ONLINE SUPPLEMENTAL MATERIAL

CENTRAL ANG-(1-7) ENHANCES BAROREFLEX GAIN IN CONSCIOUS RABBITS WITH HEART FAILURE

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Methods

Surgical Instrumentation

Rabbits were instrumented as described previously.[1] In brief, through a left thoracotomy, a platinum pacing electrode was sutured to the left ventricle and a reference electrode was secured to the left atrium. During the same surgery, a radiotelemetry transducer (DataSciences, Inc. Minneapolis MN) was implanted into the right femoral artery to monitor pulsatile and mean arterial blood pressure (MAP) and HR using Data Sciences and ADInstruments (Colorado Springs, CO) Powerlab data acquisition systems.

Two weeks after the insertion of telemetry, a brain cannula was inserted into the lateral cerebral ventricle and fixed with dental cement to the surface of the skull. The brain cannula was attached by a short microrenathane catheter to an osmotic minipump (Alzet 2001, Cupertino, CA) filled with aCSF which was infused at a rate of 1 µl/ hr.

Induction of CHF

Following the control (prepace) experiments, CHF was induced in each rabbit by chronic ventricular pacing as previously described.[1] Left ventricular pacing (360-380 bpm) was performed for 2 to 3 weeks. Cardiac function was assessed weekly by echocardiography (Accuson Sequoia 512 C; Siemens, Malvern, PA). Heart failure was characterized by a reduction in ejection fraction (EF) to approximately 45%, a 2 mm dilation of the left ventricle in systole and diastole, and clinical signs of CHF such as ascites and pulmonary edema. The experimental protocols were repeated in each rabbit after they reached an EF below 45%. All experiments were begun after the pacemaker was turned off for approximately 30 min.

Heart Rate Variability

ECG was recorded directly from the cardiac pacing leads. A five minute baseline recording of ECG was used to determine heart rate variability (HRV). HRV was analyzed using the HRV module for LabChart software (ADInstruments, Inc.). The baseline recording was analyzed for the standard deviation of normal R-wave-R-wave intervals (SDNN) and the standard deviation of the differences between adjacent intervals (SD ΔRR). Ectopic beats were removed using cycle-length cutoffs of <150 and >350 ms for ectopics and <100 and >400 ms for artifacts.

Specificity of Ang-(1-7)

To document the specificity of the Ang-(1-7) effects on baroreflex sensitivity, in a subset of CHF rabbits (n=3, representative protocol shown in Figure S1) an ICV cannula was implanted attached to an osmotic minipump containing a mixture of Ang-(1-7) (2 nmol/ µl/ hr) and A779 (Bachem, 8 nmol/1 µl/ hr). This dose of A779 was chosen based on a previous study showing that a 4:1 molar ratio of A779 to Ang-(1-7) mixture was able to block the improvement in baroreflex function by ICV infusion of Ang-(1-7) in rats.[2] Hemodynamics, baseline MAP, HR, and RSNA, the response to smoke, and baroreflex function were assessed 3-4 days following this infusion.

At the end of all experimental protocols, 1 µl of concentrated Evan's Blue dye was injected through the ICV cannula, the animal was euthanized, and the brain was removed. The placement of the cannula was confirmed by staining of all four ventricles.

Arterial Baroreflex Analysis

Arterial baroreflex curves were constructed by taking points for HR or RSNA frequency every 5 sec from the lowest to highest MAP following infusions of SNP and PE, respectively. Individual logistic regression curves, as described by Kent et al.[3] were fit to the data points by using the following equation:

$$HR \text{ or } RSNA = A/\{1+\exp[B(MAP - C]\}+D \quad (1)$$

where A is HR or RSNA range, B is the slope coefficient, C is the pressure at the midpoint of the range (BP_{50}), D is the minimum HR or RSNA, and MAP is mean arterial pressure. The peak slope (or maximum gain) was determined by taking the first derivative of the baroreflex curve as described by

$$Slope = A \times B \times \exp[B(MAP - C)]\}/(\{1+\exp[B(MAP - C)]\}2) \quad (2)$$

The mean value of each parameter from the individual curves in each group of rabbits was used to derive composite baroreflex curves.

References

1. Liu JL, Irvine S, Reid IA, Patel KP, Zucker IH. Chronic exercise reduces sympathetic nerve activity in rabbits with pacing-induced heart failure: A role for angiotensin II. *Circulation*. 2000;102:1854-1862.

