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Chronic Electrical Stimulation of the Carotid Sinus Baroreflex Improves LV Function and Promotes Reversal of Ventricular Remodeling in Dogs with Advanced Heart Failure

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Abstract

Background—Autonomic abnormalities exist in heart failure (HF) and contribute to disease progression. Activation of the Carotid sinus baroreflex (CSB) has been shown to reduce sympathetic outflow and augment parasympathetic vagal tone. This study tested the hypothesis that long-term electrical activation of carotid sinus baroreflex improves left ventricular (LV) function and attenuates progressive LV remodeling in dogs with advanced chronic HF.

Methods and Results—Studies were performed in 14 dogs with coronary microembolization-induced HF (LV ejection fraction, EF ~25%). Eight dogs were chronically instrumented for bilateral CSB activation using the *Rheos*® *System* (CVRx® Inc., Minneapolis, MN) and 6 were not and served as controls. All dogs were followed for 3 months and none received other background therapy. During follow-up, treatment with CSB increased LV EF 4.0 ± 2.4 % compared to a reduction in control dogs of −2.8 ± 1.0% ($p<0.05$). Similarly, treatment with CSB decreased LV end-systolic volume −2.5 ± 2.7 ml compared to an increase in control dogs of 6.7 ± 2.9 ml ($p<0.05$). Compared to control, CSB activation significantly decreased LV end-diastolic pressure and circulating plasma norepinephrine, normalized expression of cardiac β_1-adrenergic receptors, β-adrenergic receptor kinase and nitric oxide synthase and reduced interstitial fibrosis and cardiomyocyte hypertrophy.

Conclusions—In dogs with advanced HF, CSB activation improves global LV function and partially reverses LV remodeling both globally and at cellular and molecular levels.

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Disclosures

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Keywords

heart failure; ventricular remodeling; gene expression; baroreflex function

Autonomic dysfunction occurs in heart failure (HF) and is characterized by enhanced sympathetic activity, peripheral adrenoceptor downregulation and reduced efferent parasympathetic heart rate control. The sustained increase in sympathetic outflow in HF along with activation of the renin-angiotensin-aldosterone system and the ensuing vasoconstriction precipitates a positive feedback mechanism that leads to worsening of HF with progressive deterioration of left ventricular (LV) function, progressive LV remodeling, end-organ damage and death (1–5). The mechanism responsible for sustained sympathetic excitation in HF is not fully understood. It is generally believed, however, that the arterial reflexes, including the carotid sinus baroreflex (CSB), that are normally inhibitory to this system, have reduced sensitivity in HF and, therefore, allow sympathetic outflow to proceed unchecked (6–10). Several studies have shown an abnormally depressed arterial baroreflex control in HF (7,9,11–13). There is some evidence to suggest that digitalis-mediated augmentation of baroreflex function in HF can lead to decreased sympathetic outflow (14–16); a physiological change considered important in modifying the natural history of this syndrome.

Studies in conscious resting normal dogs by Vatner et al. showed that activation of the carotid sinus reflex through electrical stimulation can decrease heart rate and can also decrease sympathetic constrictor tone during exercise (17). Studies in patients by Eckberg and colleagues showed that electrical stimulation of the carotid baroreflex can prolong the R-R interval secondary to augmented parasympathetic activity (18). These modulation of heart rate and sympathetic tone are at present recognized as important therapeutic targets in HF. Chronic carotid sinus nerve stimulation has also been shown to be effective in the reversal of systemic arterial hypertension (19) and the relief of angina pectoris (20). In the present study, we tested the hypothesis that chronic activation of the CSB through electrical stimulation of the carotid sinus nerve, will lead to improved LV systolic function, attenuation of progressive LV remodelling, decreased sympathetic outflow and normalization of components of the cardiac β-adrenergic receptor and nitric oxide signal transduction pathways.

