Inhibiting Mitochondrial Na\(^+\)/Ca\(^{2+}\) Exchange Prevents Sudden Death in a Guinea Pig Model of Heart Failure

Ting Liu, Eiki Takimoto, Veronica L. Dimaano, Deeptankar DeMazumder, Sarah Kettlewell, Godfrey Smith, Agnieszka Sidor, Theodore P. Abraham, Brian O’Rourke

**Rationale:** In cardiomyocytes from failing hearts, insufficient mitochondrial Ca\(^{2+}\) accumulation secondary to cytoplasmic Na\(^+\) overload decreases NAD(P)H/NAD(P)\(^+\) redox potential and increases oxidative stress when workload increases. These effects are abolished by enhancing mitochondrial Ca\(^{2+}\) with acute treatment with CGP-37157 (CGP), an inhibitor of the mitochondrial Na\(^+\)/Ca\(^{2+}\) exchanger.

**Objective:** Our aim was to determine whether chronic CGP treatment mitigates contractile dysfunction and arrhythmias in an animal model of heart failure (HF) and sudden cardiac death (SCD).

**Methods and Results:** Here, we describe a novel guinea pig HF/SCD model using aortic constriction combined with daily β-adrenergic receptor stimulation (ACi) and show that chronic CGP treatment (ACi plus CGP) attenuates cardiac hypertrophic remodeling, pulmonary edema, and interstitial fibrosis and prevents cardiac dysfunction and SCD. In the ACi group 4 weeks after pressure overload, fractional shortening and the rate of left ventricular pressure development decreased by 36% and 32%, respectively, compared with sham-operated controls; in contrast, cardiac function was completely preserved in the ACi plus CGP group. CGP treatment also significantly reduced the incidence of premature ventricular beats and prevented fatal episodes of ventricular fibrillation, but did not prevent QT prolongation. Without CGP treatment, mortality was 61% in the ACi group <4 weeks of aortic constriction, whereas the death rate in the ACi plus CGP group was not different from sham-operated animals.

**Conclusions:** The findings demonstrate the critical role played by altered mitochondrial Ca\(^{2+}\) dynamics in the development of HF and HF-associated SCD; moreover, they reveal a novel strategy for treating SCD and cardiac decompensation in HF. (Circ Res. 2014;115:44-54.)

**Key Words:** calcium ■ energy metabolism ■ heart failure ■ mitochondria ■ oxidative stress ■ reactive oxygen species ■ sudden cardiac death

---

The clinical syndrome of heart failure (HF), which can occur after acute myocardial ischemic injury or after longstanding chronic cardiovascular disease, is a progressive disease often involving a phase of compensatory hypertrophy followed by a transition to impaired contractility (decompensation) with systolic or diastolic dysfunction. Although mortality in the later stages of the disease is most often attributed to impaired pump function, sudden cardiac death (SCD) is the leading cause of mortality in patients with mild to moderate HF, accounting for 35 to 50% of deaths,\(^1,2\) prompting an increase in the use of implantable cardiac defibrillators as prophylactic devices.\(^3\) The mechanisms underlying SCD, and the transition from compensated to decompensated HF, are incompletely understood, but neurohormonal abnormalities, ion channel/transporter changes, Ca\(^{2+}\) handling alterations, the activation of intra- and extracellular signaling pathways, and oxidative stress have all been implicated in the pathophysiology.\(^4\)

---

Original received November 19, 2013; revision received April 23, 2014; accepted April 29, 2014. In March 2014, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 12.63 days.

From the Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD (T.L., E.T., V.L.D., D.D., A.S., T.P.A., B.O.R.); and Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom (S.K., G.S.).

This manuscript was sent to Steven Houser, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

The online-only Data Supplement is available with this article at http://circres.ahajournals.org/lookup/suppl/doi:10.1161/CIRCRESAHA.115.303062/-/DC1.

Correspondence to Brian O’Rourke, PhD, Division of Cardiology, Department of Medicine, Johns Hopkins University, 720 Rutland Ave, 1060 Ross Bldg, Baltimore, MD 21205-2195. E-mail bor@jhmi.edu

© 2014 American Heart Association, Inc.

* Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.115.303062
Mitochondrial NADPH is crucial because it provides the reducing equivalents that drive oxidative phosphorylation and energy supply and demand,17,18 as well as ROS balance,19 in failing heart cells. The pathway of contractile and electric dysfunction involves a vicious cycle whereby increased cytoplasmic Na⁺ ([Na⁺]c), along with impaired sarcoplasmic reticulum (SR) Ca²⁺ release, leads to blunted Ca²⁺ signaling to the mitochondria during increased work.17-20 Consequently, the failure to stimulate Ca²⁺-dependent dehydrogenases of Krebs cycle21-25 results in the net oxidation of the matrix NADH pool. The decreased NADH/NAD⁺ redox potential, on the one hand, results in a deficiency of reducing equivalents that drive oxidative phosphorylation and ATP production and, on the other hand, compromises the ability to scavenge ROS, owing to the interdependence of the NADH and NADPH redox pools20 (see schema in Online Figure I). Mitochondrial NADPH is crucial because it provides the reducing power to maintain the reduced glutathione, thioredoxin, and glutaredoxin pools, all of which are vital for ROS scavenging and the prevention of protein thiol oxidative damage.26

Mitochondrial Ca²⁺ ([Ca²⁺]ₘ) is determined by the balance of influx through the [Ca²⁺]ₘ uniporter and efflux through the mitochondrial Na⁺/Ca²⁺ exchanger (mNCE); hence a change in either of these pathways can disrupt [Ca²⁺]ₘ dynamics. The Kᵣ of mNCE for Na⁺ is ≈5 to 10 mmol/L, falling within the range of [Na⁺]c levels in cardiomyocytes.27 Therefore, the elevated [Na⁺]c consistently observed in HF can significantly accelerate the [Ca²⁺]ₘ efflux rate. Studies of isolated heart mitochondria have shown that an increase of extramitochondrial Na⁺ leads to decreased [Ca²⁺]ₘ and compromises mitochondrial energetics, including oxidation of the NADH pool and decreased oxidative phosphorylation rate and ATP levels.24,25 Similarly, elevated [Na⁺]c isolated myocytes from failing guinea pig hearts19 (or ouabain-treated myocytes)30 causes insufficient [Ca²⁺]ₘ accumulation during increased work, net oxidation of NAD(P)H, and a massive increase of intracellular ROS,31 ultimately resulting in abnormal Ca²⁺ regulation and arrhythmias.30 The adverse effects of high [Na⁺]c are abolished by CGP-37157 (CGP), an inhibitor of mNCE.19,30,31

In the present study, we examine whether in vivo treatment with CGP prevents the development of HF and SCD using a novel guinea pig model of HF induced by left ventricular (LV) pressure overload combined with a daily β-adrenergic challenge. We show that partial inhibition of mNCE attenuates cardiac remodeling, preserves cardiac contractile function, and protects against HF-associated arrhythmias and SCD, significantly improving overall survival. The results highlight mNCE as a novel target for HF treatment.

