham,  $n=4$ ; CHF / CHF+CBD,  $n=8$ .

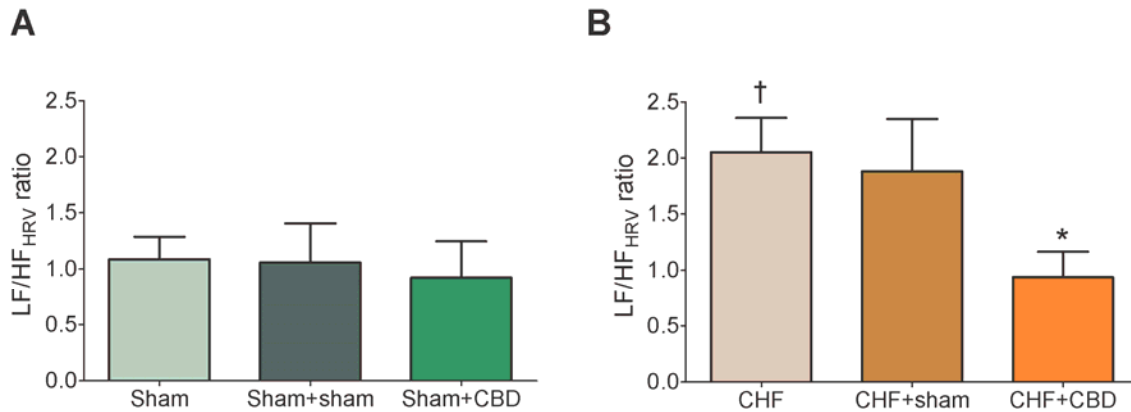
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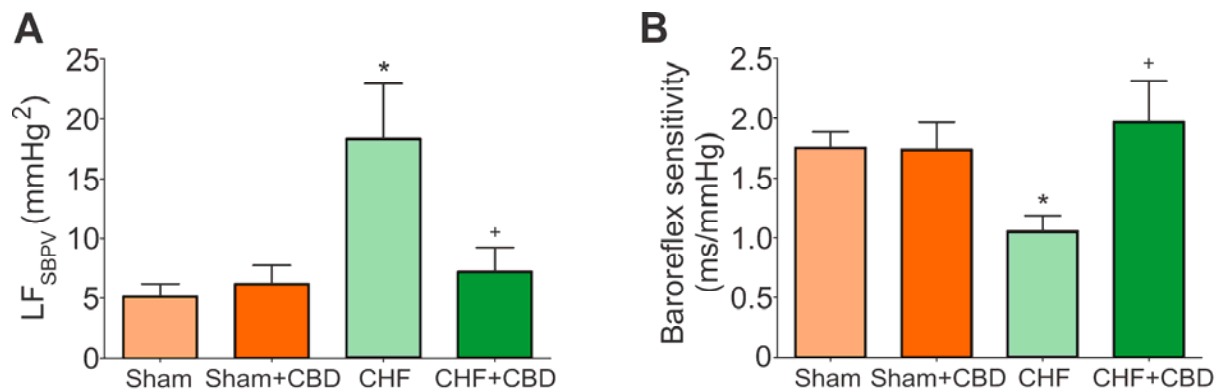
**Supplemental Figure 6.** Effects of CBD on the ventilatory response to hypercapnia (Protocol 1). **(A)** Effects of sham and CBD surgeries on the ventilatory responses to hypercapnia in Sham rats (notation as in Figure 2). CBD did not affect resting ( $F_iCO_2 \sim 0\%$ ) and hypercapnic ( $F_iCO_2 \sim 7\%$ ) ventilation in Sham rats. **(B)** Effects of CBD on the hypercapnic ventilatory response in CHF rats. CHF increased both resting ( $F_iCO_2 \sim 0\%$ ) and hypercapnic ( $F_iCO_2 \sim 7\%$ ) ventilation compared to Sham rats. CBD reduced resting ventilation in CHF rats; however, CBD did not affect the ventilatory response to acute hypercapnia in CHF rats. † $P < .05$  Sham vs. CHF, ‡ $P < .01$  Sham vs. CHF; \* $P < .05$ , CHF vs. CHF+CBD. Sham / Sham+CBD,  $n=6$ ; Sham+sham / CHF+sham,  $n=4$ ; CHF / CHF+CBD,  $n=8$ .