Methods

The canine model of coronary microembolization-induced chronic HF used in this study was previously described in detail (21,22). In the present study, 14 healthy mongrel dogs, weighing between 20 to 30 kg, underwent serial coronary microembolizations to produce HF. Embolizations were performed 1 to 2 weeks apart and were discontinued when LV EF, determined angiographically, was approximately 25%. All the procedures were performed during cardiac catheterization under general anesthesia and sterile conditions. Induction of anesthesia was initiated with intravenous administration of hydromorphone (0.22 mg/kg) and diazepam (0.17 mg/kg) and a plane of anesthesia was maintained with 1–2% isofluorane. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health "Guide and Care for Use of Laboratory Animals".

Carotid Sinus Stimulation and Study Protocol

Eight of 14 dogs were selected at random and chronically instrumented for CSB activation using the *Rheos System* (CVRx, Inc., Minneapolis, MN) and 6 were not and served as controls. The implant procedure and stimulation of the CSB was carried out as previously

described (10,23). Briefly, stimulating electrodes were implanted circumferentially around both carotid sinuses and connected to the implantable pulse generator. Efficacy of the stimulation algorithm and proper placement of the electrodes were confirmed at the time of surgery by 3 to 4 acute stimulation runs performed 3–5 minutes apart and each confirming an acute drop of blood pressure and a reduction of heart rate (HR). A period of at least 2 weeks was allowed to ensure that the electrodes had healed into place. Dogs assigned to the CSB treatment group received a predetermined voltage with 0.5–1.0 msec square wave pulses at 50–100Hz at a duty cycle of 9 minutes ON and one minute OFF. This was maintained unchanged for the 3 month duration of the therapy. Hemodynamic, ventriculographic, echocardiographic, Doppler, electrocardiographic and plasma norepinephrine measurements were made before initiating therapy (pre-treatment) and after completing 3 months of therapy or follow-up (post-treatment). All hemodynamic and ventriculographic measurements were made during cardiac catheterization. After completing the last catheterization, and while under general anesthesia, the dog's chest was opened and the heart rapidly removed and LV tissue prepared for histologic and biochemical evaluation.

Hemodynamic, Ventriculographic and Electrocardiographic Measurements

In all instances, CSB therapy was turned-off for the duration of the cardiac catheterization for hemodynamic evaluation. Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments, Houston, TX). Left ventriculograms were obtained with the dog placed on its right side and recorded on 35 mm cine film at 30 frame/sec during the injection of 20 ml of contrast material (RENO-M-60 Squibb, Princeton, NJ). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the LV. LV end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated from LV silhouettes using the area-length method (24) and LV EF was calculated as previously described (21). Stroke volume was calculated as the difference between EDV and ESV. LV end-diastolic and end-systolic sphericity indexes, measures of LV shape change, were calculated from LV angiographic silhouettes as the ratio of the major-to-minor axis at end-diastole (EDSI) and end-systole (EDSSI) as previously described (25). Cardiac output was calculated as the product of stroke volume and heart rate. Extrasystolic and post-extrasystolic beats were excluded from any of the angiographic analysis. Ventriculograms were evaluated unblinded because of device visualization. To minimize bias, random ventriculograms were selected for review by a second reader for concordance. All dogs underwent a pre-treatment and a post-treatment 24 hour ambulatory ECG Holter monitoring study. Full Holter disclosures were used to measure maximum, minimum and average heart rate and exclude any pro-arrhythmic potential of CSB therapy. Levels of norepinephrine in plasma extracted from peripheral venous blood samples was measured by competitive ELISA (26).

Echocardiographic and Doppler Flow Measurements

Echocardiographic and Doppler studies were performed using a 77030A ultrasound system (Hewlett-Packard) with a 3.5 MHZ transducer. All echocardiographic measurements were made with the dog placed in the right lateral decubitus position and recorded on a Panasonic 6300 VHS recorder for subsequent off-line analysis. LV end-diastolic circumferential wall stress (EDWS) was calculated as previously described (27). Trans-mitral inflow velocity was measured using pulsed-wave Doppler echocardiography. The velocity waveforms were used to calculate the ratio between peak mitral flow velocity in early diastole (E wave) and peak mitral inflow velocity during left atrial contraction (A wave), early mitral inflow deceleration time (DT) and presence and severity of functional mitral regurgitation (MR) as previously described (27). All echocardiograms were evaluated blinded to the intervention by a sonographer not involved in the actual echocardiographic recordings.