### Methods

#### Results

**Daily β-Adrenergic Challenge Accelerates the Transition From LV Hypertrophy to HF**

A guinea pig HF model was developed by combining ascending aortic constriction (AC) with daily administration of the β-adrenergic agonist, isoproterenol, via intraperitoneal injection (ACI). The rationale was to expose the pressure-overloaded heart to a period of increased work each day to simulate β-adrenergic stress. The isoproterenol dose was chosen so that it would have minimal effects on cardiac hypertrophic remodeling in the absence of AC. There was no evidence of cardiac hypertrophic remodeling in sham-operated animals exposed to the 4-week isoproterenol challenge (SHAMI) as compared with the sham-operated, untreated group (SHAM). However, in the presence of AC, the same isoproterenol treatment significantly exacerbated the decline in cardiac fractional shortening (FS) evoked by AC alone (Online Figure II). In AC, FS decreased from 46.5±1.0% at week 0 (prebending) to 30.5±1.5% at week 4, whereas with AC alone, FS was not significantly decreased by week 4 (44.2±1.0% at week 0 versus 43.4±2.2% at week 4). Significant cardiac dysfunction in the AC group was observed only after 6 weeks of pressure overload (FS, 38.4±1.2%), whereas at week 8, FS decreased to 29.3±3.3%, which was similar to the functional decline evident at week 4 in ACi (Online Figure II). Thus, the major effect of the daily isoproterenol challenge was to accelerate the transition from LV hypertrophy to decompensated HF.

**Inhibition of mNCE Restores [Ca²⁺]ₘ Accumulation and Prevents Energetic Mismatch and Oxidative Stress in Myocytes From ACi Hearts During a Rapid Increase in Work**

Impaired [Ca²⁺]ₘ signaling secondary to [Na⁺]c overload is a major cause of energy/redox imbalance in myocytes from pressure overload–induced failing hearts, and interventions that raise [Ca²⁺]ₘ above a required threshold prevent cellular ROS overload.19,31 Therefore, we first examined whether the same

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-AR</td>
</tr>
<tr>
<td>[Ca²⁺]ₘ</td>
</tr>
<tr>
<td>[Na⁺]c</td>
</tr>
<tr>
<td>AC</td>
</tr>
<tr>
<td>CGP</td>
</tr>
<tr>
<td>FS</td>
</tr>
<tr>
<td>HF</td>
</tr>
<tr>
<td>HR</td>
</tr>
<tr>
<td>mNCE</td>
</tr>
<tr>
<td>PVB</td>
</tr>
<tr>
<td>ROS</td>
</tr>
<tr>
<td>SCD</td>
</tr>
<tr>
<td>SR</td>
</tr>
<tr>
<td>VF</td>
</tr>
<tr>
<td>VT</td>
</tr>
</tbody>
</table>
defect was present in the ACi model and whether acute CGP treatment could mitigate the problem in vitro. [Na\textsuperscript{+}] overload was also observed in the ACi model. [Na\textsuperscript{+}] in cardiomyocytes isolated from ACi heart was increased by 208% compared with that in myocytes from SHAMi hearts (4.8±1.2 mmol/L in SHAMi versus 14.8±1.1 mmol/L in ACi; Online Figure III). [Ca\textsuperscript{2+}] dynamics, monitored with MityCam fluorescence, showed that mitochondria take up Ca\textsuperscript{2+} on a beat-to-beat basis (Figure 1A). On stimulation (0.1 Hz), [Ca\textsuperscript{2+}] increased abruptly followed by decay during diastole. An increase of work from 0.1 to 1 Hz stimulation resulted in a further increase in MityCam signal (1-F/F0; Figure 1A). Measurement of peak MityCam signal indicated that accumulation of [Ca\textsuperscript{2+}]m during increased work was significantly less in myocytes from ACi hearts than SHAMi hearts, whereas 1 μmol/L CGP treatment fully restored [Ca\textsuperscript{2+}]m in ACi myocytes (Figure 1A and 1B).

Isolated cardiomyocytes were challenged with a rapid increase in work from the resting state to 4 Hz stimulation in the presence of 100 nmol/L isoproterenol. NAD(P)H autofluorescence was strongly oxidized during 4 Hz stimulation in ACi cardiomyocytes (Figure 1C). NAD(P)H decreased from 67±4.4% at rest to 33±5.7% at the end of the stimulation period, whereas in SHAMi myocytes subjected to the same protocol, NAD(P)H levels were well maintained (67±5.1% at prestimulation versus 66±4.8% at the end of stimulation). Notably, the oxidation of NAD(P)H in ACi myocytes was prevented by treatment with 1 μmol/L CGP (68±5.5% at prestimulation versus 64±9.1% at the end of stimulation; Figure 1C). CGP had no effect on sarcolemmal Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange current at concentrations ≤10 μmol/L (Online Figure IVA), confirming its specificity for mNCE.

The oxidation of the NAD(P)H pool during increased work in ACi cells was associated with increased oxidative stress. ROS accumulation was monitored with the H\textsubscript{2}O\textsubscript{2}-sensitive fluorescent probe 5-(and -6)-chloromethyl-2\textsuperscript{′},7\textsuperscript{′}-dichlorodihydrofluorescein diacetate (CM-DCF). CM-DCF oxidation rate in SHAMi myocytes was low, with no significant change induced by the increased work (Figure 1D). In contrast,
ROS accumulation in ACi cells was markedly increased on stimulation (Figure 1D). During stimulation, the CM-DCF oxidation rate in ACi cells was ≈10-fold higher than that in SHAMi cells. The enhanced intracellular ROS accumulation rate in ACi cells was abolished by CGP treatment (Figure 1D).

**Effects of Chronic CGP Treatment on ACi-Induced Cardiac Remodeling and Functional Deterioration**

Significant cardiac hypertrophy, pulmonary congestion, and interstitial fibrosis were present in the ACi group by the end of week 4. Heart weight/tibia length was 53% higher in ACi (0.75±0.04 g/cm) versus SHAM (0.49±0.02 g/cm) and lung weight/tibia length was 66% higher in ACi (5.69±0.43 g/cm) versus SHAM (3.41±0.08 g/cm; Figure 2A). Histological analysis showed that interstitial fibrosis in ACi hearts was 12.5-fold higher than in SHAM (Figure 2B). Isoproterenol challenge in the absence of pressure overload did not significantly increase fibrosis, although there was a trend toward more fibrosis in the SHAMi group relative to the SHAM controls (percent fibrotic tissue area was 0.53±0.2 in SHAM versus 1.1±0.5 in SHAMi; P=0.052) after 4 weeks of injections (Figure 2B). CGP treatment protected against HF-associated cardiac remodeling: the ACi plus CGP group developed less cardiac hypertrophy (heart weight/tibia length, 0.64±0.04 g/cm; P<0.05) and pulmonary congestion (lung weight/tibia length, 4.04±0.22 g/cm; P<0.001) at 4 weeks compared with the ACi group (Figure 2A). CGP treatment also attenuated interstitial fibrosis, which was decreased by 50% in ACi plus CGP group compared with that in ACi group (Figure 2B).

