Calcitonin Gene-Related Peptide In Vivo Positive Inotropy Is Attributable to Regional Sympatho-Stimulation and Is Blunted in Congestive Heart Failure

Tatsuo Katori, Donald B. Hoover, Jeffrey L. Ardell, Robert H. Helm, Diego F. Belardi, Carlo G. Tocchetti, Paul R. Forfia, David A. Kass, Nazareno Paolocci

Abstract—Calcitonin gene-related peptide (CGRP) is a nonadrenergic/noncholinergic (NANC) peptide with vasodilatative/inotropic action that may benefit the failing heart. However, precise mechanisms for its in vivo inotropic action remain unclear. To assess this, dogs with normal or failing (sustained tachypacing) hearts were instrumented for pressure–dimension analysis. In control hearts, CGRP (20 pmol/kg per minute) enhanced cardiac contractility (eg, +33±4.2% in end-systolic elastance) and lowered afterload (−14.2±2% in systemic resistance, both P<0.001). The inotropic response was markedly blunted by heart failure (+6.5±2%; P<0.001 versus control), whereas arterial dilation remained unaltered (−19.3±5%). CGRP-positive inotropy was not attributable to reflex activation because similar changes were observed in the presence of a ganglionic blocker. However, it was fully prevented by the β-receptor antagonist (timolol), identifying a dominant role of sympatho-stimulatory signaling. In control hearts, myocardial interstitial norepinephrine assessed by microdialysis almost doubled in response to CGRP infusion, whereas systemic plasma levels were unchanged. In addition, CGRP receptors were not observed in ventricular myocardium but were prominent in coronary arteries and the stellate ganglia. Ventricular myocytes isolated from normal and failing hearts displayed no inotropic response to CGRP, further supporting indirect sympatho-stimulation as the primary in vivo mechanism. In contrast, the peripheral vasodilatative capacity of CGRP was similar in femoral vascular rings from normal and failing hearts in dogs. Thus, CGRP-mediated positive inotropy is load-independent but indirect and attributable to myocardial sympathetic activation rather than receptor-coupled stimulation in canine hearts. This mechanism is suppressed in heart failure, so that afterload reduction accounts for CGRP-enhanced function in this setting. (Circ Res. 2005;96:234-243.)

Key Words: calcitonin gene-related peptide ■ sympathetic efferent fibers ■ heart failure ■ norepinephrine ■ contractility

Calcitonin gene-related peptide (CGRP) is a nonadrenergic/noncholinergic (NANC) peptide with potent vasodilator activity. Its role as a counterbalance to vascular sympathetic nerve discharge is supported by the presence of hypertension in mice lacking CGRP. Exogenous administration of CGRP to patients with congestive heart failure (CHF) increases cardiac output, although whether this reflects cardiac inotropy or peripheral vasodilation is unknown. CGRP receptor components (receptor activity-modifying protein 1 and calcitonin receptor-like receptor, RAMP1 and CRLR, respectively) are upregulated in failure models, whereas circulating plasma levels are decreased and this might suggest a potential efficacy for exogenously administered CGRP. Recently, this notion received further attention with the discovery that the reduced form of nitric oxide (HNO/NO−) is a potent cardiac stimulant in normal and failing hearts, which appears in part coupled to CGRP signaling.

Direct evidence for cardiotropic effects of CGRP have only been obtained in isolated hearts and tissues. CGRP increases atrial contractility in various species including humans by stimulating specific myocardial CGRP receptors coupled to adenylate cyclase. CGRP effect on ventricular contractility is less clear, because recent studies of isolated human trabeculae reported little direct response to CGRP or no effect at all. Furthermore, although mRNAs for all components of the CGRP receptor have been detected in human ventricle, definitive evidence for functional myocyte receptors remains lacking. One alternative mechanism suggested by studies performed in isolated ventricle from guinea pig is that CGRP can stimulate catecholamine release from distal sympathetic nerve terminals to enhance contractility.
However, relevance of this mechanism in vivo, its translation to other species, and whether such release is organ-specific or coupled to local sympathetic stimulation are all unknown.