Histomorphometric Measurements

From each heart, 3 transverse slices (approximately 3 mm thick) one each from basal, middle and apical thirds of the LV, were obtained. From each slice, transmural tissue blocks were obtained and embedded in paraffin blocks. Transmural tissue blocks were also obtained from the free wall segment of the slice, mounted on cork using Tissue-Tek embedding medium, and rapidly frozen in isopentane pre-cooled in liquid nitrogen and stored at −70°C until used. For each histomorphometric measure, the multiple slices obtained from each animal were averaged and that single average was used to represent each animal in the analysis. The volume fraction of replacement fibrosis (VFRF), volume fraction of interstitial fibrosis (VFIF), myocyte cross-sectional area (MCSA), a measure of cardiomyocyte hypertrophy, capillary density (CD), and oxygen diffusion distance (ODD) were measured as previously described (28,29). LV tissue from 6 normal dogs was processed in an identical manner as above and the results used for comparisons.

mRNA Expression

mRNA expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cardiac β_1-adrenergic receptor, and β_2-adrenergic receptor, β-adrenergic receptor kinase, adenylyl cyclase, angiotensinogen, endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) was measured in LV tissue from all study dogs and from LV tissue of 6 normal dogs for comparison. Total RNA with an absorbance ratio (260 nm/280 nm) above 1.7 was isolated from frozen LV tissue, and approximately 4 μg to 10 μg RNA was reverse-transcribed into cDNA in an assay volume of 80 μl as described previously (30,31). Fluorescent band intensity was quantified using a Bio-Rad GS-670 imaging densitometer and expressed in densitometric units.

Statistical Analysis

Within group comparisons of hemodynamic, ventriculographic, echocardiographic, Doppler and electrocardiographic variables were made between measurements obtained at pre-treatment and measurements made at post-treatment after completion of 3 months of therapy using Wilcoxon signed rank tests with a significance set at $p<0.05$. To ensure that all study measures were similar at pre-treatment, between or inter-group comparisons were made using a Wilcoxon rank sum test with alpha set at 0.05. To assess treatment effect, the change (Δ) in each measure from pre-treatment to post-treatment was calculated for each of the two study arms. To determine whether significant differences in Δ were present between the control group and the CSB treatment groups, Wilcoxon rank sum tests were performed with alpha set at 0.05. Histomorphometric and mRNA expression results for normal, control and CSB treated groups were compared using Kruskal-Wallis tests with alpha set at 0.05. If significance was attained by overall ANOVA, pairwise comparisons were performed using the Wilcoxon rank sum tests. This corresponds to the Fisher's protected least significant difference (LSD) multiple comparisons approach. For all pairwise comparisons, a probability value ≤0.05 was considered significant. All data are reported as the mean ± STD.

Results

Within Group Changes of Hemodynamic, Ventriculographic and Plasma Norepinephrine Measures

Hemodynamic, ventriculographic and plasma norepinephrine results obtained at pre-treatment and post-treatment in CSB-treated dogs and controls are shown in Table 1. There were no significant differences between the 2 study groups in any of the measurement obtained at pre-treatment. Comparisons of hemodynamic, angiographic, echocardiographic, Doppler and neurohormonal measures between pre-treatment and post-treatment in control

dogs and dogs treated with CSB are shown in Table 1. In untreated control dogs LV EDV and ESV measured from ventriculograms increased and EF, also measured from ventriculograms, decreased significantly at post-treatment compared to pre-treatment (Table 1). In contrast, in dogs treated with CSB LV EDV was unchanged, ESV decreased and EF increased significantly at post-treatment compared to pre-treatment (Table 1).