Pressure overload with daily β-adrenergic challenge induced LV hypertrophy and progressive cardiac dilation and impaired contractility within 4 weeks of surgery (Figure 2C). Compared with SHAM, FS in the ACi group decreased by 36% (28.9±1.6% in ACi versus 44.9±0.9% in SHAM; P<0.001), and diastolic LV internal dimension increased by 51% (1.07±0.04 cm in ACi versus 0.71±0.01 cm in SHAM;...
Contractile impairment was also confirmed by in vivo hemodynamic studies: \( +\frac{dP}{dt} \) normalized to pressure \( +\frac{dP}{dt_{ip}} \) in the ACi group decreased by 32% compared with that of SHAM controls (73.5±4.0 in SHAM versus 49.9±2.1 in ACi; \( P<0.001 \); Figure 2D). In contrast, animals treated with CGP displayed well-maintained cardiac function without LV dilation. No significant differences in FS, LV internal dimension, or \( +\frac{dP}{dt_{ip}} \) between the SHAM and ACi plus CGP groups were observed. \( \beta \)-Adrenergic treatment alone (SHAMi versus SHAM) had no effect on cardiac hemodynamic parameters.

Effects of \( \beta \)-Adrenergic Challenge, Pressure Overload, and CGP on Heart Rate

Heart rate (HR) responses to the daily isoproterenol injection were recorded in a subset (3–6 guinea pigs) of animals from each experimental group by means of implanted biopotential telemetric transmitters. Baseline and maximal HR, determined by analysis of ECG acquired during and after isoproterenol injections, were compared at 1 and 4 weeks postsurgery (Online Figure V). Baseline HR was unaffected by chronic \( \beta \)-adrenergic treatment in the absence of pressure overload (SHAMi versus SHAM); however, the ACi group had higher baseline HR at weeks 1 and 4 after pressure overload compared with SHAMi controls (Online Figure V). Chronic treatment with CGP (ACi plus CGP), administered continuously at a dose of 0.015 mg/kg per hour by means of an implanted osmotic pump, attenuated but did not completely eliminate the increase of baseline HR at week 1 but abolished it at week 4 (baseline HR at week 4: 218.8±9.0 beats per minute in SHAMi versus 206.2±7.5 beats per minute in ACi plus CGP). After injection of isoproterenol, HR rapidly increased to a maximum of \( \approx390 \) to 400 beats per minute in all groups and then slowly declined back to the baseline rate in 4 to 5 hours (Online Figure V). The peaks and kinetics of the normalized chronotropic response to isoproterenol were not significantly different between the experimental groups.

**Figure 3.** The antiarrhythmic effect of CGP-37157 (CGP). **A** and **B,** Left: Representative telemetric ECG recordings at wk 1 (A) and wk 4 (B) after aortic constriction showing baseline records before isoproterenol (ISO) injection, after ISO injection (post-ISO), and during recovery 4 to 5 h after ISO injection. Black arrows indicate premature ventricular beats (PVBs); white arrows indicate ventricular fibrillation. **Right:** PVB incidence at baseline and after ISO injection in wk 1 and wk 4. *\( P<0.05 \) compared with SHAMi group, †\( P<0.05 \) ACi plus CGP compared with ACi group. **C,** Left: Representative ECG recordings at baseline for each group at wk 1 and wk 4; **right:** summary measurements of QTc at wk 1 and wk 4 showing that long QT was induced by pressure overload. Neither CGP treatment nor ISO challenge had any effect on QTc.
Antiarrhythmic Effects of CGP

Telemetry studies revealed that animals in the ACi HF group had a high incidence of premature ventricular beats (PVBs; Figure 3). PVB frequency was significantly decreased by CGP treatment. To evaluate the antiarrhythmic effects of CGP, PVBs were counted in a 2-hour period at baseline and a 4-hour period after isoproterenol injection at weeks 1 (Figure 3A) and 4 (Figure 3B) after surgery. At baseline, occasional PVBs were detected in all groups, but the incidence of PVB was low in SHAM and SHAMi groups. Moreover, the rates did not change from week 1 to week 4 (week 1, 3.3±2.0 counts per hour; week 4, 4.0±2.5 counts per hour). In contrast, at baseline, the ACi group had a much higher incidence of PVB (23.5±5.3 counts per hour in week 1) and the frequency increased as HF developed (51.3±10.1 counts per hour in week 4). CGP treatment significantly reduced the baseline frequency of PVB as compared with ACi (ACi plus CGP PVB frequency: 9.2±6.3 counts per hour in week 1 and 13.0±3.7 counts per hour in week 4). Isoproterenol increased PVB frequency in all groups. At week 1, PVB frequency in SHAmi after isoproterenol (1 mg/kg) was 104.0±36.2 counts per hour, whereas in ACi, PVB frequency increased to 388.4±35.1 counts per hour after isoproterenol injection (Figure 3A and 3B, right panels). CGP treatment inhibited the arrhythmogenic effect of the β-adrenergic challenge: PVB frequency after isoproterenol injection in the ACi plus CGP group was 219.8±31.2 counts per hour, a decrease of 43% compared with the ACi group. Although, as mentioned above, the HR response to isoproterenol was similar in all of the groups, the increase in PVB frequency after the injection was much less in week 4 than in week 1 (30.6±8.1 counts per hour in SHAMi, 105.9±12.5 counts per hour in ACi, and 32.5±8.0 counts per hour in ACi plus CGP; Figure 3A and 3B, right panels), despite the isoproterenol dose being higher (2 mg/kg). Increased intrinsic sympathetic drive related to postoperative stress in week 1 or β-adrenergic receptor desensitization in week 4 might explain this difference.

The antiarrhythmic effect of CGP was greater in week 4, both at baseline and after isoproterenol injection (Figure 3A and 3B). CGP treatment (comparing ACi plus CGP with ACi) suppressed baseline PVB frequency by 60% in week 1 and 75% in week 4. In the context of acute isoproterenol injection, CGP decreased PVB frequency by 43% in week 1 and 67% in week 4. Analysis of ECG data revealed prolonged QT intervals (QTc is corrected for HR) in both ACi and ACi plus CGP groups (Figure 3C). There was no difference in QTc between ACi and ACi plus CGP groups at either week 1 or week 4, indicating that CGP treatment did not reverse QT prolongation in the HF model (Figure 3C; right panel). QTc was also unaffected by the daily β-adrenergic challenge; there was no difference in QTc interval between SHAM and SHAMi groups.

Prevention of HF-Associated SCD by CGP and Overall Mortality Benefit

The ACi model was associated with a remarkably high incidence of SCD. Here 61.3% of ACi animals died by the end of 4 weeks (Figure 4) without overt HF symptoms (eg, cyanosis, labored breathing, inactivity, piloerection, or cachexia). ECG analysis showed that death was preceded by sustained polymorphic ventricular tachycardia or fibrillation (VT/VF; Figure 3A and 3B, left panels). Interestingly, SCD did not occur during the peak of the HR response after isoproterenol injection, but usually happened several hours after the injection (5/6 SCD events occurred between 2 and 5 hours postinjection, 1 occurred 9 hours postinjection), when the HR had recovered almost to baseline. CGP treatment (ACi plus CGP) significantly reduced the 4-week death rate to just 14.3%, which was not significantly different from that of the SHAMi control (Figure 4). Moreover, all of the deaths in CGP-treated group and in the SHAMi group occurred in the first week after surgery, perhaps attributable to the postoperative stress making the animals more susceptible to arrhythmias. Survival analysis of the ACi group indicated that the death rate was higher in week 1 (22.4%) and week 4 (26.9%) compared with that in week 2 (15.8%) and week 3 (18.8%). Similar to the other groups, the higher death rate in week 1 in ACi may reflect the overall effects of postoperative stress, along with a higher susceptibility to isoproterenol-induced arrhythmias. Considering that the PVB frequency after isoproterenol injection in week 4 is only 27% of that in week 1, the higher death rate (26.9%) in week 4 suggests that PVBs may be more life-threatening because of HF-associated changes in the arrhythmogenic substrate.