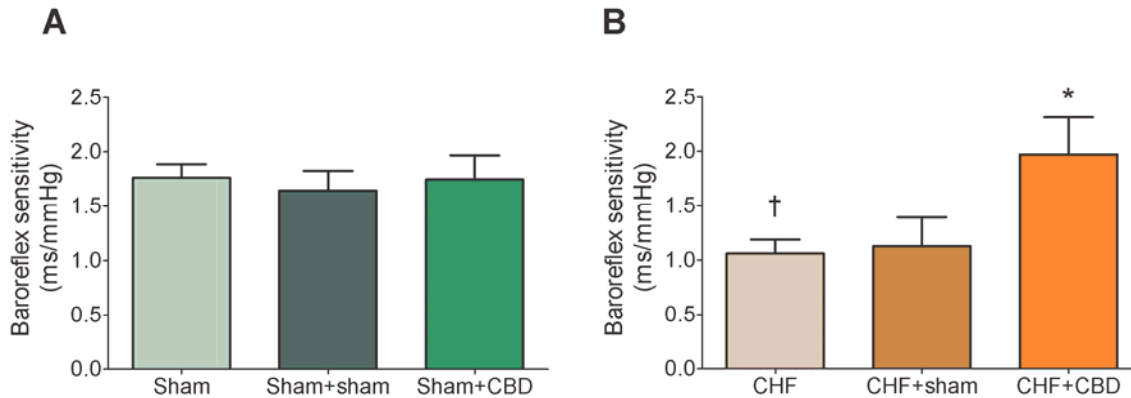
**Supplemental Figure 7.** Effects of CBD on the frequency of spontaneous sighs during resting breathing in Sham and CHF rats (Protocol 1). The frequency of spontaneous sighs remained unchanged among all groups. Sham / Sham+CBD, n=6; CHF / CHF+CBD, n=8.



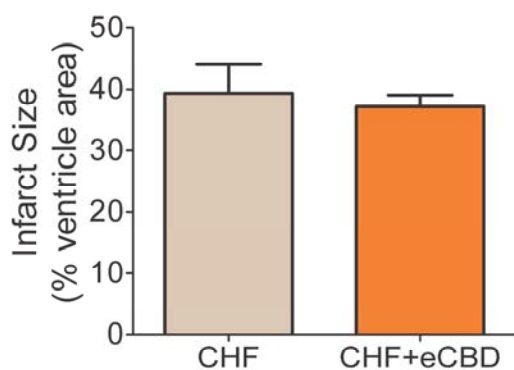
**Supplemental Figure 8.** Heart rate variability after sham or CBD surgeries in Sham and CHF rats (Protocol 1). **(A)** and **(B)** Effects of sham and CBD surgeries on the LF/HF ratio of the heart rate variability (HRV) in Sham (A) and CHF (B) rats, respectively. CHF increased LF/HF<sub>HRV</sub> compared to that in Sham rats. CBD restored the LF/HF<sub>HRV</sub> ratio in CHF rats to a level comparable to that of Sham rats. <sup>†</sup>P<.05 Sham vs. CHF. \*P<.05, CHF vs. CHF+CBD. Sham / Sham+CBD, n=6; Sham+sham / CHF+sham, n=4; CHF / CHF+CBD, n=8.



**Supplemental Figure 9.** Selective CBD and indices of sympatho-mediated vasomotor tone and baroreflex gain in CHF. **(A)** Changes in the low frequency (LF) component of the systolic blood pressure variability (SBPV) in CHF. CHF rats exhibited a significant increase in the LF-SBPV compared to sham rats. Ablation of the CB returned LF-SBPV to levels comparable to Sham rats. **(B)** Baroreflex gain was impaired in CHF rats, and selective CBD increased baroreflex gain toward normal levels. \* $p < 0.05$  vs. Sham, <sup>+</sup> $P < .05$  vs. CHF. Sham / Sham+CBD,  $n=6$ ; CHF / CHF+CBD,  $n=8$ .



**Supplemental Figure 10.** Effects of CBD on spontaneous baroreflex sensitivity in Sham and CHF rats (Protocol 1). **(A)** and **(B)** Effects of sham and CBD surgeries on spontaneous baroreflex sensitivity in Sham **(A)** and CHF **(B)** rats, respectively. CHF depressed spontaneous baroreflex sensitivity compared to that in Sham rats. CBD enhanced baroreflex sensitivity in CHF rats to a level comparable to that of Sham rats. † $P < .05$  Sham vs. CHF. \* $P < .05$ , CHF vs. CHF+CBD. Sham / Sham+CBD,  $n=6$ ; Sham+sham / CHF+sham,  $n=4$ ; CHF / CHF+CBD,  $n=8$ .



**Supplemental Figure 11.** Effects of early CBD (2 weeks after coronary ligation, eCBD) on infarct size in hearts from CHF rats (Protocol 2). CBD did not affect infarct size. CHF / CHF+eCBD, n=6.



## Supplemental Tables

**Supplemental Table 1.** Body weight, left ventricular volumes, ejection fraction and fractional shortening in Sham and CHF rats before and one week after CB denervation (Protocol 1).

	<i>Sham</i>		<i>CHF</i>	
	<i>Before CBD</i>	<i>After CBD</i>	<i>Before CBD</i>	<i>After CBD</i>
	n = 6	n = 6	n = 8	n = 8
BW (g)	476 ± 14	482 ± 11	474 ± 11	481 ± 10
LVs Vol (µl)	82.7 ± 15.7	88.7 ± 18.9	329.9 ± 54.8*	326.8 ± 64.3*
LVd Vol (µl)	275.8 ± 22.0	281.3 ± 17.3	565.6 ± 72.1*	547.6 ± 77.9*
EF (%)	70.8 ± 3.8	69.4 ± 5.3	44.1 ± 3.7*	42.6 ± 4.7*
FS (%)	41.7 ± 3.3	41.0 ± 4.9	23.4 ± 2.4*	22.2 ± 2.6*

Values are means ± S.E.M. BW, body weight; HW, heart weight; LVs Vol, left ventricular end-systolic volume; LVd Vol, left ventricular end-diastolic volume; EF, ejection fraction; FS, fractional shortening. \*P < .05 compared to Sham.