This study tested the hypotheses that in vivo cardiotoric effects of CGRP are indirect, mediated by β-adrenergic neural-dependent mechanisms rather than direct myocyte interaction, and decreased in failing hearts. Studies were conducted in conscious dogs chronically instrumented for pressure–dimension analyses, and data were measured in normal hearts and in those with CHF induced by sustained tachypacing. We report for the first time to our knowledge that in vivo CGRP-mediated inotropy is primarily attributable to local cardiac β-stimulation rather than direct CGRP receptor/agonist signaling on myocytes, and is markedly down-regulated in CHF. This contrasts to a direct CGRP capacity to dilate, in vivo arteries and ex vivo vascular rings, that is preserved in heart failure (HF). These data provide important new insights regarding the nature of CGRP modulation of the intact heart and its influence in late-stage CHF.

Materials and Methods

In Vivo Experimental Preparation and Protocol

Adult male mongrel dogs (22 to 25 kg) were chronically instrumented for pressure–dimension analysis as described.11,21 Animals were anesthetized with 1% to 2% halothane after induction with sodium thiopental (10 to 20 mg/kg, intravenous). The surgical/experimental animal protocol was approved by the Johns Hopkins University Animal Care and Use Committee. The surgical preparation involved placement of an left ventricle (LV) micromanometer (P22; Konigsberg Instruments, Pasadena, Calif), sonomicrometers to measure anteroposterior LV dimension, an inferior vena cava perivascular occluder to alter cardiac preload, aortic pressure catheter, ultrasound coronary flow probe (proximal circumflex artery), and epicardial-pacing electrodes for atrial pacing. Cardiac failure was induced by rapid ventricular pacing for 3 weeks as described.11,21

Cardiovascular effects of CGRP were assessed in 14 normal control dogs and 6 with CHF. Data were acquired in conscious animals, standing quietly in a sling, with ventricular pacing suspended at least 30 minutes before the study in dogs with HF. CGRP (4 to 40 pmol/kg/min × 10 to 20 minutes) was infused intravenously, with heart rate (HR) maintained constant by atrial pacing (140 to 150 beats per minute). These rates were needed to assure matching before and after CGRP, and between control and failing animals. To test the role of baroreflex activation on CGRP effects, studies were performed in the presence of ganglionic blockade (hexamethonium chloride 5 mg/kg every 15 minutes intravenous; n

Isolated Myocyte Studies

Adult canine ventricular myocytes were isolated from freshly excised LVs of normal (n=3) or CHF (n=3) dogs. Hearts were removed under ice-cold cardioplegia (100 mEq K+/Plegisol, Abbott Labs), and a section of the LV was dissected and perfused at constant flow (25 mL/min) and pressure (90 mm Hg) with warmed (37°C) calcium-free Krebs-Henseleit (K-H) solution, followed by EGTA-free K-H containing collagenase (type I, 178 U/mL; Worthington Biochem) and protease (type XIV, 0.12 mg/mL). Perfusate was then switched to a modified Tyrode solution containing 125 μmol/L Ca2+ for 10 minutes, and then heart tissue was mechanically disaggregated. All solutions were oxygenated with 95%O2–5%CO2 and warmed to 37°C. Cells were imaged with an inverted microscope equipped for simultaneous assessment of sarcomere shortening (IonOptix) and INDO-1-AM fluorescence to measure the calcium transient.

Plasma CGRP Assay

Arterial, venous, and coronary sinus blood plasma were sampled and analyzed for CGRP concentration by RIA following manufacturer’s instructions (Peninsula Labs). CGRP antiserum, code RAS 6012, was used, with a dynamic range of 1 to 128 pg per 300 μL.25