Antiarrhythmic Effects of CGP on the Isolated Failing Heart

We next determined whether arrhythmia susceptibility and cardiac function in ACi failing hearts could be rescued by acute treatment with 1 μmol/L CGP in isolated perfused hearts. Langendorff-perfused SHAMi and ACi hearts, isolated at week 4, were challenged with 10 μmol/L isoproterenol for 15 minutes. Cardiac contractility and ECG were analyzed...
before, during, and after isoproterenol challenge. Baseline contractility before isoproterenol was significantly reduced in ACi hearts (Figure 5A); LV diastolic pressure, +dP/dt, and –dP/dt of ACi hearts were only 37%, 38%, and 36%, respectively, of values in SHAMi hearts (Table 1). Administration of isoproterenol increased cardiac contractility by a similar percentage in both ACi and SHAMi groups. However, ACi hearts were more sensitive to the stress of isoproterenol challenge; contractility of ACi hearts was depressed more after isoproterenol washout. In the postisoproterenol state, LV diastolic pressure, +dP/dt, and –dP/dt of ACi hearts were 12%, 16%, and 16% of values in SHAMi hearts (Table 1). Treatment with CGP had no statistically significant effects on cardiac contractility during preisoproterenol or isoproterenol states, but attenuated contractile dysfunction after isoproterenol treatment (Table 1). Treatment with CGP improved LV diastolic pressure, +dP/dt, and –dP/dt recovery in the postisoproterenol state by 205%, 135%, and 120%, as compared with ACi hearts without CGP treatment (Table 1).

HR variability analysis was applied to quantify changes in the ECG in the SHAMi and ACi hearts under different experimental conditions. Treatment with 10 μmol/L isoproterenol did not induce overt arrhythmias during the period of maximum LVPD in either group. There were no differences in RR interval, standard deviation of RR interval, root mean square of successive difference, short-term dispersion, or long-term dispersion between SHAMi, ACi, and ACi plus CGP treatment groups during preisoproterenol and isoproterenol states (Table 2). However, after washout of isoproterenol, HR variability of ACi hearts was dramatically increased compared with that of SHAMi hearts (clusters of points are indicative of ectopic activity in the Poincaré plots in Figure 5B), whereas CGP treatment completely inhibited the arrhythmias in ACi hearts in the postisoproterenol state (Figure 5B; Table 2). There were no differences in RR interval, standard deviation of RR interval, root mean square of successive difference, short-term dispersion, or long-term dispersion between SHAMi and ACi plus CGP–treated hearts (Table 2).

### Table 1. Measurements of Cardiac Contractility in Isolated Perfused Heart

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP (mmHg)</td>
<td>66.7±11.1</td>
<td>89.1±14.0</td>
<td>46.2±12.4</td>
<td>24.5±11.6</td>
<td>33.1±13.6*</td>
<td>5.6±1.1*</td>
<td>41.5±9.1</td>
<td>51.2±3.8*</td>
<td>17.1±2.8*</td>
</tr>
<tr>
<td>+dP/dt (mmHg)</td>
<td>1255±137</td>
<td>3372±218</td>
<td>1114±160</td>
<td>483±198*</td>
<td>988±373*</td>
<td>181±36*</td>
<td>759±122*</td>
<td>1514±86*</td>
<td>425±65*</td>
</tr>
<tr>
<td>–dP/dt (mmHg)</td>
<td>−983±155</td>
<td>−2247±352</td>
<td>−927±354</td>
<td>−355±139*</td>
<td>−730±323*</td>
<td>−151±33</td>
<td>−563±102</td>
<td>−938±101*</td>
<td>−330±48†</td>
</tr>
</tbody>
</table>

ISO indicates isoproterenol; and LVDP, left ventricular diastolic pressure.

*P<0.05 when compared with control.
†P<0.05 when compared with ACi.
circles in function and pathology of HF, whereas isoproterenol
-AR agonist administration.42 In our study, the
-AR signal activation on the heart depends on the dose and
-AR blockade. The impact of selective
-AR to impairment of the inotropic, chronotropic, and lusitropic
-AR is, to our knowledge, one of the few animal models of HF-
-AR) signaling is known
-AR) agonist administration.40,41 In addition to impairment of the inotropic, chronotropic, and lusitropic
effects of β-AR stimulation in HF, chronic hyperactivation of the
receptor results in receptor desensitization, detrimental
effects on cardiac function, and structural remodeling, which
can be mitigated by β-blockade. The impact of selective β-
AR signal activation on the heart depends on the dose and
duration of β-AR agonist administration.42 In our study, the
chronic effects of isoproterenol on cardiac remodeling were
minimized by selecting an isoproterenol dose that had no
independent hypertrophic effects in sham-operated animals.

TABLE 2. Heart Rate Variability Analysis

<table>
<thead>
<tr>
<th></th>
<th>SHAMI</th>
<th>ACi</th>
<th>ACi Plus CGP-37157</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time domain results</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean RR, ms</td>
<td>260±18</td>
<td>336±25</td>
<td>307±36</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>3.85±1.39</td>
<td>42.08±8.51</td>
<td>2.76±1.69†</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>0.88±0.29</td>
<td>65.60±15.60*</td>
<td>1.14±0.59†</td>
</tr>
<tr>
<td><strong>Nonlinear results</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD1, ms</td>
<td>0.626±0.207</td>
<td>46.418±11.048*</td>
<td>0.804±0.415†</td>
</tr>
<tr>
<td>SD2, ms</td>
<td>5.934±1.968</td>
<td>36.542±6.167*</td>
<td>3.812±2.347†</td>
</tr>
</tbody>
</table>

RMSSD indicates root mean square of the successive differences; SD1, short-term dispersion; SD2, long-term dispersion; and SDNN, standard deviation of normal RR interval.

*P<0.05 when compared with control.
†P<0.05 when compared with ACi.

**Discussion**

The major findings of the present study were that: (1) the transition from compensated pressure overload hypertrophy to HF is exacerbated by a daily β-adrenergic challenge; (2) SCD is a major contributor to mortality in the ACi HF model; (3) the mNCE inhibitor, CGP, prevents cardiac contractile decompensation, blunts hypertrophic remodeling, decreases arrhythmia incidence, and prevents SCD associated with HF.