**Supplemental Table 2.** Baseline tidal volume, respiratory rate, blood pressure and heart rate in Sham and CHF rats before and one week after CB denervation (Protocol 1).

	<i>Sham</i>		<i>CHF</i>	
	<i>Before CBD</i> n = 6	<i>After CBD</i> n = 6	<i>Before CBD</i> n = 8	<i>After CBD</i> n = 8
$V_T$ (ml/min/100g)	0.49 ± 0.03	0.47 ± 0.01	0.80 ± 0.07*	0.69 ± 0.06**
RR (bpm)	75.6 ± 1.2	67.7 ± 2.8 <sup>#</sup>	98.8 ± 4.1*	72.4 ± 3.3 <sup>+++</sup>
MAP (mmHg)	78.8 ± 8.1	79.8 ± 7.9	79.7 ± 8.9	75.9 ± 7.2
HR (bpm)	313.6 ± 21.1	285.6 ± 7.0	310.0 ± 10.6	282.8 ± 12.8

Values are means ± S.E.M.  $V_T$ , tidal volume; RR, respiratory rate; MAP, mean arterial pressure; HR, heart rate. \*P<.05 compared to Sham, <sup>#</sup>P<.05 compared to Sham before CBD; \*\*P<.05 compared to CHF before CBD, <sup>+++</sup>P<.001 compared to CHF before CBD.

**Supplemental Table 3.** Body and heart weight and echocardiographic variables in early CBD (second week post-infarct) rats (Protocol 2).

	<i>CHF</i>	<i>CHF+eCBD</i>	<i>CHF</i>	<i>CHF-eCBD</i>	<i>CHF</i>	<i>CHF-eCBD</i>
<i>Post-infarct</i>	<i>Week 2</i>	<i>Week 2</i>	<i>Week 6</i>	<i>Week 6</i>	<i>Week 16</i>	<i>Week 16</i>
	n = 7	n = 11	n = 7	n = 11	n = 7	n = 11
BW (g)	393 ± 16	335 ± 21	420 ± 13	404 ± 15 <sup>†</sup>	478 ± 14 <sup>**,#</sup>	492 ± 20 <sup>†††,‡‡</sup>
HW (mg)	-----	-----	-----	-----	2,633 ± 290	2,420 ± 498
HW/BW (mg g <sup>-1</sup> )	-----	-----	-----	-----	5.29 ± 0.36	4.94 ± 1.09
LVDs (mm)	6.8 ± 0.5	7.4 ± 0.3	8.0 ± 0.5	8.1 ± 0.3	9.0 ± 0.3 <sup>**</sup>	8.9 ± 0.4 <sup>††</sup>
LVDd (mm)	8.8 ± 0.5	9.3 ± 0.4	10.1 ± 0.4	10.1 ± 0.3	10.8 ± 0.3 <sup>**</sup>	10.8 ± 0.4 <sup>†</sup>
LVs Vol (μl)	229.0 ± 53.9	295.7 ± 28.0	342.1 ± 56.8	357.8 ± 28.5	453.4 ± 34.3 <sup>**</sup>	450.8 ± 49.0 <sup>†</sup>
LVd Vol (μl)	410.4 ± 38.2	494.4 ± 40.9	566.9 ± 95.9	587.3 ± 39.8	675.5 ± 49.4 <sup>**</sup>	678.8 ± 59.5 <sup>†</sup>
EF (%)	44.6 ± 2.1	40.5 ± 2.2	40.1 ± 1.9	39.2 ± 2.1	32.7 ± 2.1 <sup>**,#</sup>	34.0 ± 2.5

Values are means ± S.E.M. BW, body weight; HW, heart weight; LVDs, left ventricular end-systolic diameter; LVDd, left ventricular end-diastolic diameter; LVs Vol, left ventricular end-systolic volume; LVd Vol, left ventricular end-diastolic volume; EF, ejection fraction; FS, fractional shortening. \*\*P<.01 compared to CHF week 2; #P<.05 compared to CHF week 6; †P<.05, ††P<.01, †††P<.001 compared to eCBD week 2; ‡‡P<.01 compared to eCBD week 6.

**Supplemental Table 4.** Baseline tidal volume, respiratory rate, blood pressure and heart rate in early CBD (Protocol 2).

	<b>CHF</b>	<b>CHF+eCBD</b>
	n = 4	n = 4
V <sub>T</sub> (ml/min/100g)	0.84 ± 0.08	0.60 ± 0.06*
RR (bpm)	96.4 ± 13.8	87.8 ± 9.2
MAP (mmHg)	72.5 ± 6.4	60.1 ± 5.1
HR (bpm)	298.2 ± 9.2	269.3 ± 3.0

Values are means ± S.E.M. VT, tidal volume; RR, respiratory rate; MAP, mean arterial pressure; HR, heart rate. \*P<.05 compared to CHF.