Diabetes mellitus is a widespread chronic disease affecting ≥285 million adults worldwide, projected to reach 439 million by 2030. Cardiovascular disease is among the most common causes of mortality in diabetic patients. Furthermore, diabetes mellitus increases heart failure (HF) risk in humans 2.5-fold, even when adjusted for comorbidities such as concomitant coronary artery disease, obesity, dyslipidemia, or age.2–4 Diabetic cardiomyopathy is characterized by early onset of left ventricular (LV) diastolic dysfunction, often preceding systolic dysfunction,2–4 accompanied by adverse structural changes such as LV hypertrophy, increased cardiac fibrosis and cardiomyocyte apoptosis. These characteristics are a prognostic indicator of mortality in diabetic patients,4 hence development of new pharmacological targets to treat LV dysfunction and remodeling in the diabetic heart is paramount.

Background—Nitroxyl (HNO), a redox congener of nitric oxide (NO•), is a novel regulator of cardiovascular function, combining concomitant positive inotropic, lusitropic, and vasodilator properties. Moreover, HNO exhibits myocardial antihypertrophic and superoxide-suppressing actions. Despite these favorable actions, the impact of chronic HNO administration has yet to be reported in the context of cardiomyopathy. Diabetic cardiomyopathy is characterized by early diastolic dysfunction and adverse left ventricular (LV) structural remodeling, with LV superoxide generation playing a major causal role. We tested the hypothesis that the HNO donor 1-nitrosocyclohexylacetate (1-NCA) limits cardiomyocyte hypertrophy and LV diastolic dysfunction in a mouse model of diabetes mellitus in vivo.

Methods and Results—Diabetes mellitus was induced in male FVB/N mice using streptozotocin. After 4 weeks, diabetic and nondiabetic mice were allocated to 1-NCA therapy (83 mg/kg per day IP) or vehicle and followed up for a further 4 weeks. Diabetes mellitus–induced LV diastolic dysfunction was evident on echocardiography-derived E and A wave velocities, E:A ratio, deceleration, and isovolumic relaxation times; LV systolic function was preserved. Increased LV cardiomyocyte size, hypertrophic and profibrotic gene expression, and upregulation of LV superoxide were also evident. These characteristics of diabetic cardiomyopathy were largely prevented by 1-NCA treatment. Selectivity of 1-NCA as an HNO donor was demonstrated by sensitivity of acute 1-NCA to l-cysteine but not to hydroxocobalamin in the normal rat heart ex vivo.

Conclusions—Our studies provide the first evidence that HNO donors may represent a promising strategy for treatment of diabetic cardiomyopathy and implies therapeutic efficacy in settings of chronic heart failure. (Circ Heart Fail. 2015;8:572-581. DOI: 10.1161/CIRCHEARTFAILURE.114.001699.)

Key Words: cardiomyopathies • diabetes mellitus • diastolic heart failure • left ventricular hypertrophy • nitrates
sGC–mediated vasodilator actions. Moreover, HNO exhibits antihypertrophic and superoxide-suppressing actions in the myocardium, but these have yet to be extended to an in vivo setting. HNO donors may thus offer favorable actions in a range of cardiac pathologies. Indeed, the recent reports that a pure HNO donor CXL-1020 reproduces these acute inotropic and lusitropic benefits in failing canine and human myocardium highlight the exciting therapeutic potential offered by HNO. CXL-1020 is superior to classical donors because it only releases HNO (without generation of other by-products) and does not require an alkaline vehicle.

The chronic effects of any HNO donor, either CXL-1020 or older donors (Angeli salt, IPA-NO), have never been reported in any cause, likely because of short-acting effects in vivo. Moreover, efficacy of HNO in the distinctive context of diabetic cardiomyopathy also remains unresolved. Given the diabetic heart is characterized by cardiac remodeling and diastolic dysfunction, downstream of LV superoxide upregulation, we hypothesized that chronic administration of an HNO donor limits cardiomyocyte hypertrophy and LV diastolic dysfunction in a mouse model of diabetes mellitus in vivo. An alternative putative HNO donor, 1-NCA, has been described, reportedly releasing HNO at physiological pH. Vasodilation and cardiomyocyte contractility are enhanced by 1-NCA in vitro; both HNO and NO- may, however, contribute to its actions. Although the half-life of 1-NCA has only been reported in cell-free settings, this has been reported as ≈1–1.5 hours in a methanol/neutral buffer mixture considerably longer than that offered by CXL-1020 or conventional HNO donors. On this basis, we selected 1-NCA for this in vivo study.