Comparisons of Treatment Effect

Between-group comparisons of the change (Δ) between pre-treatment and post-treatment measurements are shown in Table 2. There were no significant differences in HR (obtained under anesthesia) and mean aortic pressure among the 2 study groups. Compared with controls, CSB activation decreased LV end-diastolic pressure, and increased both stroke volume and cardiac output. CSB activation therapy also significantly decreased EDV and ESV measured from ventriculograms and increased LV EF, also measured from ventriculograms, compared to control. It also significantly increased both EDSI and ESSI indicating some physiologic restoration of LV shape. Along with improvement in LV systolic function and global LV remodeling, active CSB therapy also improved LV diastolic function as evidenced by a significant reduction of EDWS, a significant increase of PE/PA and an increase of DT but the latter did not reach statistical significance. Compared to control, CSB therapy also significantly decreased functional MR and circulating plasma norepinephrine levels (Table 2).

Ambulatory ECG Holter Monitoring

Twenty four hour ambulatory ECG Holter monitoring obtained at pre-treatment and post-treatment showed no de-novo ventricular arrhythmias in dogs treated with CSB activation therapy. In control dogs, maximum HR increased from 119 ± 40 to 158 ± 32 beats/min, average HR increased from 69 ± 22 to 76 ± 15 beats/min and minimum HR increased from 39 ± 11 to 45 ± 11 beats/min. None of these increases reached statistical significance. In CSB-treated dogs, maximum HR decreased from 161 ± 40 to 122 ± 47 beats/min, average HR decreased from 92 ± 19 to 64 ± 18 beats/min and minimum HR decreased from 54 ± 18 to 38 ± 11 beats/min. None of these reductions of HR reached statistical significance. Treatment effect comparisons between groups showed a marked reduction of maximum, average and minimum HR following CSB therapy compared to control but again this reduction did not reach statistical significance.

Histomorphometric Findings

Histomorphometric findings are shown in Table 3. Compared to normal dogs, control HF dogs showed a significant increase in VFRF, VFIF, ODD and MCSA and a significant decrease in CD (Table 3). Compared to control, treatment with CSB activation resulted in a significant reduction of VFRF, VFIF, ODD and MCSA and a significant increase in CD (Table 3). Results from mRNA expression analyses are shown in Table 4. As expected, expression of GAPDH was the same in controls and CSB-treated dogs. Compared to normal dogs, mRNA expression of cardiac β_1-adrenergic receptor, adenylyl cyclase and eNOS decreased significantly in untreated HF control dogs while expression of cardiac β-adrenergic receptor kinase, angiotesinogen and iNOS increased significantly. There was no difference in the expression of cardiac β_2-adrenergic receptor between normal dogs and HF controls. Treatment with CSB activation partially normalized expression of all the dysregulated genes (Table 4).

Discussion

The sustained increase in sympathetic outflow in HF along with the ensuing vasoconstriction has long been thought to precipitate a positive feedback mechanism that

leads to worsening of HF evidence by progressive deterioration of left ventricular (LV) function, progressive LV remodeling, end-organ damage and ultimately death (1–5,32). Therapies that target a reduction of sympathetic overdrive and enhance parasympathetic drive, are desirable in the management of chronic HF. Results of this study indicate that long-term therapy with CSB improves global LV systolic and diastolic function in dogs with advanced HF. The improvement of LV function was accompanied by improvement in global LV remodeling evidence by reduced LV size and partial restoration of LV shape to one that is more ellipsoidal rather than spherical.

In the present study, chronic carotid sinus electrical stimulation or CSB therapy in dogs with chronic HF resulted in partial normalization of components of the cardiac beta-adrenergic signal transduction specifically, up-regulation of β1-adrenergic receptors and adenylyl cyclase and down-regulation of β-adrenergic receptor kinase. This was accompanied by a significant reduction of circulating plasma norepinephrine. Chronic CSB therapy also resulted in down regulation of angiotensinogen and, hence, a possible partial de-activation of the vasoconstrictive influence of the tissue renin-angiotensin-adosterone system. Interestingly, CSB therapy also normalized nitric oxide signaling evidenced by normalization of both eNOS and iNOS. Normalization of the nitric oxide pathway can have important benefits on the progression of HF through vascular effects related to afterload reduction and reduced myocardial oxygen consumption. In the present study, normalization of signal transduction pathways was associated with improved systolic and diastolic global LV function and partial reversal of LV global and cellular remodeling. The latter also evidenced by a reduction of VFRF, VFIF, ODD and MCSA and an increase of capillary density. CSB therapy also tended to decrease HR in conscious HF dogs, providing some supportive evidence for CSB as modulator of parasympathetic activity (33).