Cardiac Interstitial Fluid Norepinephrine

Intersitial myocardial norepinephrine in response to CGRP was determined by microdialysis in 4 normal anesthetized dogs. Eight additional animals served as time controls. All the procedures and experimental protocols were reviewed and approved by the East Tennessee State University Institutional Committee on Animal Care and conformed to the Animal Welfare Act according to the Public Health Policy on Humane Care and Use of Laboratory Animals. Anesthesia was induced by sodium thiopental (15 mg/kg intravenous) and maintained with isofluorane (2% inhalation). Hearts were exposed by median sternotomy, and three microdialysis probes (Clirans; Terumo, Tokyo, Japan) were inserted into the anterior LV myocardium at base, middle, and apical regions.24 The inflow capillary tube for each probe was connected via a larger deactivated silica tube to a gas-tight glass syringe filled with normal saline and perfused at 2.5 μL/min. Effluent (dialysate) was collected from the outflow tube in EGTA and reduced glutathione and immediately frozen (−80°C) until analyzed.24 Data were collected at least 2 hours after surgical instrumentation to assure stable basal norepinephrine levels. CGRP (20 pmol/kg per minute) was infused into a central vein for 15 minutes, and interstitial fluid and blood from aorta and coronary sinus were collected before, during, and after drug infusion. Norepinephrine levels were determined by radio-enzymatic assay (Amersham Pharmacia Biotech).

CGRP Receptor Autoradiography

Fresh ventricular full-thickness myocardium and stellate ganglia were frozen and cut into 20-μm sections, thaw-mounted onto separate chrome alum-gelatin–coated slides, dried, and stored at −80°C. CGRP receptors were labeled using [3H]CGRP (2200 Ci/mmol; PerkinElmer Life Science Products, Boston, Mass) as described.25 Autoradiography films were processed after exposure for 3 or 7 days at 4°C, and digitized images were analyzed using image quantitation software (MCID; Imaging Research, Ontario, Canada) to convert relative optical density values to amounts of radioligand bound as fmol/mg tissue. Several measurements were made for each tissue region and averaged to yield total, nonspecific, and specific (difference between first two) binding for each animal.

Ex Vivo Arterial Response to CGRP

Studies of the vascular responsiveness to CGRP were performed in fresh canine femoral arterial segments obtained from failing and normal animal hearts at the time of euthanization. A 2-cm segment of femoral artery was dissected free of fat and connective tissue, cut into 2-mm vascular rings, and placed in ice-cold Krebs buffer (concentrations in mM: 118 NaCl, 4.7 KCl, 1.6 CaCl2, 1.2 KH2PO4, 25 NaHCO3, 1.2 MgSO4, and 11.1 glucose). Rings were suspended...
between two wire stirrups and immersed in organ chambers containing Krebs buffer maintained at 37°C, pH 7.4, bubbled with 95% O2–5% CO2. Rings were stretched to 3 grams of developed tension over a 1-hour period to optimize contractile response to KCl. A single concentration of KCl (60 mmol/L) was used to assess vascular smooth muscle viability. Rings were then washed, preconstricted with 10–6 M phenylephrine, and exposed to increasing concentrations of CGRP (10–11 to 10–7 M).26 Data were collected using the MacLab system and analyzed using Dose Response Software (AD Instruments).

Chemicals
β-CGRP, CGRP8–37, timolol, hexamethonium, and protease were purchased from Sigma (St. Louis, Mo) and dissolved in saline just before use.

Statistical Analysis
Data are presented as mean±SEM. Analysis was performed by paired t test, one-way analysis of variance, or repeated measures ANOVA with a Tukey test for post-hoc comparisons.

Results
Hemodynamic Effects of CGRP in Control and Failing Hearts
Figure 1A shows an example of pressure–dimension data before and during CGRP infusion. End-systolic pressure–dimension relation shifted leftward and had a steeper slope, indicating a positive inotropic effect. At 20 pmol/kg per minute, this amounted to a 34.2±4.1% \( E_{es} \) increase and 40.9±5.0% increase in \( D_{EDD} \) (both \( P<0.001 \); Table 1). CGRP also reduced arterial resistance (16.3±1.6%; \( P<0.001 \)) but with little net decline in systolic pressure. For comparison, the extent of CGRP-induced arterial dilation in failing preparations (19±4.5% in total resistance; \( P<0.001 \)) is not far from the response to a maximal dose (limited by arrhythmias) of a nitric oxide (NO) donor (diethylamine NONOate, 2.0 μg/kg per minute, –29.5±11%, \( n=5, P<0.05 \) versus base, unpublished data). Diethylamine NONOate also re-
Role of Reflex and \( \beta \)-Adrenergic Receptor Stimulation