Methods

Animal Models

Studies were conducted in accordance with National Health and Medical Research Council of Australia guidelines, and approved by Alfred Medical Research and Education Precinct Animal Ethics Committee. The majority of studies used male FVB/N mice in vivo (Alfred Medical Research and Education Precinct Animal Services, Melbourne, Australia). At 6 to 7 weeks old, mice received 5 consecutive daily injections of citrate (0.1 mol/L IP; pH 4.5) or streptozotocin (55 mg/kg, in 0.1 mol/L citrate). Diabetes mellitus was confirmed by forcibly tail blood glucose from saphenous vein (ACCU-CHEK Advantage; Roche, Basel, Switzerland). In mice, blood glucose ≥26 mmol/L was considered diabetic and <12 mmol/L nondiabetic; mice with blood glucose 12 to 25.9 mmol/L were excluded. Upper limit of detection for blood glucose readings was 33.3 mmol/L; HI readings were recorded as 33.3 mmol/L. After 4 weeks, 1-NCA (83 mg/kg per day; EpiChem Pty Ltd, Murdoch, WA) or equivalent vehicle (55 mg/kg, in 0.1 mol/L citrate) was administered intraperitoneally to nondiabetic and diabetic mice for 4 weeks. After total 8-week diabetic mellitus, LV function was determined, before tissue collection.27–29 Mice were anesthetized (ketamine/xylazine/atropine, 60/6/0.6 mg/kg IP, respectively) at study end point, and echocardiography performed with blood glucose 12 to 25.9 mmol/L were excluded. Upper limit of detection for blood glucose readings was 33.3 mmol/L; HI readings were recorded as 33.3 mmol/L. After 4 weeks, 1-NCA (83 mg/kg per day; EpiChem Pty Ltd, Murdoch, WA) or equivalent vehicle (0.25% methanol, 200 μL/day) were administered intraperitoneally to nondiabetic and diabetic mice for 4 weeks. After total 8-week diabetes mellitus, LV function was determined, before tissue collection.27–29

Tissue Collection and Histology

Cardiac puncture was performed in anesthetized mice for heparginized blood collection. Heart and lungs were excised, and wet weights were recorded. Tibia bones were removed to measure length. A small LV portion was used for lucigenin chemiluminescence in fresh tissue, with remaining tissue cut into 3 at the horizontal short-axis plane. These were allocated to a cryomold (Sakura Finetek, Torrance, CA) containing Tissue-Tek OCT (Grale Scientific, Ringwood, Australia) on dry ice (with subsequent storage at −80°C), paraffin-embedding, or snap-freezing in liquid nitrogen (stored at −80°C). Paraaffin-embedded sections were H&E-stained for determination of cardiacmyocyte width and cross-sectional area, measures of cardiomyocyte hypertrophy.

Analysis of LV Gene Expression in Mice

RNA was extracted from frozen tissues, and cDNA generated from DNase-treated RNA via reverse transcription (Taqman Reverse-Transcription reagents; Applied Biosystems, Mulgrave, Victoria, Australia). SYBR Green chemistry was used to determine expression of β-myosin heavy chain (Myh7), connective tissue growth factor, and pro-oxidant mitochondrial uncoupling protein 3, in addition to sarcoplasmic reticulum Ca2+-ATPase-2a (SERCA2a), using the Applied Biosystems ABI Prism 7700 Sequence Detection System. Ribosomal 18S was the endogenous control. Primers were generated using murine-specific sequences derived from Genbank. Relative fold increases in expression compared with vehicle-treated nondiabetic mice were calculated using the comparative delta C method.