2. Oliveira DR, Santos RAS, Santos GFP, Khosla MC, Campagnole-Santos MJ. Changes in the baroreflex control of heart rate produced by central infusion of selective angiotensin antagonists in hypertensive rats. *Hypertension*. 1996;27:1284-1290.

3. Kent BB, Drane JW, Blumenstein BB, Manning JW. A mathematical model to assess changes in the baroreceptor reflex. *Cardiology*. 1972;57:295-310.

Table S1. Baseline Hemodynamics in Sham and CHF Rabbits before and after Ang-(1-7) Infusion for Rabbits Subject to RSNA analysis

Variable	Sham+aCSF	Sham+Ang-(1-7)	CHF+aCSF	CHF+Ang-(1-7)
n	4	4	4	4
Body Weight, kg	3.6±0.1	3.6±0.1	3.4±0.1	3.2±0.1
Baseline MAP, mmHg	75.9±2.6	68.9±2.9	73.6±5.4	64.4±2.2
Baseline HR, bpm	208.2±1.7	202.6±5.3	234.5±11.4 *	210.5±2.9 †
LVEDD, mm	16.3±0.3	16.6±0.5	16.6±1.0	13.6±4.0
LVSD, mm	7.1±1.4	9.4±1.4	13.6±1.0 *	11.1±3.3 *
LVd Vol, ml	7.5±0.4	7.8±0.6	6.2±0.9	8.0±1.3
LVs Vol, ml	2.2±0.1	2.6±0.4	6.7±1.9 *	5.9±0.6 *
FS %	34.8±2.3	35.1±2.0	18.1±1.0 *	17.8±1.3 *
Ejection Fraction, %	70.2±0.9	70.5±0.8	40.5±1.9 *	40.0±2.5 *

Values are mean±SEM. LV indicates left ventricle; MAP, mean arterial pressure; LVEDD, left ventricular end-diastolic diameter; LVSD, left ventricular systolic diameter; LVd Vol, left ventricular diastolic diameter; LVs Vol, left ventricular systolic diameter; FS, fractional shortening.*P<0.05 vs. Sham+aCSF, †P<0.05 vs. CHF+aCSF.

Table S2. Effect of Central Ang-(1-7) on Time-Domain Parameters of Heart Rate Variability

Variable	Sham+aCSF	Sham+Ang-(1-7)	CHF+aCSF	CHF+Ang-(1-7)
SD ΔRR, ms	5.7±0.9	5.0±1.4	3.5±0.4 *	7.1±2.8 †
SDNN, ms	11.6±1.7	8.1±1.7 *	7.9±1.3*	15.4±1.8 †
RMSSD, ms	5.7±0.9	4.9±1.5	3.5±0.4*	7.1±2.7†

Values are mean±SEM. SD ΔRR, standard deviation of differences between adjacent NN (normal sinus rhythm intervals); SDNN, standard deviation of normal NN. *P<0.05 vs. Sham+aCSF, †P<0.05 vs. CHF+aCSF.

Table S3. Effect of Autonomic Blockade on Baseline MAP, HR, and Logistic Parameters of Baroreflex Curves for Control of HR following Ang-(1-7) Infusion

Group	MAP, mmHG	HR, bpm	Range, bpm	Min HR, bpm	BP_{50} , mm Hg	Peak Slope, bpm/ mm Hg
Sham+aCSF						
Control (n=13)	74.3±1.9	198.5±4.6	284.2±11.6	62.1±7.3	101.5±1.9	5.6±0.5
Atropine (n=6)	76.3±3.5	239.3±3.4*	48.3±10.2*	249.6±7.4*	70.8±12.7	1.7±1.7*
Metoprolol (n=6)	67.2±3.9	187.9±10.1*	168.0±8.1*	62.6±10.5	94.5±11.8	3.9±0.2
Sham+Ang-(1-7)						
Control (n=11)	69.1±3.1	186.8±4.5	285.8±17.6	57.33±5.3	99.6±4.1	5.0±0.5
Atropine (n=6)	77.5±2.5	226.8±6.2*	51.0±13.6*	259.3±13.4*	73.2±7.8	1.9±1.0*
Metoprolol (n=6)	66.9±1.2	192.5±4.8	153.5±4.5*	71.3±7.3	98.2±5.1	3.0±0.2
CHF+aCSF						
Control (n=11)	73.4±3.0	248.0±5.5	129.9±15.1	182.4±12.8	87.5±4.5	2.6±0.3
Atropine (n=6)	83.2±4.0*	245.2±5.3	50.7±17.8*	241.6±10.1*	56.9±17.2	0.5±0.1*
Metoprolol (n=6)	58.7±2.2*	201.1±9.6*	67.7±2.5*	150.5±8.8*	80.5±5.1	1.8±0.4
CHF+Ang-						