The present study was motivated by our earlier findings that [Na+] overload, by increasing the driving force for mNCE-mediated Ca2+ extrusion from the mitochondrial matrix, results in insufficient [Ca2+]i accumulation during increased work.17,19,30 If the Ca2+ signal to the mitochondria falls below a critical threshold level,19 Ca2+-dependent activation of Krebs cycle enzymes is disrupted,29 causing the mitochondrial pyridine nucleotide redox potential (NAD(P)H/NAD(P)+) to become more oxidized. The consequent decrease in reducing equivalents driving oxidative phosphorylation (NADH) and the antioxidant pathways (NADPH) causes both an impairment of energy supply and ROS overload (Figure 1C and 1D). This pathological mechanism was demonstrated in normal myocytes overloaded with [Na+]i,17 myocytes treated with the Na+/K+ inhibitor ouabain,30 and in myocytes isolated from failing (AC model) guinea pig hearts.19 In the present study, we demonstrated that the net oxidation of NAD(P)H pool and increased oxidative stress during rapid pacing (4 Hz) in the presence of isoproterenol is also present in the ACi model. The increase in NAD(P)H oxidation, ROS accumulation, and arrhythmias associated with [Na+]i overload can be prevented by either lowering [Na+]i19 or by inhibition of mNCE with CGP19,30,31 (Figure 1C and 1D).

The present findings are the first to demonstrate that chronic treatment with CGP prevents the progressive decline in contractile function and SCD in vivo in an animal model of HF. The ACi model was developed to incorporate both hemodynamic stress (LV pressure overload) and chronic sympathetic hyperstimulation. 2 factors that are known to contribute to HF and SCD in humans.32,33 Compared with pressure overload alone, the ACi model accelerated the decline in function and pathology of HF, whereas isoproterenol treatment in the absence of pressure overload (SHAMI) had no significant effects on cardiac function and remodeling in control animals.

A unique feature of the ACi model is the high incidence of SCD (>60% at 4 weeks), which is manifested as an increased death rate even in the first week post-AC, when function is largely preserved, and continues during the course of HF progression. Notably, the ACi model shows QTc prolongation even after only 1 week of pressure overload, before overt contractile dysfunction, as well as a large increase in spontaneous PVBs. The PVB rate and the incidence of VT/VF were both suppressed by CGP treatment; however, the QTc prolongation was not reversed, suggesting that the alterations in sarcolemmal ionic currents during hypertrophy, by themselves, are not sufficient to account for the increased automaticity and tendency toward re-entry underlying SCD. Presumably, the combined effects of ion channel remodeling, oxidative stress, and the change in the myocardial substrate were all involved in setting the stage for VT/VF. In this light, it is still not clear whether long QTc is a useful predictor of SCD in human HF.

Breidthardt et al34 reported prolonged QTc interval (≥440 ms) in 72% of patients with acute destabilized HF, but 720-day all-cause mortality was no different in patients with prolonged versus normal QTc intervals. Patients with prolonged QRS interval, however, had a significant 2-fold increase in mortality. Similarly, QTc was determined to have no independent prognostic value on 1-year mortality in a subset of patients with congestive HF in a recent clinical trial.35 In general, though, the overall risk of malignant arrhythmias increases with the magnitude of QTc prolongation,36 and more refined analyses, such as QTc variability, may have predictive value for SCD,37 perhaps as a better indicator of QT dispersion.38

The present results suggest that prolonged QTc may provide the substrate for potentially fatal arrhythmias, but that a secondary event—we propose an oxidative stress event secondary to an energy/redox supply and demand mismatch—would be required to initiate SCD. The nonlinear nature of the secondary event could, therefore, account for the unpredictability of SCD.

Apart from genetically engineered animals,39 the ACi model is, to our knowledge, one of the few animal models of HF-associated acquired long QT that displays a high incidence of spontaneous SCD. The accelerated transition between the compensated and decompensated states should be useful for investigating the mechanisms behind progressive systolic and diastolic dysfunction, as well as vulnerability to arrhythmias, in future studies.

Altered β-adrenergic receptor (β-AR) signaling is known to be a key player in the development of HF.40,41 In addition to impairment of the inotropic, chronotropic, and lusitropic effects of β-AR stimulation in HF, chronic hyperactivation of the receptor results in receptor desensitization, detrimental effects on cardiac function, and structural remodeling, which can be mitigated by β-blockade. The impact of selective β-AR signal activation on the heart depends on the dose and duration of β-AR agonist administration.42 In our study, the chronic effects of isoproterenol on cardiac remodeling were minimized by selecting an isoproterenol dose that had no independent hypertrophic effects in sham-operated animals.
and by administrating isoproterenol with repeated injections that induced only transient activation of β-AR signaling. The β-AR challenge had detrimental functional effects (arrhythmias and impaired postisoproterenol contractility) on LV pressure–overloaded animals, but not on controls, suggesting that the pressure-overloaded heart is more vulnerable to β-AR activation. This is consistent with other animal studies. For example, in spontaneously hypertensive rats with LV hypertension, infusion of isoproterenol initiated a transition from compensated hypertrophy to HF, whereas the same infusion had no significant effects on cardiac function and remodeling in control rats.43 Genetically modified mouse lines that have constitutively increased β-AR drive with normal cardiac function and structure are also more susceptible to pressure overload.44,45 Our finding that CGP treatment protects pressure-overloaded hearts (both in vivo and ex vivo) from the detrimental effects of β-AR challenge supports the hypothesis that blunted [Ca\(^{2+}\)]\(_c\) accumulation leading to oxidative stress is a key event underlying increased vulnerability of the failing heart to β-AR–mediated cardiac dysfunction.

In addition to exacerbating contractile dysfunction, β-AR challenge also predisposed the pressure-overloaded heart to VT/VF, and CGP protected ACi animals from both high PVB rate and SCD. Increased sympathetic activity during physical or emotional stress is known to provoke VT/VF in catecholaminergic polymorphic VT, and elevated diastolic Ca\(^{2+}\) and hyperactive ryanodine receptors are thought to be critical factors leading to SCD in animal models of catecholaminergic polymorphic VT.46,47 β-AR stimulation can contribute to arrhythmias by increasing Ca\(^{2+}\) current and SR Ca\(^{2+}\) ATPase activity, both of which increase SR Ca\(^{2+}\) load to increase vulnerability to spontaneous Ca\(^{2+}\) release. In HF, although the Ca\(^{2+}\) transient amplitude and SR load are decreased, the prolonged duration of the action potential and Ca\(^{2+}\) transient, together with increased Ca\(^{2+}\) leak via the ryanodine receptors, could favor spontaneous afterdepolarizations. For example, Desantiago et al48 demonstrated that increased SR Ca\(^{2+}\) by β2-AR activation results in enhanced arrhythmogenesis in myocytes from human and rabbit failing hearts. Interestingly, in the ACi model, the proarrhythmic effect of β-AR challenge was not evident during the peak of the response, but was revealed only after recovery from the increased work. We propose that CGP is protecting against damage induced by mitochondrial ROS accumulation by preserving the pyridine nucleotide/antioxidant capacity during β-AR activation. Among the potential ROS targets, the local oxidation of the ryanodine receptors by mitochondrial ROS49 could underlie spontaneous Ca\(^{2+}\) release and ectopic activity. Ryanodine receptor channels have multiple cysteine residues that sense the local redox state, and their oxidation is responsible for increased spontaneous SR Ca\(^{2+}\) leak in HF, which can be normalized by dithiothreitol.15 In this light, we have previously demonstrated that CGP, by increasing the [Ca\(^{2+}\)]\(_m\) response and preserving NAD(P)H redox potential during increased work, can prevent delayed afterdepolarizations triggered by ouabain-induced Na\(^{+}\) overload in myocytes, hearts, and intact animals.30 This mechanism is the likely explanation for the CGP-mediated protection against postisoproterenol ectopic activity observed in the isolated perfused ACi hearts (Figure 5).