Detection of LV Superoxide Generation in Mice

Superoxide generation was quantitated first using lucigenin (5 μmol/L)-enhanced, NADPH-driven chemiluminescence in fresh heart tissue, normalized to tissue weight. Dihydroethidium fluorescence was used as a second measure of superoxide detection. LV sections were mounted on superfrosted slides, before 45-minute incubation, 37°C, with dihydroethidium alone (Invitrogen, Mulgrave, Australia; 2 μmol/L in ice-cold phosphate-buffered saline) or dihydroethidium+sупeroxide dismutase-polyethylene glycol (PEG-SOD; Sigma-Aldrich, Sydney, Australia; 500 U/mL), to quench superoxide. Images were taken with a Zeiss LSM 510 Meta confocal microscope under ×40 magnification using 568 nm/585 nm excitation/emission wavelengths. Three images/section were analyzed using Image J software (version 1.44; National Institutes of Health, Bethesda, MD).

Changes in Coronary Flow in the Isolated Rat Heart

Isolated Langendorff rat heart studies were undertaken for insight into the relative contribution of HNO versus NO in 1-NCA actions. Hearts isolated from anesthetized male Sprague–Dawley rats (ketamine/xylazine, 100/12 mg/kg IP, respectively) were perfused with Kreb (pH 7.4), bubbled with 95% O2/5% CO2 at 37°C, under...
constant pressure. Coronary flow was detected using the STH Pump Controller (ADInstruments Pty Ltd). After 30-minute equilibration, U46619 (10−5.5 mol/L, 0.1–2.5 mL/min) was used to preconstrict coronary vessels by ≈50%. A single bolus dose of vehicle (2% methanol) was administered just above the aorta, followed by a 1-NCA dose–response curve (10−7−10−1 mol, in 2% methanol). In parallel series of hearts, selective scavengers of HNO (L-cysteine, 4 mmol/L) or NO (hydroxocobalamin, 50 μmol/L) were added to perfusion buffer from the last 15 minutes of equilibration, for the remaining duration. All materials for rat heart studies were obtained from Sigma-Aldrich unless indicated otherwise.

Statistical Analysis
All data are presented as mean±SD, with n representing number of animals. Statistical analysis was performed using 1- or 2-way ANOVA as indicated, followed by Tukey post hoc test for in vivo studies in mice (unless otherwise specified). Two-way repeated measures ANOVA followed by Bonferroni’s post hoc test was used for rat isolated heart studies (GraphPad Prism 6; GraphPad Software Inc, La Jolla, CA). P<0.05 was considered significant.

Results
Systemic Characteristics
Successful diabetes mellitus induction was shown by significantly higher blood glucose in vehicle- and 1-NCA–treated diabetic, compared with nondiabetic, mice (Table 1; P<0.0001 2-way ANOVA). There were no significant differences in final bodyweight, tibia length, systolic BP, or diastolic BP induced by diabetes mellitus and 1-NCA, alone or in combination.

1-NCA Protected the Heart Against Diabetes Mellitus–Induced Cardiac Dysfunction
Representative transmitral pulsed-wave Doppler echocardiography flow patterns are shown in Figure 1A. Diabetes mellitus significantly increased peak A wave velocity and reduced peak E wave velocity (Figure 1B and 1C). 1-NCA treatment for the last 4 weeks of diabetes mellitus significantly improved peak A wave velocity. On 2-way ANOVA, each of diabetes mellitus (P=0.0036), 1-NCA (P=0.0427), and their interaction (P=0.0165) were associated with significant differences in peak A wave velocity. A similar trend for 1-NCA to ameliorate impaired peak E wave velocity was evident (P=0.06 versus vehicle-treated diabetic mice, unpaired t test), but the interaction was not significant on 2-way ANOVA. Moreover, the additional markers of diastolic function, reduced E:A ratio (P=0.0027; Figure 2A), and prolonged deceleration time (P=0.0005; Figure 2B) and isovolumic relaxation times (P=0.0084; Figure 2C) were observed, in diabetic