The present study cannot provide a definitive mechanism for the observed improvement of LV systolic and diastolic function from chronic CSB therapy. A paramount observation of this study was a reduction in sympathetic activity and normalization of the cardiac β-adrenergic receptor signal transduction pathway. Cardiac sympathetic dysfunction is a prime therapeutic target in HF and successful use of β-adrenergic blockers in HF is a testament to that (10,22). Another possible mechanism is attenuation of the enhanced activity of the peripheral renin-angiotensin-aldosterone-system. In a study in dogs with pacing-induced HF, Zucker and colleagues suggested that the improvement in survival seen in their study may also be due, in part, to enhanced endothelial function mediated by chronic CSB therapy (10). They postulated that this benefit may have resulted from reduced levels of circulating plasma norepinephrine and angiotensin-II seen also in their study. In the present study, we observed a normalization in the expression of cardiac endothelial and inducible nitric oxide synthases, enzymes that catalyze the production of nitric oxide from L-arginine. Nitric oxide plays a vital role in several biologic processes that include modulation of the immune system and vasodilation of blood vessels. Finally, another possible contributor to the improvement of LV function with chronic CSB therapy is the observed reduction of heart rate in treated dogs. Even though this reduction of heart rate did not reach statistical significance, the magnitude of the reduction is in-line with reduction seen following chronic therapy with β-blockers in patients with HF.

There are limitations to the study that warrant discussion. Whereas the absence of background therapy with angiotensin converting enzyme inhibitors, beta adrenergic receptor blockers and/or aldosterone antagonists focus attention on the direct effects of CSB therapy, it does limit extrapolation of the results to patients who would surely be receiving established HF therapy. The present study is also limited by the absence of data on the effects of CSB on exercise tolerance, a measure often used to assess the efficacy of therapy in patients with HF.

In conclusion, the results of this study indicate that long-term therapy with CSB improves global LV systolic and diastolic function in dogs with advanced HF. The improvement of LV function was accompanied by improvement in global LV remodeling evidence by reduced LV size and partial restoration of LV shape to one that is more ellipsoidal rather than spherical. Global LV remodeling was also accompanied by structural remodeling at the cellular level as well as by correction, albeit in part, of molecular abnormalities characteristic of the HF state as evidenced by normalized expression of cardiac β_1-adrenergic receptor and nitric oxide signal transduction pathways along with and reduced interstitial fibrosis and cardiomyocyte hypertrophy. These experimental results support the initiation of clinical trials in patients with systolic HF. At present, the CSB *Rheos*® *System* used in this study is being tested in a clinical trial in patient with refractory hypertension titled “Device-based Therapy for Hypertension (DEBuT-HT) and in a trial in patients with heart failure and preserved LV ejection fraction title “Rheos Health Outcomes Prospective Evaluation for Heart Failure with EF≥40% (HOPE4HF).

CLINICAL PERSPECTIVE

Despite major advances in the development of new drugs for the treatment of chronic heart failure, the mortality and morbidity from this disease syndrome remains unacceptably high. In some patients the use of life saving standard pharmacologic therapy such as angiotensin-converting enzyme inhibitors, beta-adrenergic receptor blockers and aldosterone antagonists is suboptimal because of poor tolerability. Even in patients with heart failure who have over the years tolerated and benefited from these pharmacologic therapies, a time is reached when their effectiveness wanes and symptomatology from the disease increases and are in need to new therapeutic interventions. In the present study we tested a novel device-based therapy that can potentially be used in the treatment of such patients. The approach, termed chronic activation of the carotid sinus baroreflex (CSB) that acts via electrical stimulation of the carotid sinus nerve, was tested as monotherapy in dogs with experimental heart failure. Compared to no therapy at all, chronic CSB treatment resulted in improved left ventricular (LV) systolic and diastolic function and attenuation of LV remodeling. The improvement in ventricular function was associated with reduced LV filling pressure, reduced LV size and lowering of heart rate along with normalization of components of the renin-angiotensin-aldosterone system, the sympathetic nervous system and the nitric oxide signalling pathway. All of these benefits argue in favour of CSB therapy that is likely to provide benefit to patients with advanced chronic heart failure. Translation of the results of this study to clinical setting, however, must be used with care given that the experimentation was conducted in the absence of standard background medical therapy as would certainly be the case in patients.

