The nitric oxide donor molsidomine rescues cardiac function in rats with chronic kidney disease and cardiac dysfunction

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CHRONIC KIDNEY DISEASE (CKD) is associated with a strongly increased risk for cardiovascular disease, including left ventricular (LV) hypertrophy, LV systolic dysfunction (LVSD), and heart failure (20). The prognosis of patients with CKD is poorer when LVSD is present, and, conversely, the outcome in patients with heart failure is worse when renal dysfunction is present (15, 16, 47). This coexistence of cardiac and renal failure was designated as (severe) cardiorenal syndrome (9, 36). We recently developed a rat model of combined CKD and LVSD based on the CKD model of subtotal (5/6th) nephrectomy (SNX). LVSD was induced in SNX rats with a low-dose of nitric oxide (NO) synthase (NOS) inhibition given up to 8 wk after SNX, and ejection fraction (EF) was almost half of that in rats with SNX alone (8). This was associated with strongly decreased NO production and worsened proteinuria. When NOS inhibition was stopped, systemic NO availability remained suppressed, and this was accompanied by persistent LVSD.

Reduced NO availability has a negative impact on cardiac function, especially in failing hearts, but it is also a common feature in CKD (3, 39). Our previous study suggested that the cardiac dysfunction observed in our model was related to NO availability. However, the effect of changes in NO availability on cardiac function in CKD has not yet been fully established. We thus hypothesized that the persistence of LVSD in our model is due to low NO availability and that supplementing NO in vivo with an NO donor could reverse cardiac dysfunction and ameliorate cardiorenal failure. N-(ethoxycarbonyl)-3-(4-morpholino)sydnone-imine [molsidomine (Mols)] is an NO-releasing prodrug (18, 38) that has been previously shown to have beneficial effects in SNX and cholesterol-fed rats (1, 5) but also in patients with heart failure (27). We studied the in vivo cardiorenal effects of Mols given as a rescue therapy during the last 4 wk of the protocol.

METHODS

The study protocol was approved by the Utrecht University Committee on Animal Experiments and conformed with Dutch Law on Laboratory Animal Experiments. Male inbred Lewis rats (Lew/Crl, 180–200 g) were purchased from Charles River and housed in a climate-controlled facility with a 12:12 h light-dark cycle.

Experimental setup and SNX. Rats were divided into two groups with similar initial body weights: sham-operated rats (control group) and SNX rats treated with 20 mg/l N-nitro-l-arginine (l-NNA, Sigma-Aldrich, St. Louis, MO) in drinking water (SNX + l-NNA group). Animals were pretreated with l-NNA or normal (acidified) drinking water for 2 wk and fed standard pellet rodent chow (CRM-E, Special Diet Services, Essex, UK). On week −1, two-stage SNX by resection or a sham operation was performed (8). After 1 wk of recovery, rats were restarted on l-NNA or normal water, and all groups were fed standard powdered chow (CRM-FG, Special Diet Services) supplemented with 6% (wt/wt) NaCl. This was done to accelerate the progression of CKD and results in stable CKD comparable with the model of Vercauteren et al. (43) in the same rat strain. Normal Lewis rats are resistant to high-salt (8%) diets with respect to blood pressure and the development of proteinuria (25).

In vivo measurements of renal and cardiac function (see below) were performed on week 8 in a selection of animals, after which l-NNA treatment was stopped. After a 3-wk washout period, cardiac function, renal function, and creatinine clearance was increased by Mols (both week 15 and week 16). On week 15, LV hemodynamics and pressure-volume relationships were measured invasively, and rats were killed to quantify histological damage. On week 16, blood pressure was mildly reduced and creatinine clearance was increased by Mols (both P < 0.05). Mols treatment improved ejection fraction (53 ± 3% vs. 37 ± 2% in Veh-treated rats, P < 0.001) and stroke volume (324 ± 33 vs. 255 ± 15 μl in Veh-treated rats, P < 0.05). Rats with Mols treatment had lower end-diastolic pressures (8.5 ± 1.1 mmHg) than Veh-treated rats (16.3 ± 3.5 mmHg, P < 0.05) and reduced time constants of relaxation (21.9 ± 1.8 vs. 30.9 ± 3.3 ms, respectively, P < 0.05). The LV end-systolic pressure-volume relationship was shifted to the left in Mols compared with Veh treatment. In summary, in a model of cardiorenal failure with low NO availability, supplementing NO significantly improves cardiac systolic and diastolic function without a major effect on afterload.