| Table 1. Systemic Characteristics at Study End Point (Mean±SD) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Vehicle—Treated | Vehicle—Treated | 1-NCA—Treated | 1-NCA—Treated |
|                 | Nondiabetic Mice | Diabetic Mice   | Nondiabetic Mice | Diabetic Mice   |
| Blood glucose, mmol/L | 10.5±1.7       | 32.2±2.3*       | 10.6±1.2       | 32.4±1.2*       |
| Bodyweight, g     | 27.0±2.2       | 28.5±4.2       | 27.3±3.2       | 25.9±1.8       |
| Heart weight, mg   | 117±14         | 117±24         | 115±8          | 115±7           |
| Lung weight, mg    | 131±24         | 153±15†        | 148±14         | 138±15†         |
| Tibia length, mm   | 16.6±0.5       | 16.9±0.5       | 16.7±0.2       | 16.8±0.3       |
| SBP, mm Hg         | 103±23         | 88±9           | 94±16          | 88±10           |
| DBP, mm Hg         | 67±19          | 62±8           | 64±11          | 61±7            |

*P<0.0001 and †P<0.05 vs nondiabetic, vehicle-treated sham; ‡P<0.05 vs diabetic, vehicle-treated mice.
compared with nondiabetic mice on 2-way ANOVA. These diabetes mellitus–induced impairments in diastolic function were all significantly ameliorated by 1-NCA (which had no impact on diastolic function in nondiabetic mice). Each of diabetes mellitus \((P<0.005)\), 1-NCA \((P<0.05)\), and their interaction \((P<0.05)\) were associated with significant differences on all 3 parameters, suggesting 1-NCA prevented diastolic dysfunction.

Diabetes mellitus did not elicit any significant differences in LV systolic function (using M-mode echocardiography; Table 2), consistent with both previous studies using this model and with the clinical context of earlier stages of diabetic cardiomyopathy.\(^2\)\(^-\)\(^4\)\(^,\)\(^23\)\(^-\)\(^28\) 1-NCA significantly reduced LV end-systolic dimension \((P=0.0121)\) and increased fractional shortening \((P=0.0009)\), with a similar trend on velocity of circumferential fiber shortening, in diabetic mice selectively. There were no significant differences in heart rate or in other LV dimensions, induced by diabetes mellitus and 1-NCA (Table 2).

### 1-NCA Protected Against Diabetes Mellitus–Induced Cardiomyocyte Hypertrophy

As shown in Figure 3A, diabetes mellitus induced a modest but significant increase in lung weight normalized to tibia length; this was prevented by 1-NCA \((P=0.0094)\). Neither diabetes mellitus nor 1-NCA affected echocardiography-derived LV mass, or heart weight (both normalized to tibia length; Figure 3B and 3C). Despite the absence of net cardiac hypertrophy, diabetes mellitus significantly induced cardiomyocyte hypertrophy (Figure 4), on cardiomyocyte width and area; 1-NCA significantly prevented these changes. Diabetes mellitus–induced cardiomyocyte hypertrophic gene expression was also observed \((P=0.0043;\) Figure 5A), accompanied by a trend for increased profibrotic gene expression (Figure 5B) and downregulated SERCA expression \((P=0.0175;\) Figure 5C). All 3 parameters of LV remodeling were significantly ameliorated by 1-NCA in diabetic mice, but the HNO donor had no impact in nondiabetic mice.