In addition to ROS-driven dysfunction, impaired mitochondrial energetics in HF also contribute to cardiac decompensation.50 For example, limitations of ATP delivery could impair diastolic function by affecting the Ca\(^{2+}\) removal capacity via SR Ca\(^{2+}\) ATPase when energy demand increases during β-AR stimulation. This has been observed in studies of energetically deficient animal models. In the creatine kinase–deficient mouse, the reduced energy reserve leads to a decreased capacity of SR Ca\(^{2+}\) ATPase to remove cytosolic Ca\(^{2+}\).51,52 A peroxisome proliferator–activated receptor γ coactivator 1β–knockout mouse also displays reduced respiration rate and ATP production53; these animals showed increased susceptibility to catecholamine-induced arrhythmias.54 Cardiomyocytes from these mice have normal Ca\(^{2+}\) transients at rest, but elevated [Ca\(^{2+}\)]\(_c\) and spontaneous diastolic Ca\(^{2+}\) transients were induced after isoproterenol administration.55 By improving energy supply, CGP treatment could thus help to maintain the activity of SR Ca\(^{2+}\) ATPase or other energy-dependent processes during increased work to preserve systolic and diastolic function.

The present findings highlight the central role of oxidative stress in both the functional deterioration and arrhythmias associated with HF progression. In addition to direct damage to the myocyte, mounting evidence indicates that ROS modulate various signaling pathways involved in hypertrophic growth, apoptotic and necrotic cell death, and proliferation of cardiac fibroblasts.11,55,56 ROS are also downstream mediators of neurohumoral stimuli implicated in HF, such as angiotensin II, endothelin 1, and tumor necrosis factor α. Increased ROS can also directly activate signaling cascades including tyrosine kinases, mitogen-activated protein kinases, protein kinase C, and phosphoinositide 3-kinase,57 which are potent regulators of cardiac growth. The effects of CGP on these downstream effectors of ROS signaling remain to be determined, but could also contribute to the observed protection. Nevertheless, given that the hypertrophic response was only partially decreased by CGP treatment, the major beneficial effect of CGP may be to prevent the redox modification of proteins (eg, ion channels, myofilament proteins, and mitochondrial enzymes) that lead to contractile dysfunction and deficient energetics. Recent studies confirm the pre-eminent role of mitochondrial ROS in HF; for example, a mitochondrially targeted antioxidant peptide (SS-31) protects animals from angiotensin II–induced cardiac hypertrophy and fibrosis,14 and overexpression of mitochondrial, but not cytosolic, catalase prevents changes in the proteome of mice subjected to pressure overload–induced HF.15 CGP treatment intervenes in the mechanism leading to the mitochondrial ROS overload while preserving energy supply and demand balance, which may have advantages compared with strategies that simply attempt to increase the ROS scavenger capacity.

In summary, CGP preserves cardiac function, attenuates remodeling and fibrosis, and prevents SCD in the guinea pig HF and SCD model produced by pressure overload combined with β-AR stimulation. The beneficial effects of CGP can be attributed to the effects of restored [Ca\(^{2+}\)]\(_m\) dynamics by CGP on mitochondrial energetics and redox balance. Additional
studies will be needed to characterize all of the HF-associated modifications affected by CGP treatment, including cellular redox status, ROS balance, the electrophysiology of the heart, excitation–contraction coupling, energy metabolism, and post-translational protein modifications. More work will also be required to determine whether CGP treatment can reverse established LV hypertrophic remodeling to prevent decompensation and SCD in this model. In addition, given the known variations in [Na\(^+\)] in different species, the efficacy of CGP may be different in other models of HF and in humans. Nevertheless, the findings support the hypothesis that [Ca\(^{2+}\)\(_{\text{m}}\)] dynamics plays a critical role in the development of HF and HF-associated SCD and implicate mNCE as a novel therapeutic target for HF.

**Sources of Funding**

This work was supported by National Institutes of Health grants R01HL101235, R01HL105216, and P01HL077180 and the Fondation Leducq Transatlantic Networks of Excellence Program.

**Disclosures**

None.

**References**

What Is Known?

- Sudden cardiac death (SCD) accounts for ≈50% of deaths in patients with heart failure and, in many cases, can be attributed to paroxysmal ventricular arrhythmias.
- The mechanisms underlying SCD are unknown, but ion channel remodeling, action potential prolongation, and abnormal cytoplasmic and mitochondrial Ca2+ handling have been implicated.
- Energy metabolism is also known to be altered in heart failure, but the links between impaired energy supply, oxidative stress, and contractile and electric dysfunction are incompletely understood.

What New Information Does This Article Contribute?

- A novel guinea pig model of heart failure/SCD is described, combining pressure overload and daily catecholamine challenge, which displays impaired contractility, long QT, and a high incidence of SCD over a 4- to 8-week time span.
- Treatment with an inhibitor of the mitochondrial Na+/Ca2+ exchanger prevents contractile decompensation and SCD in vivo but does not prevent QT prolongation.
- Mitochondrial Na+/Ca2+ exchanger inhibition prevents NAD(P)H oxidation and reactive oxygen species overload during increased work in myocytes from failing hearts and decreases the incidence of arrhythmias after a catecholamine challenge in perfused failing hearts.
- Impaired mitochondrial Ca2+ balance, and the consequent impairment of NAD(P)H availability, plays a key role in heart failure progression and vulnerability to fatal arrhythmias by compromising energy supply and antioxidant capacity in the failing heart.

SCD is a major cause of death in patients with heart failure, but the mechanisms underlying this increased vulnerability are poorly understood. In cardiomyocytes from failing hearts, it was previously shown that insufficient mitochondrial Ca2+ accumulation, secondary to cytoplasmic Na+ overload, decreases NAD(P)H/NAD(P)+ redox potential, increases oxidative stress, and disrupts Ca2+ handling when workload increases. Here, we show that chronic in vivo treatment with an inhibitor of the mitochondrial Na+/Ca2+ exchanger (CGP-37157) prevents the progression from compensated hypertrophy to heart failure and eliminates the high incidence of spontaneous SCD. The findings highlight the critical role played by mitochondrial Ca2+ dynamics in maintaining redox and energy balance in the failing heart and support the feasibility of targeting mitochondria for therapeutic intervention in heart failure.
Inhibiting Mitochondrial Na\(^+\)/Ca\(^{2+}\) Exchange Prevents Sudden Death in a Guinea Pig Model of Heart Failure

Ting Liu, Eiki Takimoto, Veronica L. Dimaano, Deeptankar DeMazumder, Sarah Kettlewell, Godfrey Smith, Agnieszka Sidor, Theodore P. Abraham and Brian O’Rourke

Circ Res. 2014;115:44-54; originally published online April 29, 2014; doi: 10.1161/CIRCRESAHA.115.303062

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/115/1/44

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2014/04/29/CIRCRESAHA.115.303062.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
Supplemental Materials

Methods

Animal model: Hartley guinea pigs (~300 g; HillTop Lab Animals) were housed in an animal facility at the Johns Hopkins University. This study conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Johns Hopkins Animal Care and Use Committee.