(1-7)						
Control (n=9)	67.3±3.1	209.6±4.9	236.7±4.7	64.2±7.2	104.3±3.7	6.6±1.0
Atropine (n=4)	89.5±5.1*	229.5±190.4*	35.8±10.1*	234.4±7.2*	62.1±11.4*	1.2±0.7*
Metoprolol (n=4)	69.7±1.5	190.4±6.9*	45.1±12.8*	168.41±7.1*	87.0±6.9	2.4±0.8*

*P<0.05 vs. Control

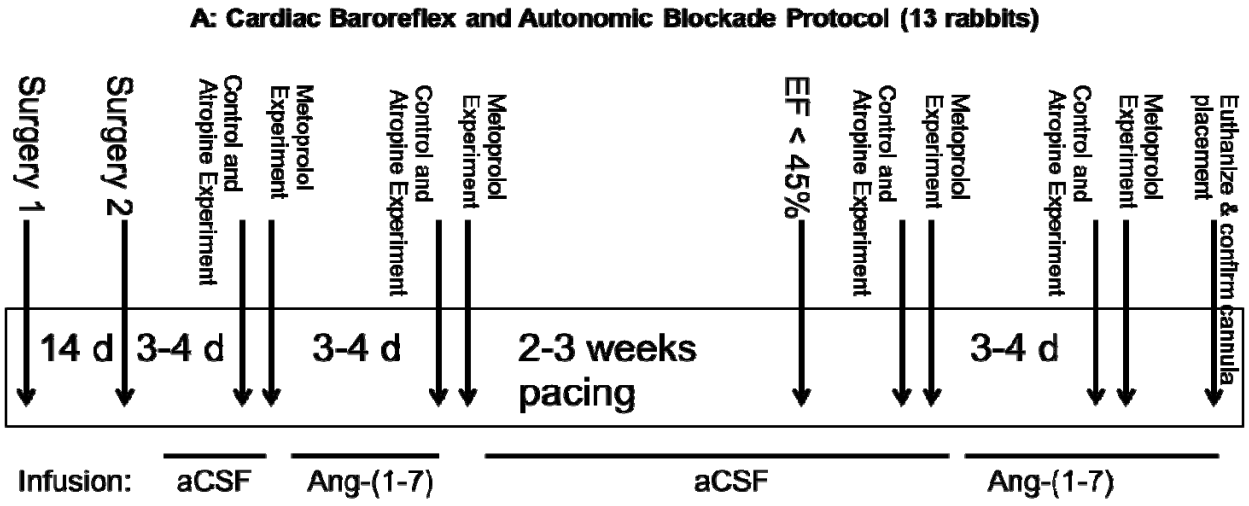
Table S4. Logistic Parameters of Baroreflex Curves for Control of RSNA following ICV Ang-(1-7) Infusion

Group	**Range, % max**	**Min RSNA, % max**	**BP_{50} , mm Hg**	**Peak Slope, % max**
Sham+aCSF (n=4)	99.8±0.2	1.4±1.0	59.9±5.3	5.8±1.0
Sham+Ang-(1-7) (n=4)	98.6±1.4	7.3±1.0	58.2±2.0	7.3±1.0
CHF+aCSF (n=4)	80.6±12.4	18.7±11.9	60.2±4.0	3.7±0.6 *
CHF+Ang-(1-7) (n=4)	99.4±0.6	1.3±0.8	48.4±1.1	6.8±1.0 †
CHF+Ang-(1-7)+A779 (n=3)	62.4±5.7	41.0±6.0	68.8±6.5	2.4±0.9 *