### Impact of 1-NCA on Diabetes Mellitus–Induced LV Superoxide Generation

Diabetes mellitus significantly increased LV superoxide generation, on both lucigenin-enhanced chemiluminescence \((P=0.0145;\) Figure 6A) and PEG-SOD–sensitive dihydroethidium fluorescence \((P=0.0001;\) Figure 6B); expression of mitochondrial uncoupling protein 3 was also markedly upregulated \((P=0.0196;\) Figure 6C). On 2-way ANOVA, diabetes mellitus was associated with a significant difference on all 3 parameters. None of these diabetes mellitus–induced

#### Table 2. LV Dimensions and Function on M-Mode Echocardiography (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle-Treated Nondiabetic Mice (n=8)</th>
<th>Vehicle-Treated Diabetic Mice (n=9)</th>
<th>1-NCA–Treated Nondiabetic Mice (n=8)</th>
<th>1-NCA–Treated Diabetic Mice (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>370±363</td>
<td>391±88</td>
<td>387±41</td>
<td>437±80</td>
</tr>
<tr>
<td>External LV dimension, mm</td>
<td>5.42±0.26</td>
<td>5.54±0.35</td>
<td>5.39±0.20</td>
<td>5.37±0.29</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.55±0.39</td>
<td>3.65±0.32</td>
<td>3.58±0.20</td>
<td>3.45±0.29</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.09±0.31</td>
<td>2.11±0.35</td>
<td>2.33±0.25</td>
<td>1.76±0.33</td>
</tr>
<tr>
<td>LVFW, mm</td>
<td>0.85±0.11</td>
<td>0.86±0.18</td>
<td>0.73±0.13</td>
<td>0.89±0.14</td>
</tr>
<tr>
<td>FS, %</td>
<td>41.3±2.8</td>
<td>42.5±5.4</td>
<td>35.6±4.3</td>
<td>49.2±6.3*</td>
</tr>
<tr>
<td>Vcfc, circ/s</td>
<td>7.4±1.5</td>
<td>7.6±2.2</td>
<td>6.0±1.2</td>
<td>9.2±2.4*</td>
</tr>
</tbody>
</table>

FS indicates fractional shortening; LV, left ventricular; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; LVFW, LV posterior wall thickness; and Vcfc, velocity of circumferential fiber shortening.

*P<0.05 vs vehicle-treated diabetic mice.

---

Figure 2. 1-Nitrosocyclohexylacetate (1-NCA) prevents diabetes mellitus–induced left ventricular diastolic dysfunction. Diabetes mellitus (A) decreases E:A and prolongs (B) deceleration time, and (C) isovolumic relaxation times (IVRT) in mice. 1-NCA prevents these diabetes mellitus–induced changes. Results are presented as mean±SD. **P<0.01, ***P<0.001, and ****P<0.0001 vs nondiabetic vehicle, #P<0.05, ###P<0.001, ####P<0.0001 vs diabetic vehicle.
increases were evident after treatment with 1-NCA; the HNO donor tended to nonsignificantly reduce all 3 parameters in diabetic mice (all $P \leq 0.1$ versus diabetic vehicle on unpaired $t$ test), but had no impact in nondiabetic mice.

Relative Selectivity of 1-NCA as a Donor of HNO
The relative contributions of HNO and NO$^\bullet$ to the acute actions of 1-NCA were determined in the normal, Langendorff rat heart. As shown in Figure 7, dose-dependent coronary vasodilator responses induced by serial 1-NCA was significantly attenuated by L-cysteine ($P<0.0001$ on 2-way repeated-measures ANOVA) but was unaffected by hydroxocobalamin, suggesting a greater contribution of HNO than NO$^\bullet$ to the actions of 1-NCA.