Given the potential impact of hypotension-induced reflex activation of sympathetic efferents on cardiac inotropy, we tested whether the response to exogenous CGRP was prevented by ganglionic blockade in control. The adequacy of blockade was confirmed using a previously reported method.\(^{27}\) Under control conditions, Ees increased after a preload decline (+baroreflex, +52.7±18.9; \( P<0.05 \)), and this was eliminated by hexamethonium (+1.7±3.2\%; \( P<0.03 \) versus baseline). Despite reflex blockade, CGRP-mediated inotropy and vasodilation were unchanged (Figure 2). However, blockade of downstream \( \beta \)-adrenergic receptors by timolol eliminated the inotropic response to CGRP (Ees: +3.2±4.3\%; \( D_{500} \): −3.6±2.8\%, \( P=\text{NS} \) versus timolol alone) but still had no impact on CGRP-mediated systemic vasodilation (Figure 2). The timolol dose fully blocked inotropic (Ees, +71.6±12.5\% versus −8.1±1.1\%) and chronotropic (HR, +39.9±35.7\% versus −0.5±0.5\%) effects of high-dose isoproterenol infusion (0.4 \( \mu g/\text{kg per minute} \), \( n=2 \)).

To test whether CGRP triggered cardiac-specific versus diffuse sympathetic efferent activation, norepinephrine content was measured in LV myocardial interstitial fluid by microdialysis. Norepinephrine nearly doubled from 5.08±0.55 to 9.94±1.56 nmol/L with CGRP infusion (Figure 3), peaking after 10 minutes and persisting \( \approx \)10 minutes after the infusion was terminated. In contrast, systemic plasma norepinephrine concentration was unchanged (1.31±0.07 versus 1.49±0.10 nmol/L).

**CGRP Receptor Binding Distribution**

To better define the potential tissue targets for CGRP stimulation, we performed autoradiography using \([125\text{I}]\)CGRP (Figure 4). Binding was undetectable in left ventricular myocardium of hearts of normal dogs and those with HF. Conversely, binding was very abundant in coronary arteries (Figure 4A to 4C, Table 2) and arteries in and around the stellate ganglia in both groups (Figure 4D to 4F, Table 2). Specific CGRP binding was also present in regions of stellate ganglia that contain sympathetic efferent neurons.

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**TABLE 1. Hemodynamic Effects of CGRP Before and After CHF**

<table>
<thead>
<tr>
<th>Dose, pmol/kg per minute</th>
<th>Control (N=14)</th>
<th>HF (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline  20</td>
<td>Baseline  20</td>
</tr>
<tr>
<td>( E_{\text{es}} ), mm Hg/mm</td>
<td>11.1±0.7</td>
<td>14.8±0.9*</td>
</tr>
<tr>
<td>( D_{500} ), mm Hg/sec per mm</td>
<td>128.6±12.4</td>
<td>177.3±14.9*</td>
</tr>
<tr>
<td>PRSW, mm Hg</td>
<td>75.8±3.9</td>
<td>88.2±4.3*</td>
</tr>
<tr>
<td>Tau, msec</td>
<td>33.4±0.9</td>
<td>32.9±1.8</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>35.5±1.8</td>
<td>35.5±1.8</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>28.0±2.2</td>
<td>27.2±2.2*</td>
</tr>
<tr>
<td>LVESP, mm Hg</td>
<td>128.0±3.9</td>
<td>120.6±3.5**</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8.5±0.8</td>
<td>9.5±1.1</td>
</tr>
<tr>
<td>SAP, mm Hg</td>
<td>135.2±5.2</td>
<td>120.7±4.3</td>
</tr>
<tr>
<td>DAP, mm Hg</td>
<td>106.8±3.8</td>
<td>95.6±3.4*</td>
</tr>
<tr>
<td>MCF, mL/min</td>
<td>31.0±8.5</td>
<td>31.3±8.1</td>
</tr>
<tr>
<td>RT, mm Hg/mm per second</td>
<td>7.6±0.6</td>
<td>6.4±0.5*</td>
</tr>
<tr>
<td>Cardiac output, m/min</td>
<td>1.12±0.11</td>
<td>1.25±0.12‡</td>
</tr>
</tbody>
</table>