*P<0.05 vs. Sham+aCSF

†P<0.05 vs. CHF+aCSF

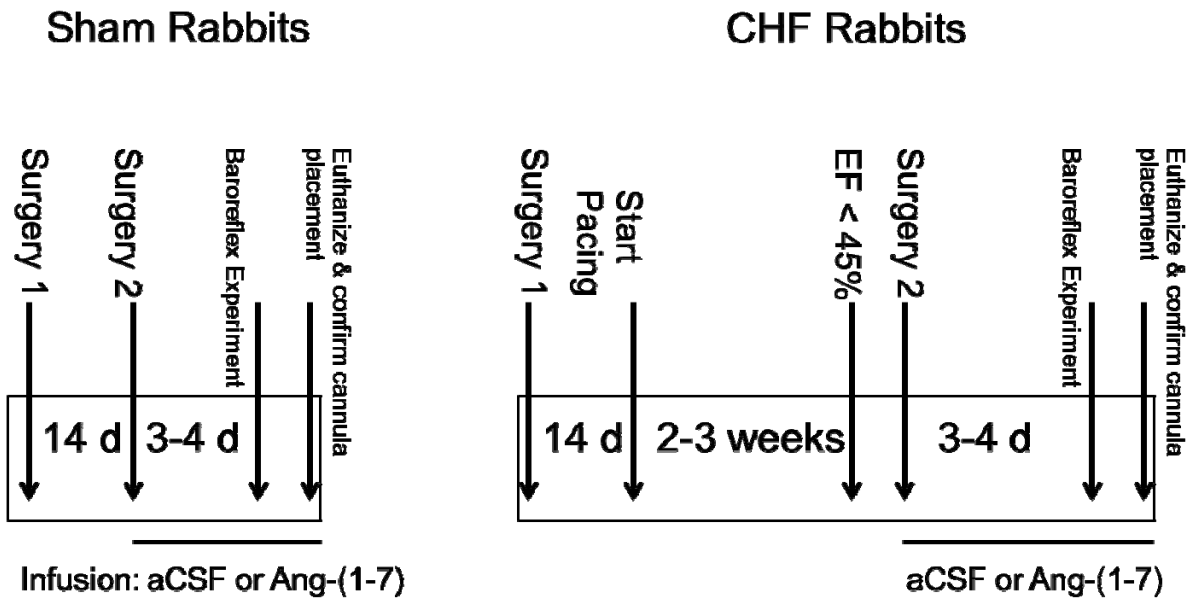
Figure S1



Surgery 1: Implantation of left ventricular pacing electrodes, radiotelemetry in the right femoral artery

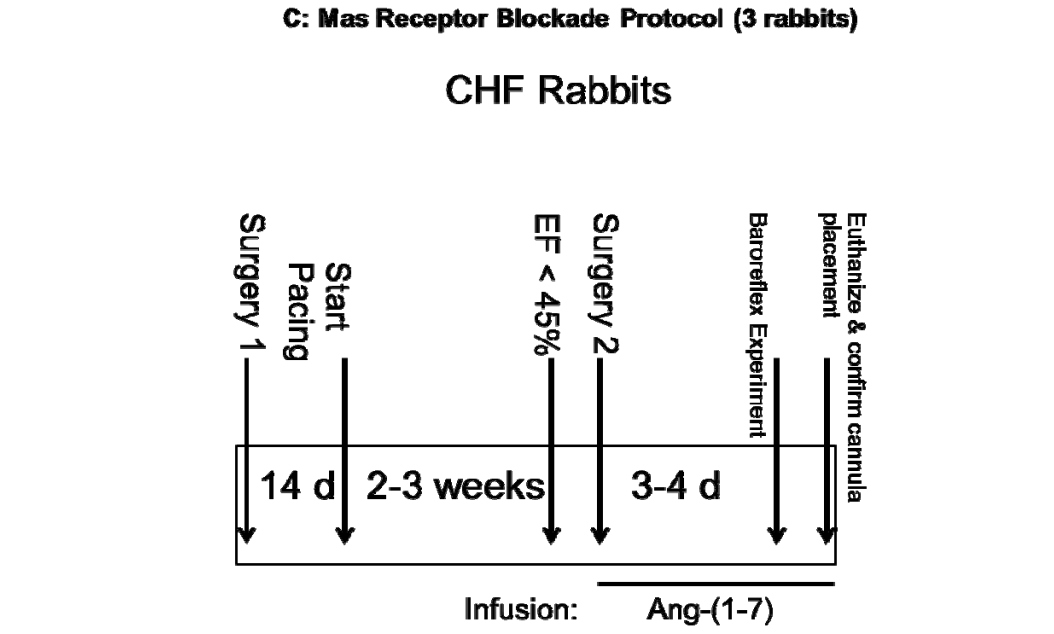
Surgery 2: Placement of ICV cannula attached to aCSF pump

B: RSNA Measurement Protocol (16 rabbits)



Surgery 1: Implantation of left ventricular pacing electrodes, radiotelemetry in the right femoral artery

Surgery 2: Placement of ICV cannula attached to aCSF or Ang-(1-7) osmotic minipump and renal nerve recording electrodes



Surgery 1: Implantation of left ventricular pacing electrodes, radiotelemetry in the right femoral artery

Surgery 2: Placement of ICV cannula attached to Ang-(1-7) osmotic minipump and renal nerve recording electrodes

Figure S1: Representative time lines for the animals used in this study. A: measurement of cardiac baroreflex function with and without autonomic blockade (13 animals). B: measurement of baseline RSNA and baroreflex control of RSNA (13 animals). C: analysis of baroreflex function after mas receptor blockade with A779 (3 animals).