Male animals were anesthetized with 4% isoflurane in a closed box for 4min, and then intubated. Animals were ventilated with oxygen and 2% isoflurane. Ascending aortic constriction (AC) was produced by tying a suture around the ascending aorta using an 18-gauge needle as a spacer, which was then removed. Sham-operation was performed following the same procedure without tying the suture. When animals were breathing spontaneously after the procedure, bupronex (0.05mg/kg) was administrated via intramuscular injection for analgesia and animals were observed until full recovery. Isoproterenol was administrated daily by i.p. injection at 1 mg/kg for the first week after surgery and at 2 mg/kg for another 3 weeks. In the group with CGP treatment, CGP was introduced by i.p. injection (~40mg/kg) followed by implantation of an osmotic pump in the abdominal cavity (delivery rate: 0.015 mg/kg/hr) for extended delivery of the compound.

Echocardiography: Transthoracic echocardiography was performed in guinea pigs at 4 weeks after surgery. Following sedation with isoflurane inhalation (1 L/min, 1% to effect), the anterior chest area was shaved and the guinea pig was placed on a thermal pad in supine position with its limbs restrained. Electrocardiogram leads were fastened to the two front limbs and the left rear limb. Temperature was monitored via a rectal probe maintained at 37°C using a warming pad and a heating lamp. Heart rate was monitored, and echocardiography was performed using a 5-12 MHz ultraband transducer interfaced with Vivid 7 System only when heart rate was above 220 BPM. Two-dimensionally directed M-mode images were obtained from the long axis views. Echocardiographic measurements were made on 3 consecutive cardiac cycles by the leading edge-to-leading edge method. LV end-diastolic and end-
systolic dimensions, and LV end-diastolic posterior wall thickness, were measured from the M-mode images, and left ventricular fractional shortening was calculated with the software (VisualSonics V1.3.8).

**Cardiac myocyte isolation:** Cardiac myocytes were isolated from SHAMI and ACi guinea pig hearts by enzymatic digestion as described previously.

**Fluorescence recordings of mitochondrial Ca\(^{2+}\) dynamics, NADH, and ROS in isolated myocytes:** Myocytes were loaded into a heated field-stimulation chamber (37°C) on the stage of a fluorescence microscope (Nikon Eclipse TE300) and superfused with Tyrode's solution containing (in mmol/L): NaCl 130, KCl 5, MgCl\(_2\) 1, Na-HEPES 10, CaCl\(_2\) 2, glucose 10, pyruvate 2, ascorbic acid 0.3; pH 7.4. \([Ca^{2+}]_m\) was measured with a genetically-encoded, mitochondrially-targeted Ca\(^{2+}\) indicator, MityCam\(^2\). Adenovirus-mediated MityCam gene transduction was performed in vivo by intramuscular injection of 100µl adenovirus suspension (1.5X10\(^{10}\) virus particles) into the LV free wall of ACi or SHAMI guinea pigs at week 3 after aortic constriction surgery. Cardiomyocytes were then isolated at 5-7 days later. In the presence of 100nM isoproterenol, cells with MityCam expression were imaged every 25ms at resting state followed by 0.1 and 1Hz stimulation, respectively, and then returned to resting state. MityCam fluorescence was measured with Image J (NIH) and expressed as 1-F/F0 (F0 was the baseline fluorescence prior to stimulation). The level of \([Ca^{2+}]_m\), accumulation was taken as the peak 1-F/F0 of MityCam fluorescence during 1hz stimulation. The endogenous autofluorescence of NAD(P)H was recorded with 360nm excitation and emission at 450nm. NAD(P)H redox potential was expressed as percent reduction of the NAD(P)H/NAD(P)+ pool, calibrated by applying the mitochondrial uncoupler FCCP (5 µM; 0% reduced) and the cytochrome oxidase inhibitor NaCN (4 mM; 100% reduced) at the end of each recording. To monitor ROS production, myocytes were loaded with 2µM CM-DCFDA, CM-DCF fluorescence was excited 485nm, and its emission was measured at 525nm. In the presence of 100nM isoproterenol, fluorescence was recorded for 100s in the resting state and 100s at 4 Hz stimulation followed by another 100s recording in the resting state. For CGP-37157 treatment, cells were perfused with 1 µM CGP-37157 for 5 min before recording.

**In vivo hemodynamic study:** Guinea pigs were anesthetized and ventilated as described above. The external jugular vein was cannulated with 30-gauge needle for volume administration. Fluid
supplementation (25% human albumin in saline) was provided at 100 µl/min. The apex portion of the heart was exposed via a substernal-transverse incision. The pericardium was opened at the apex, and an apical stab will be made with a 25-gauge needle to place a 1.4-F, four-electrode pressure-volume catheter (modelSPR-719, Millar Instruments) along the long axis. The pressure-volume catheter was connected to a custom-designed conductance system producing a constant current of 30 µA at a frequency of 2 or 20 kHz. Correct catheter positioning was confirmed by on-line visualization of the pressure-volume loops and placement of the distal electrode within the chamber. After a short stabilization period, left ventricular (LV) pressure–volume loops were recorded at baseline for 5 s with the ventilator stopped (typically 20-30 cardiac cycles), and then the pressure-volume relationship was measured by transiently occluding the inferior vena cava. At the end of each experiment, a bolus of hypertonic saline was rapidly injected into jugular vein to determine the parallel conductance volume coefficient (Vp) and absolute volume, and aortic flow was measured by insertion of an ultrasonic perivascular flow probe connected to a flow meter (probe: model 1RB, flow meter: T106, Transonics, Ithaca, NY) around the abdominal aorta, which was used to determine stroke volume. Data were collected and analyzed using custom-developed software.

**Histological study:** Hearts were excised and rapidly immersed into ice-cold saline solution. The aorta was then cannulated with a 16G needle, retrogradely perfused with 4% phosphate-buffered paraformaldehyde, and immersion-fixed overnight in the same fixative. Following fixation, the specimens was embedded in paraffin and 5 µm sections were cut perpendicular to the long axis of the heart. 6 sections were collected from the mid-ventricular region of each heart, and stained with Masson’s Trichrome to assess the interstitial fibrosis. Interstitial collagen fraction was determined using computer-assisted image analysis (NIH Image J).

**Electrocardiogram analysis:** ECG data were collected with a telemetry system (DSI, St. Paul MN). An ECG transmitter (EA-F20) was implanted in the abdominal cavity of the animal and the leads were secured in a lead II placement at the end of AC surgery. ECG signals were collected and transmitted via radio-frequency signals to a receiver that was connected to a computer, and the data was exported and analyzed with the Dataquest OpenART software system.
**Hemodynamics and ECG study in Langendorff-perfused hearts:** The ex vivo study was performed 4 weeks after surgery. Recordings of hemodynamics and ECGs were made in Langendorff-perfused ACi or SHAMi hearts as previously described. After a 10 min equilibration period, hearts were subjected to the following protocol: 10 minute baseline recording while the heart was perfused with control buffer, application of 10 μM isoproterenol and 15 minutes recording, washout with control buffer with recording for another 20 minutes. In the CGP treated group, 1 μM CGP was added to the buffer at the beginning of baseline recording. The measurements of hemodynamic parameters were determined by taking the average during the last 5 minutes before isoproterenol application (pre-ISO), for 3 minutes during the maximal effect on LV developed pressure (LVDP) of isoproterenol (ISO), and for 5 minutes at 15 minutes after isoproterenol washout (post-ISO). Simultaneously recorded ECG data during the same time periods were used for blinded HRV analysis.