| DAP indicates diastolic arterial pressure; \( D_{500} \), dp/dt-end-diastolic dimension relation; \( E_{\text{es}} \), end-systolic elastance; HF, heart failure; LVEDD, left ventricular end-diastolic dimension; LVESP, left ventricular end-systolic pressure; LVEDD, left ventricular end-systolic dimension; LVEDP, left ventricular end-diastolic pressure; SAP, mean coronary flow; PRSW, prerecruitable stroke work; RT, total resistance; TIM, systolic arterial pressure.

*\( P<0.001 \) vs baseline; †\( P<0.01 \); ‡\( P<0.05 \); §\( P<0.005 \) vs control; ¶\( P<0.01 \); ††\( P<0.05 \); **\( P<0.005 \).
CGRP Does Not Alter Inotropy of Isolated Ventricular Myocytes

The preceding findings suggested that CGRP-mediated positive inotropy was coupled to regional myocardial catechol-amine release rather than direct interaction of the peptide with receptors on cardiomyocytes. Canine ventricular myocytes were isolated from normal and failing hearts and sarcomere shortening, as well as whole-cell calcium transients, were recorded in response to 10 nmol/L CGRP. Such doses had been previously reported to enhance contractility in isolated rat ventricular myocytes and to induce relaxation of isolated coronary arteries. CGRP did not alter sarcomere shortening or calcium transient in myocytes from hearts of either condition (Figure 5). Positive control data with 10 nmol/L isoproterenol are provided to confirm inotropic reserve.

Preserved CGRP Vasodilation in Ex Vivo Vascular Rings

The similarity of CGRP-induced systemic vasodilation in controls, HF animals, and dogs treated with ganglionic or \( \beta \)-blockade suggested a direct and unaltered CGRP dilator effect. To test this, we exposed preconstricted femoral artery rings to incremental doses of CGRP. CGRP vasorelaxation was dose-dependent and was similar in rings from failing or control animals (Figure 6).

Discussion

This study provides the first in vivo evidence that exogenous infusion of the NANC-peptide CGRP induces a load-independent increase in cardiac contractility and reveals that this response is attributable to localized cardiac sympatho-stimulation mediated by \( \beta \)-receptors rather than to a direct myocyte CGRP effect. This mechanism is blunted in animals with dilated cardiac failure characterized by \( \beta \)-adrenergic downregulation. The study provides the first demonstration that CGRP induces selective arterial but no apparent venodilation, and that it does so similarly in animals with or without \( \beta \)-blockade or cardiac failure.

CGRP Receptor Distribution and Signaling

Previous studies have established the existence of CGRP receptors in cardiac tissue, and in rodents, agonist-receptor binding triggers cAMP and positive inotropy in isolated muscle and myocytes. However, controversy remains whether this is species-specific and how such data translate to in vivo CGRP contractility modulation. In vitro contractile CGRP responses have been inconsistent even in the same species (eg, human) and tissue (ventricular trabeculae). The latter is paralleled by disparate data regarding the presence of CGRP receptors in human ventricular myocytes showing moderate CRLR-like immunoreactivity but virtu-
ally no CGRP binding sites by autoradiography. Co-expression of CRLR and RAMP1 leads to the formation of functional heterodimeric receptors for CGRP. Along the same line, Sugiyama et al first reported that immunoreactive CGRP fibers primarily targeted coronary arteries and not myocardial tissue in the canine heart, and they found minimal inotropy or chronotropy in isolated papillary muscle and sinoatrial node preparations from canine hearts in response to CGRP. Given the very similar distribution of CGRP receptors and fibers in humans and dogs, it is likely the current results are more pertinent to humans and, importantly, provide a novel mechanism for in vivo CGRP inotropy that does not require direct myocyte receptor/CGRP interaction. Our results further show the necessity of taking an integrative approach to properly identify neurohumoral and myocyte interactions.