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Table 1

Hemodynamic, Ventriculographic, Echocardiographic and Doppler Measures at Pre-Treatment and Post-Treatment in Control Dogs and Dogs treated with Carotid Sinus Baroreflex (CSB) Activation

	Control (n=6)			CSB Activation (n=8)		
	PRE	**POST**	**P-Value**	**PRE**	**POST**	**P-Value**
HR (BPM)	81±2	86±7	0.156	86±8	86±8	0.750
mAoP (mmHg)	71±14	84±15	0.156	79±12	76±8	1.000
LVEDP (mmHg)	14±3	15±2	0.500	17±2	12±1	0.008
CO (L/min)	1.43±0.15	1.43±0.13	1.000	1.69±0.27	1.92±0.37	0.039
SV (ml)	17.8±1.7	16.7±0.5	0.250	19.6±3.0	22.8±4.2	0.016
LVEF (%)	25.0±1.3	22.2±0.8	0.031	25.9±2.4	29.9±3.3	0.016
LV EDV (ml)	69.7±4.1	75.2±4.4	0.031	75.1±7.3	75.8±7.2	0.484
LV ESV (ml)	51.8±3.5	58.5±4.0	0.031	55.5±5.2	53.0±3.7	0.047
LV ESSI	1.54±0.08	1.49±0.08	0.094	1.50±0.07	1.53±0.07	0.086
LV EDSI	1.55±0.10	1.48±0.06	0.094	1.42±0.11	1.441±0.13	0.266
DT (msec)	79.0±7.0	73.3±8.2	0.063	81.6±13.1	92.3±14.6	0.102
PE/PA	1.88±0.31	1.76±0.34	0.094	2.01±0.24	2.30±0.49	0.039
LV EDWS (g/cm^2)	71.8±9.6	82.9±15.2	0.063	73.8±7.5	62.9±10.7	0.016
MR (%)	16.7±6.0	21.8±2.5	0.063	18.3±4.9	14.2±3.9	0.039
PNE (pg/ml)	173±74	243±158	0.250	248±89	163±99	0.023

HR = heart rate; mAoP = mean aortic pressure; LVEDP = left ventricular (LV) end-diastolic pressure; CO = cardiac output; SV = stroke volume; LVEF = LV ejection fraction from ventriculograms; EDV = end-diastolic volume from ventriculograms; ESV = end-systolic volume from ventriculograms; ESSI = end-systolic sphericity index from ventriculograms; EDSI = end-diastolic sphericity index from ventriculograms; DT = early mitral inflow deceleration time; PE/PA = ratio of peak mitral flow velocity in early diastole (PE) and peak mitral inflow velocity during left atrial contraction (PA); EDWS = circumferential end-diastolic wall stress; MR = functional mitral regurgitation; PNE = plasma norepinephrine.

Table 2

Treatment Effect (Δ) Comparison Between Controls Dogs and Dogs Treated With Carotid Sinus Baroreflex Activation (CSB)

	Control (n=6)	CSB Activation (n=8)	Wilcoxon P-value
ΔHR (BPM)	5 ± 6	0 ± 6	0.154
ΔmAoP (mmHg)	13 ± 18	−2 ± 15	0.106
ΔLVEDP (mmHg)	2 ± 4	−4 ± 3	0.008
ΔCO (L/min)	0.00 ± 0.14	0.23 ± 0.23	0.045
ΔSV (ml)	−1.2 ± 1.6	3.1 ± 2.2	0.003
ΔLVEF (%)	−2.8 ± 1.0	4.0 ± 2.4	0.002
ΔLV EDV (ml)	5.5 ± 3.5	0.6 ± 2.9	0.016
ΔLV ESV (ml)	6.7 ± 2.9	−2.5 ± 2.7	0.002
ΔLV ESSI	−0.05 ± 0.05	0.03 ± 0.04	0.012
ΔLV EDSI	−0.07 ± 0.06	0.02 ± 0.04	0.014
ΔDT (msec)	−5.7 ± 5.1	10.6 ± 14.6	0.070
ΔPE/PA	−0.12 ± 0.11	0.29 ± 0.44	0.010
ΔLV EDWS (g/cm^2)	11.2 ± 9.8	−11.0 ± 11.0	0.005
ΔMR (%)	5.1 ± 4.7	−4.1 ± 4.8	0.007
ΔPNE (pg/ml)	70 ± 125	−121 ± 108	0.012