**Statistical Analysis:** Data are expressed as mean ± SEM. Comparisons between 2 groups were performed with unpaired t-test. Survival rates were analyzed with Kaplan-Meier method. Sample sizes are provided in the Figures.

**Methods used for supplemental experiments:**

**Effects of CGP-37157 on NCX current (I_{NCX}) and action potential (AP)** – Myocytes were loaded in a heated chamber (37°C) on the stage of a fluorescence microscope (Nikon Eclipse TE300) and were whole-cell patch-clamped with 2-4 MΩ pipettes. For $I_{NCX}$ measurement, cells were superfused with Cs-Tyrode’s solution, containing (in mM): NaCl 130, CsCl 5, MgCl₂ 1, Na-HEPES 10, CaCl₂ 2, glucose 10; pH 7.4. To block L-type Ca²⁺ channels and Na⁺-K⁺-ATPase, 10μM nitrendipine and strophanthidin, respectively, were applied in Cs-Tyrode’s solution. Cells were voltage-clamped and equilibrated with a pipette solution containing (in mM) CsCl 120, MgCl₂ 0.5, Na-HEPES 20, Mg-ATP 5, BAPTA 5, and CaCl₂ 3; pH 7.25. The presence of 5mM BAPTA and 3mM CaCl₂ in pipette solution buffered the intracellular Ca²⁺ to 200nM. After achievement of whole-cell conformation, cells were voltage-clamped at a holding potential of -
40mV. $I_{\text{NCX}}$ was recorded with families of pulses applied from +80 to -80 mV in 20 mV steps for 300 ms at 0.5 Hz. $I_{\text{NCX}}$ was defined as the current blocked by 5 mM NiCl$_2$ (Ni-sensitive current). To record AP, cells were superfused with normal Tyrode’s solution and were current-clamped with a pipette solution containing (in mM): K-glutamate 120, KCl 10, HEPES 10, EGTA 5, MgATP 5, and pH7.2. Cells were paced at 0.5hz. 1 and 5µM CGP was applied after steady-state AP was achieved. AP duration was measured with custom software.

**Measurement of intracellular Na$^+$**—Intracellular Na$^+$ was measured ratiometrically using SBFI as described previously. 4

**References:**


**ONLINE FIGURES:**
Online Figure I. Interactions between Ca\textsuperscript{2+} handling (green), mitochondrial oxidative phosphorylation (OxPhos; orange), and the antioxidant system (AOS; blue). During excitation-contraction coupling, L-type Ca\textsuperscript{2+} channels (L) trigger SR Ca\textsuperscript{2+} release channels (RyR) and activation of myofilament myosin ATPase activity. Increases in work (ATP demand) are associated with increases in mitochondrial Ca\textsuperscript{2+} uptake via the calcium uniporter MCU, leading to activation of NADH production by the tricarboxylic acid (TCA) cycle to match a higher NADH oxidation rate by the electron transport chain (complexes I, II, III, IV, V). NADPH in the mitochondrial matrix, derived from reactions dependent on TCA cycle intermediates and a transhydrogenase (THD), donates electrons to support the moiety-conserved glutathione, thioredoxin, and glutaredoxin antioxidant pathways. Increased cytoplasmic Na\textsuperscript{+} and blunted Ca\textsuperscript{2+} release in HF results in enhanced efflux through the mitochondrial Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (mNCE), the target of CGP-37157, resulting in insufficient NAD(P)H production to maintain both ATP production and reactive oxygen species (ROS) scavenging. Mitochondrial ROS emission increases to affect multiple redox-sensitive targets in the cell, leading to arrhythmias, Ca\textsuperscript{2+} and contraction dysfunction, and cell death. NCX: sarcolemmal Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, SERCA: SR/ER Ca\textsuperscript{2+} ATPase, GSH: Reduced glutathione, GSSG: oxidized glutathione, GR: glutathione reductase, GPX: glutathione peroxidase, TrxSS: oxidized thioredoxin, Trx-SH: reduced thioredoxin, TrxR: thioredoxin reductase, Prx: peroxiredoxin, SOD: superoxide dismutase.
Online Figure II. A daily β-adrenergic challenge exacerbates functional deterioration in a pressure-overload model of heart failure. Cardiac fractional shortening (FS) measured with echocardiography at week 0, 2, 4, 6, and 8 after ascending aortic-constriction in guinea pigs with (ACi) or without (AC) daily isoproterenol injection. In ACi group, 4 of 6 animals died following overt HF symptoms before echocardiographic imaging at week 8 and the FS of these animals was counted as 0%. *, p<0.01 as compared to week 0 of the same group.
Online Figure III

Online Figure III. Measurement of cytoplasmic Na⁺([Na⁺]ₐ). A) representative SBFI ratio images (340/380nm exc.) of a cardiomyocyte when cell was intact (baseline) and when [Na⁺]ₐ was clamped to 0, 70, and 140mM in the presence of 7.5µM gramicidin D. B) Average [Na⁺]ₐ measured in myocytes isolated from SHAMi (n=4) and ACi (n=17) hearts, *, p<0.001 as compared to SHAMi.
Online Figure IV. Effect of CGP on $I_{\text{NCX}}$, APD, and QTc. A) To determine if CGP-37157 inhibits sarcolemmal NCX, $I_{\text{NCX}}$ was recorded with perfusate containing 0, 1, 5, or 10 µM CGP. Representative recording of sarcolemmal NCX activity. CGP of various concentrations and NiCl$_2$ (Ni) were added to perfusate as indicated. B) Measurements of Ni-sensitive $I_{\text{NCX}}$ at +80mV in the presence of 0, 1, 5, and 10 µM CGP (n=4). C) Time course of APD$_{50}$ and APD$_{90}$ shows that application of CGP 1 and 5µM has no effect on APD (n=3). D) The acute effect of CGP-37157 on QT intervals was examined in Langendorff-perfused hearts. There was no difference in the average QTc of a 3-min period before and after 1µM CGP administration (n=3).
Online Figure V. The chronotropic effect of β-adrenergic challenge. Heart rates (HR) of animals in SHAM (n=3), SHAMi (n=4), ACi (n=6), and ACi+CGP (n=4) groups were analyzed at week 1 (A) and week 4 (B) post-banding. Upper panels are representative HR recordings (normalized to baseline) for 12 hours showing the baseline before isoproterenol (iso) injection, the increase of HR in response to iso, and recovery from iso effect. Arrow indicates iso injection. Lower panels show average baseline HR and maximum HR following iso injection. *, p<0.05 compared to SHAMi group; †, p<0.05 ACi+CGP compared to ACi group.