**CGRP and Sympatho-Stimulation**

Several lines of evidence support localized sympatho-stimulation by CGRP; norepinephrine increased in cardiac interstitial fluid but not...
systemic plasma, and CGRP inotropy persisted despite ganglionic blockade but was inhibited by β-blockade. The notion that CGRP could stimulate release of norepinephrine from isolated sympathetic nerves was first raised by Seyedi et al20 in isolated guinea pig hearts, and they also demonstrated this could be inhibited by the selective CGRP blocking peptide CGRP8–37. The present study is the first to our knowledge to show this as the primary mechanism for CGRP inotropy in vivo.

### TABLE 2. CGRP Receptor Abundance

<table>
<thead>
<tr>
<th>Region</th>
<th>Total Binding</th>
<th>Nonspecific Binding</th>
<th>Specific Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular myocardium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>0.258±0.051</td>
<td>0.240±0.056</td>
<td>ND</td>
</tr>
<tr>
<td>CHF (n=3)</td>
<td>0.367±0.024</td>
<td>0.343±0.092</td>
<td>ND</td>
</tr>
<tr>
<td>Coronary arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>0.966±0.038</td>
<td>0.271±0.025</td>
<td>0.695±0.057†</td>
</tr>
<tr>
<td>CHF (n=3)</td>
<td>1.36±0.579</td>
<td>0.329±0.12</td>
<td>1.03±0.456*</td>
</tr>
<tr>
<td>Stellate ganglion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=3)</td>
<td>0.102±0.041</td>
<td>0.029±0.023</td>
<td>0.073±0.024*</td>
</tr>
</tbody>
</table>

Units are fmol/mg tissue.

*P<0.001, †P<0.005 between total and nonspecific binding.

ND indicates not detectable.

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**Figure 5.** Direct effects of CGRP on isolated myocytes. CGRP had no effects on maximal sarcomere shortening (A) or calcium transients (B) in isolated myocytes from normal and failing dog hearts. As positive control, isoproterenol (ISO) revealed the expected improvement in both components but with less response in failing myocytes. *P<0.001 vs normal; †P<0.05 vs baseline.
The fibers involved with CGRP sympatho-stimulation are likely those innervating the ventricles directly, given the localized myocardial norepinephrine response. Norepinephrine can also act on sensory cardiac nerve endings and resistance vessels to attenuate CGRP release, whether such a feedback loop played a role in the current study is unclear. A similar circuit has been proposed for CGRP and CGRP receptors in the stellate ganglia and with presence of CGRP-immunoreactive cells and nerves processes, where sympathetic neurons that innervate the heart are also found. CGRP-labeled neurons are abundant in human stellate ganglia as well, and CGRP peptide abundance is enhanced after myocardial infarction or in patients with congenital heart disease. Whether CGRP release is altered in these conditions and/or whether CGRP has alternative actions at the neuronal level beyond triggering norepinephrine release from sympathetic efferent fibers remains unknown.

CGRP and the Failing Heart
CGRP enhanced cardiac function in failing hearts largely because of systemic vasodilation, not inotropy. This is consistent with a primarily sympatho-stimulatory mechanism, because cardiac failure (and in particular the tachypacing model) is accompanied by β-adrenergic downregulation. CGRP triggered release of interstitial norepinephrine, and norepinephrine interstitial level directly correlates with ventricular contractility. This CGRP paracrine effect is likely blunted in failing hearts, because previous studies have shown a reduced capacity for norepinephrine release in response to electrical stimulation in the same failure model. In this setting, alterations of norepinephrine-releasing mechanisms rather than reduced norepinephrine stores are likely responsible for this deficiency. Norepinephrine overflow occurring in response to tyramine was of a similar magnitude in failing and healthy preparations. The overall effect we obtained with exogenous CGRP in failing hearts is congruent with previous data showing attenuated LV dP/dt max to exogenous CGRP in the same setting, but, unlike the earlier study, rule out HR or differential loading as major explanations for this response.