Discussion
Compelling epidemiological and clinical evidence has confirmed the existence of diabetic cardiomyopathy, a disorder characterized by early diastolic dysfunction and adverse structural remodeling$^{2,4}$ with preclinical evidence implicating a major causal role of upregulated myocardial ROS generation. Although net LV dysfunction in diabetic patients likely reflects both increased coronary heart disease (secondary to atherosclerosis) and a specific diabetic cardiomyopathy, diabetes mellitus not only escalates risk of HF but also increases its incidence $>2.5$-fold, independent of age or concomitant obesity, dyslipidemia, or coronary heart disease.$^4$
Diabetic patients account for up to one third of patients in clinical HF trials, with diabetes mellitus an independent predictor of poor outcome. Despite these statistics, there is no specific therapy for the diabetic heart. With their concomitant positive inotropic, lusitropic, and vasodilator properties, short-acting HNO donors have recently attracted significant interest for clinical management of acute HF. Their impact with chronic administration has, however, yet to be reported in the context of cardiomyopathy. The objective of this study was to elucidate whether the putative HNO donor 1-NCA prevents LV diastolic dysfunction and cardiomyocyte hypertrophy induced by diabetes mellitus. We provide the first evidence of beneficial myocardial actions of an HNO donor over the longer term in vivo, demonstrating that chronic treatment with 1-NCA prevents diabetes mellitus–induced diastolic dysfunction, cardiomyocyte hypertrophy, and LV superoxide production in a mouse model of diabetes mellitus. These beneficial actions seem to be a direct effect on the myocardium, as 1-NCA was without BP effects. In addition, evidence supporting 1-NCA as an HNO donor was also obtained.
Advanced glycation, or other modifications of SERCA2a, result from diabetes mellitus–induced post-translational modifications to SERCA2a, including oxidative (eg, at 674Cys), nitrosative stress as a result of imbalanced upregulated ROS generation and their ineffective elimination.14,22,23,28 NADPH oxidase is one of the major sources of LV ROS. In the current study, LV NADPH-driven superoxide generation was increased in diabetes mellitus, likely secondary to hyperglycemia, consistent with previous studies.22,23,28 We have previously shown that Angeli salt and IPA-NO suppress cardiomyocyte ROS production in response to hypertrophic stimuli in cultured cardiomyocytes.18,19 In the current study, the ability of diabetes mellitus to stimulate NADPH-driven LV superoxide generation (on lucigenin-enhanced chemiluminescence, confirmed on PEG-SOD–sensitive dihydroethidium fluorescence) was absent in 1-NCA–treated diabetic mice. This trend was reproduced on LV mitochondrial uncoupling protein 3 expression. Although sGC (in its reduced, Fe²⁺-containing form) is regarded as the receptor for NO+, HNO is an equipotent sGC stimulator.19 HNO suppression of cardiomyocyte ROS production is mediated via sGC-dependent downregulation of NADPH oxidase.18,19 Insufficient mouse LV tissue precluded determination of LV cGMP content here. Unlike NO+, HNO is resistant to ROS scavenging, does not develop tolerance to its actions, and may target oxidized sGC.9,12,19,44,45 HNO also has a unique ability to enhance LV function,8,16,46,47 considered dependent on both sGC-independent and sGC-dependent mechanisms.8

1-NCA Treatment Protects Against Diabetes Mellitus–Induced Cardiomyocyte Hypertrophy

Cardiomyocyte hypertrophy is a common characteristic of diabetic cardiomyopathy that likely further impairs cardiac relaxation.12,23,27,28,46 In the present study, diabetes mellitus–induced cardiomyocyte hypertrophy was evident on cardiac fibrosis and area, as well as LV β-myosin heavy chain expression, consistent with previous studies.22,23,27,28,39–41 Increased cardiomyocyte size, however, was not reflected in net LV or heart weight at study end point (possibly as a result of cardiomyocyte apoptosis).12,43 The increased size of the remaining surviving cardiomyocytes may counterbalance reduced cardiomyocyte numbers, resulting in an absence of net increased heart mass. In the current study, all markers of diabetes mellitus–induced cardiomyocyte hypertrophy were attenuated by 1-NCA, consistent with previous short-term findings with classical HNO donors in isolated cardiomyocytes.18,19 To our knowledge, this is the first evidence to demonstrate antihypertrophic actions of HNO in a chronic setting in vivo. The clear, diabetes mellitus–induced cardiomyocyte hypertrophy was accompanied by a trend for increased profibrotic connective tissue growth factor expression, which was absent in 1-NCA–treated diabetic mice. There were, however, no significant differences in cardiac collagen deposition between the 4 experimental groups (results not shown), perhaps because of relatively shorter duration of diabetes mellitus studied here (8 weeks) versus longer studies where significant cardiac fibrosis is evident.22,23,28