HR = heart rate; mAoP = mean aortic pressure; LVEDP = left ventricular (LV) end-diastolic pressure; CO = cardiac output; SV = stroke volume; LVEF = LV ejection fraction from ventriculograms; EDV = end-diastolic volume from ventriculograms; ESV = end-systolic volume from ventriculograms; ESSI = end-systolic sphericity index from ventriculograms; EDSI = end-diastolic sphericity index from ventriculograms; DT = early mitral inflow deceleration time; PE/PA = ratio of peak mitral flow velocity in early diastole (PE) and peak mitral inflow velocity during left atrial contraction (PA); EDWS = circumferential end-diastolic wall stress; MR = functional mitral regurgitation; PNE = plasma norepinephrine.

Table 3

Histomorphometric Findings at the End of 3 Months in Normal Dogs, Control Dogs and Dogs Treated with Carotid Sinus Baroreflex Activation

	Normal (n=6)	Control (n=6)	CSB Activation (n=8)
VFRF (%)	0.0 ± 0.0	21.0 ± 2.6*	15.7 ± 3.8*†
VFIF (%)	3.7 ± 0.2	12.3 ± 1.4*	9.9 ± 0.7*†
MCSA (μm^2)	409 ± 26	736 ± 57*	585 ± 22*†
CD (cap/fiber)	1.00 ± 0.0	0.95 ± 0.05*	1.03 ± 0.05†
ODD (μm)	8.9 ± 0.5	11.6 ± 0.4*	10.5 ± 0.3*†

Cap/cell = capillary density for fiber; CD = capillary density based on capillary to fiber ratio (Cap/fiber); MCSA = myocyte cross-sectional area; ODD = oxygen diffusion distance; VFIF = volume fraction of interstitial fibrosis; VFRF = volume fraction of replacement fibrosis.

*=p<0.05 vs. Normal,

†=p<0.05 vs. Control.

Table 4

Gene Expression in Normal dogs, Untreated Heart failure Control Dogs and Heart failure Dogs Treated with Chronic Carotid Sinus Baroreflex (CSB) Activation

	Normal (n=6)	Control (n=6)	CSB Activation (n=8)
GADPH (du)	0.34 ± 0.02	0.35 ± 0.03	0.36 ± 0.02
β_1-Adrenergic Receptor (du)	0.55 ± 0.06	0.26 ± 0.04*	0.51 ± 0.08†
β_2-Adrenergic Receptor (du)	0.23 ± 0.03	0.26 ± 0.04	0.25 ± 0.03
β-Adrenergic Receptor Kinase (du)	0.27 ± 0.05	1.22 ± 0.08*	0.54 ± 0.06*†
Adenylyl Cyclase (du)	0.64 ± 0.05	0.34 ±0.04*	0.53 ± 0.06*†
Angiotensinogen (du)	0.36 ± 0.05	0.74 ± 0.06*	0.39 ± 0.03†
eNOS (du)	0.71 ± 0.07	0.44 ± 0.07*	0.56 ± 0.07*†
iNOS (du)	0.34 ± 0.06	0.72 ± 0.06*	0.43 ± 0.05*†

GADPH = glyceraldehyde-3-phosphate dehydrogenase ; eNOS = endothelial nitric oxide synthase. iNOS – inducible nitric oxide synthase ;

*=$p<0.05$ vs. Normal,

†=$p<0.05$ vs. Control.