Unlike CGRP-mediated sympatho-stimulation and positive inotropy, dilation of vascular smooth muscle was preserved in HF, and with ganglionic and β-blockade, suggesting a direct and independent mechanism. As recently reviewed, vasorelaxant effects of CGRP are attributable to multiple mechanisms. The vascular ring results indicate direct effects independent of sympathetic nerve release, and this was similar between normal and failing hearts. Furthermore, systemic norepinephrine levels were similar before and after CGRP infusion, so substantial systemic sympatoh-stimulation was not observed. The decline in basal CGRP levels in the failing heart is consistent with previous reports and may contribute to increased resting vascular tone and resistance in this disorder.

CGRP signaling has received renewed interest because of recent discoveries that nitroxyl anion (HNO/NO·), the one electron-reduced form of NO, triggers load-independent and reflex-independent inotropy associated with elevated plasma CGRP. In controls, this response was inhibited by co-infusion of the CGRP receptor blocker (CGRP8-37), but not β-blockade, and nitroxyl-induced more selective venodilation. The present findings that CGRP inotropy is prevented by β-blockade, blunted by CHF, and associated principally with arterial dilation all suggest that CGRP signaling does not explain the in vivo HNO/NO· response. Additional studies are needed to test if HNO/NO· stimulates more generalized NANC fiber signaling, involves release of alternate peptides that share CGRP cardiovascular properties, or has direct myocardial effects.

Limitations
First, the tachypacing model of cardiac failure recapitulates many important abnormalities observed in the human disease. It has admitted differences as well. More direct analysis of the present findings to human CHF would be required to establish this. Second, we did not directly assess cardiac interstitial norepinephrine release after CGRP infusion in failing preparations. This was performed given concerns over the fragility of the preparation and need for open-chest interventions to obtain these data. Further, there are existing data demonstrating that impairment of norepinephrine release from sympathetic efferent fibers remains unknown.

CGRP, Norepinephrine, and Myocardial Contractility

Figure 6. Direct effects of CGRP on isolated vascular rings. Dose response of CGRP in ex vivo femoral artery rings from normal and failing dog hearts. CGRP reduced the tension similarly in both types of tissues. *P<0.05 vs baseline.

Conclusion
Exogenous CGRP exerts dose-dependent load-independent positive inotropy in the normal in vivo canine heart attributable to local sympatho-stimulation. This “CGRP–norepinephrine axis” is likely to be relevant in species, such as dogs (and humans), that use β-adrenergic signaling as an important “reserve” mechanism to increased demand. CGRP levels increase during exercise in humans, and our results not only provide novel coupling of this response to adrenergic inotro-
pic responses in the heart but also highlight how this would be impacted by β-receptor downregulation (blockade or failure). In the light of these data, afterload reduction is the primary mechanism for improved cardiac output to exogenous CGRP in HF, and increased CGRP levels are unlikely to augment contractility in this disease in which myocardial β-receptor signaling is downregulated.

Acknowledgments

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References


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Table: Plasma CGRP levels in normal controls and animal with heart failure

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=5)</th>
<th>HEART FAILURE (n=6)</th>
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</thead>
<tbody>
<tr>
<td>dose (pmol/kg/min)</td>
<td>baseline</td>
<td>4</td>
</tr>
<tr>
<td>Artery</td>
<td>7.1±0.2</td>
<td>14.0±2.6†</td>
</tr>
<tr>
<td>Vein</td>
<td>7.5±0.3</td>
<td>13.0±2.8†</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>8.3±0.4</td>
<td>14.2±1.1†</td>
</tr>
</tbody>
</table>

pmol/L. *P < 0.01 vs. Baseline; †P < 0.05 vs. baseline; §P < 0.01 vs. control.