Potential Role of HNO-Induced LV Suppression

Development and progression of cardiovascular diabetic complications are closely associated with increased oxidative stress as a result of imbalanced upregulated ROS generation and their ineffective elimination.5,12,22,23 NADPH oxidase is one of the major sources of LV ROS. In the current study, LV NADPH-driven superoxide generation was increased in diabetes mellitus, likely secondary to hyperglycemia, consistent with previous studies.22,23,28 We have previously shown that Angeli salt and IPA-NO suppress cardiomyocyte ROS production in response to hypertrophic stimuli in cultured cardiomyocytes.18,19 In the current study, the ability of diabetes mellitus to stimulate NADPH-driven LV superoxide generation (on lucigenin-enhanced chemiluminescence, confirmed on PEG-SOD–sensitive dihydroethidium fluorescence) was absent in 1-NCA–treated mice. This trend was reproduced on LV mitochondrial uncoupling protein 3 expression. Although sGC (in its reduced, Fe²⁺-containing form) is regarded as the receptor for NO+, HNO is an equipotent sGC stimulator.19 HNO suppression of cardiomyocyte ROS production is mediated via sGC-dependent downregulation of NADPH oxidase.18,19 Insufficient mouse LV tissue precluded determination of LV cGMP content here. Unlike NO+, HNO is resistant to ROS scavenging, does not develop tolerance to its actions, and may target oxidized sGC.9,12,19,44,45 HNO also has a unique ability to enhance LV function,8,16,46,47 considered dependent on both sGC-independent and sGC-dependent mechanisms.8
HNO Contributes to the Mechanism of Action of 1-NCA

1-NCA is thought to spontaneously hydrolyze to form an unstable α-hydroxy-nitroso intermediate, which then generates cyclohexanone and HNO.24 There is, however, previous evidence implicating both HNO and NO- in its actions in vitro.24–26 We selected this donor here, however, on the basis of both its reportedly longer half-life than that offered by CXL-1020 or conventional HNO donors, as well as its stability as an HNO donor at physiological pH.24 In the present study, we sought to determine the relative contribution of the 2 redox siblings to the vasodilator properties of 1-NCA, by examining its acute sensitivity to selective scavengers of HNO (α-cysteine) and NO- (hydroxocobalamin) in the normal isolated rat heart ex vivo.

Both its reportedly longer half-life than that offered by CXL-1020 or conventional HNO donors, as well as its stability as an HNO donor at physiological pH.24 In the present study, we sought to determine the relative contribution of the 2 redox siblings to the vasodilator properties of 1-NCA, by examining its acute sensitivity to selective scavengers of HNO (α-cysteine) and NO- (hydroxocobalamin) in the normal isolated rat heart ex vivo. α-cysteine significantly reduced 1-NCA–induced increases in coronary flow, implicating HNO, at least in part, in the mechanism of action of acute 1-NCA. In previous studies, the putative selective NO-scavenger, carboxy-PTIO [2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1-H-imidazolyl-1-oxy-3-oxide, monopotassium salt], tended to also reduce a component of the vasodilator actions of 1-NCA.24 As carboxy-PTIO blunts both NO- and peroxynitrite-mediated actions,28 the current study used hydroxocobalamin (a more selective NO-scavenger) to investigate the possible contribution of NO to 1-NCA actions. Hydroxocobalamin did not affect vasodilator action of 1-NCA; this suggests that the effects of 1-NCA may be more dependent on HNO rather than NO-.

Limitations

Limited tissue availability precluded assessment of LV cGMP content, or of excitation–contraction coupling, to gain further insight into the potential mechanisms of action of 1-NCA. Moreover, the longer term nature of our study did not permit the use of HNO or NO--selective scavengers in vivo, to unequivocally demonstrate the role of HNO in the protective effects of chronic therapy. Our experimental design also precluded pharmacokinetic assessment of 1-NCA, to determine its plasma half-life and bioavailability in vivo. Although 1-NCA has been reported to have a longer half-life (≈13 hours)24 compared with other HNO donors (Angeli salt, IPA-NO, and CXL-1020, half-life 2–3 minutes),5,7,20 this has only been determined in a cell-free environment in vitro.24 Our studies, however, open the door for analogous longer term studies interrogating the impact of next-generation, pure HNO donors on diabetes mellitus–induced LV diastolic dysfunction, cardiomyocyte hypertrophy, and LV ROS generation, building on the promising results achieved with CXL-1020 in the clinical context of acute HF.8,21