Figure S2

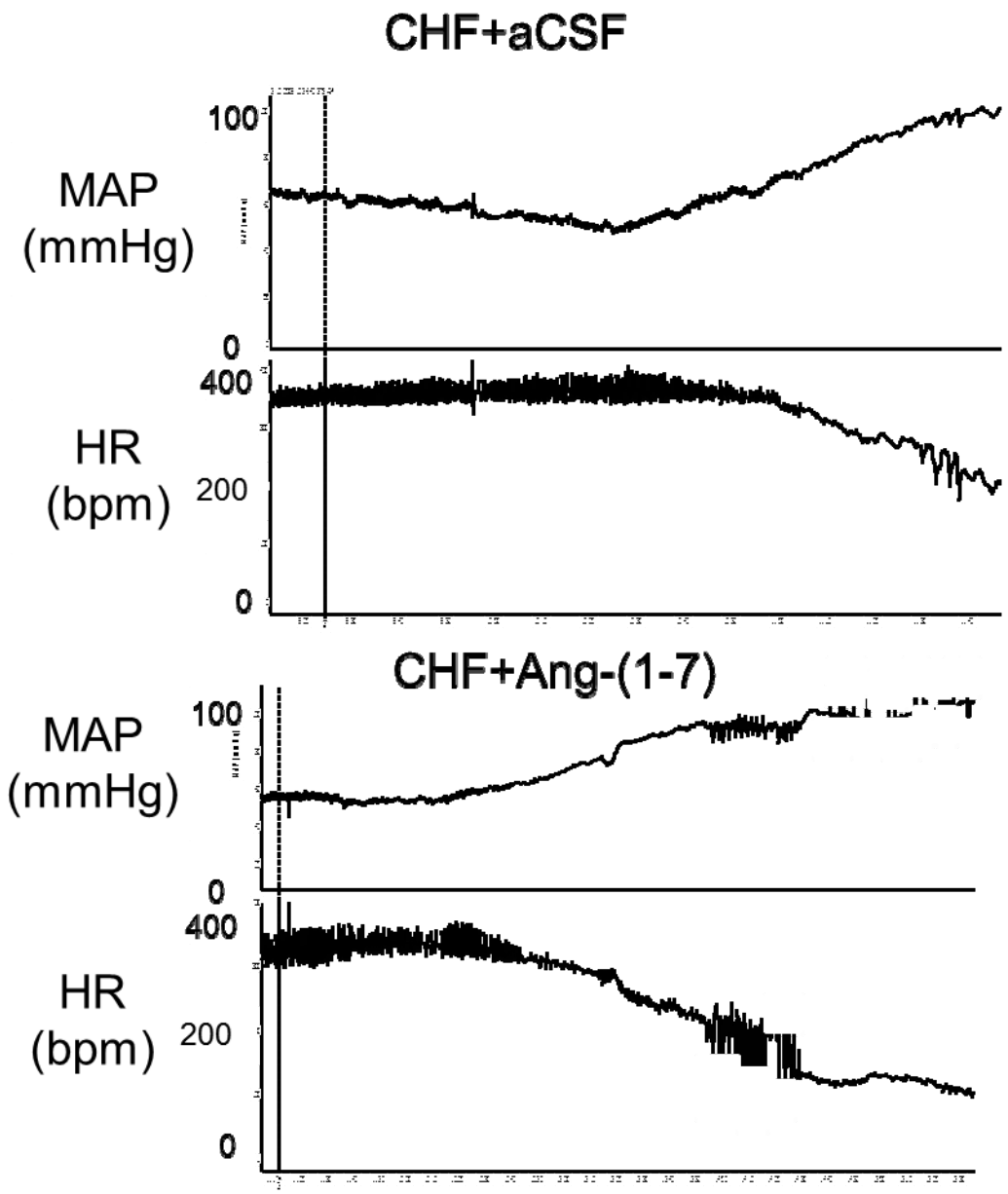


Figure S2. Original hemodynamic recordings following infusion of phenylephrine in a CHF rabbit with central aCSF or Ang-(1-7) infusion. Dashed line, intravenous injection of phenylephrine (80 μg/ kg). MAP, mean arterial pressure. HR, heart rate.