Conclusions

This study is the first to show the beneficial effects of chronic therapy with a putative HNO donor over the longer term in vivo, in any cardiomyopathy context. Moreover, it is the first interrogation of the potential actions of HNO specifically in the setting of diabetes mellitus. We have demonstrated here that 1-NCA rescued LV diastolic function, together with reduced LV ROS production and cardiomyocyte hypertrophy, in a mouse model of diabetes mellitus in vivo, without adverse effects. Collectively, our studies provide the first evidence that HNO donors may represent a promising new strategy for the treatment of diabetic cardiomyopathy and implies their therapeutic efficacy in settings of chronic HF, either as stand-alone therapy or as a supplement to standard care.

Sources of Funding

This work was supported by a Grant-In-Aid from Diabetes Australia (Y12G-RITR) and, in part, by the Victoria Government’s Operational Infrastructure Support Program. Dr Du (ID1043026) and R.H. Ritchie (ID472673 and ID1059960) are supported by Senior Research Fellowships from National Health and Medical Research Council of Australia.

Disclosures

None.

References

12. Irvine JC, Kemp-Harper BK, Widdop RE. Chronic administration of the HNO donor Angelis’s salt does not lead to tolerance, cross-tolerance, or endothelial dysfunction: comparison with GTN and DEA/NO. Antioxid Redox Signal. 2011;14:1615–1624. doi: 10.1089/ars.2010.3269.


**CLINICAL PERSPECTIVE**

Diabetes mellitus not only escalates risk of heart failure but also increases its incidence, independent of age or concomitant obesity, dyslipidaemia, or coronary disease. Furthermore, patients with diabetes mellitus and heart failure have a poorer prognosis than those without diabetes mellitus, with diabetic patients accounting for up to one third of patients in clinical trials for heart failure. Early diastolic dysfunction yet preserved ejection fraction is characteristic of diabetic patients with heart failure. Management of heart failure in this context remains a persisting unanswered clinical need. Several recent studies have revealed that the novel NO\(^*\) redox sibling, nitroxyl, acutely enhances myocardial function (an action that persists in the failing heart), while concomitantly unloading the heart and negatively regulating cardiomyocyte hypertrophy and its triggers. These have served to highlight the exciting therapeutic potential offered by nitroxyl, at least in the short term. We now show that chronic treatment with a longer-acting putative nitroxyl donor, 1-nitrosocyclohexyl acetate, during the past 4 weeks of diabetes mellitus, rescues diabetes mellitus–induced left ventricular diastolic dysfunction in a preclinical mouse model of diabetes mellitus–induced cardiomyopathy in vivo. This benefit was accompanied by significant improvements in cardiomyocyte hypertrophy, without adverse effects. Our studies provide the first evidence that nitroxyl donors may represent a promising strategy for treatment of diabetic cardiomyopathy and implies therapeutic efficacy in other settings of chronic heart failure. Development of longer-acting, small molecule nitroxyl donors suitable for human use in the clinic is thus urgently warranted.
Chronic Administration of the Nitroxyll Donor 1-Nitrosocyclo Hexyl Acetate Limits Left Ventricular Diastolic Dysfunction in a Mouse Model of Diabetes Mellitus In Vivo
Nga Cao, Yung George Wong, Sarah Rosli, Helen Kiriazis, Karina Huynh, Chengxue Qin, Xiao-Jun Du, Barbara K. Kemp-Harper and Rebecca H. Ritchie

*Circ Heart Fail. 2015;8:572-581; originally published online March 3, 2015;
doi: 10.1161/CIRCHEARTFAILURE.114.001699
*Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/8/3/572

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure 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: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/