left ventricular systolic dysfunction; rescue therapy; hemodynamics

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and renal function were reevaluated at 11 wk in all rats. Rats in the SNX + l-NNa group were stratified according to plasma urea and echocardiographic LV fractional area change [FAC = (LV end-diastolic area – LV end-systolic area/LV end-diastolic area × 100%)] and then divided into two groups: rats treated with normal water [vehicle (Veh)] or rats treated with 120 mg/l Mols (Sigma-Aldrich). Mols is metabolized in the liver to linsidomine (SIN-1), which releases NO without further enzymatic activation. Although SIN-1 can generate peroxynitrite in vitro, at in vivo levels of oxygen concentration it functions as a donor of NO (17, 38). Because Mols is sensitive to light (41), the medication was supplied in dark brown bottles. In vivo measurements were then performed again 2 and 4 wk after the start of treatment in all groups. On week 16, terminal measurements were performed, and organs were harvested. Data from the treatment phase were censored for deaths occurring in that period.

Systolic blood pressure and 24-h urine sampling. Systolic blood pressure (SBP) was measured by the tail-cuff method (24). After SBP measurements, a 24-h urine sample was collected in individual metabolic cages with 1 ml antibiotic-antimycotic solution (Sigma-Aldrich) to prevent the degradation of NO metabolites. These measurements were performed on weeks 8, 11, and 15. In metabolism cages, rats received no chow but had access to water with 2% glucose and supplemented with l-NNa or Mols if indicated. After 24 h, urinary volume was measured, and samples were stored at –80°C. Urine samples were analyzed for total protein, creatinine, and NO metabolites as previously described (8). Creatinine clearance was calculated by the standard formula.

Echocardiography and blood samples. Transesophageal echocardiography was performed as previously described (8). In brief, animals were anesthetized with isoflurane and placed in a supine position on a warming pad. A three-lead ECG system was connected to the paws, the chest was shaved, and excess hair was removed with depilatory cream. Two-dimensional B-mode cine loops with continuous ECG registration were recorded in the parasternal long-axis and midpapillary short-axis views while isoflurane anesthesia was adjusted to the lowest possible level (1.75–1.85%) to minimize effects on heart rate (HR) and blood pressure. The typical study duration was 15 min. Acquisitions were coded, and the results were decoded after analysis. Recordings were analyzed offline using software present on the system, and the variables were measured in at least three heart beats at end diastole and the corresponding end systole as previously described (8). LV volume was calculated with the prolate ellipsoid area-length method (46), and LV mass was calculated by the formula described by Litwin et al. (28). For further details on calculations, please refer to the Supplemental Material.

After echocardiography, a blood sample (500 μl) was collected from the tail vein and analyzed for urea (blood urea nitrogen) and creatinine (8).

Hemodynamic experiments. Terminal LV hemodynamic measurements were performed via the closed-chest approach on week 16. After induction with isoflurane, rats were placed on heating pads, intubated, and mechanically ventilated with 2% isoflurane at a rate of 65 breaths/min with 40% O2. Buprenorfine (0.015 mg/kg im) was injected for analgesia. The left internal jugular vein was isolated and cannulated with a polyethylene-50 catheter for the infusion of saline at a rate of 8–10 ml/kg body wt⁻¹·min⁻¹. LV hemodynamics were assessed with a 2-Fr pressure micromanometer (Millar Instruments, Houston, TX) and recorded with Chart software (AD Instruments, Spechbach, Germany). After a 15-min stabilization period, LV pressures were recorded during a short period with the ventilator switched to continuous positive airway pressure.

Hemodynamic variables were calculated using the Blood Pressure module. Data from at least three separate intervals, each consisting of at least five cardiac cycles, were averaged, and the following parameters were calculated: HR (in beats/min), end-diastolic pressure (EDP; in mmHg), end-systolic pressure (ESP; in mmHg), maximum change of pressure with time (dP/dt max; in mmHg/s), minimum first time derivative of pressure (dP/dt min; in mmHg/s), and the regression of dP/dt versus pressure [time constant of relaxation (τr; in ms)]. Carotid artery pressure was measured, and mean arterial pressure (MAP) was calculated.

Cardiac pressure-volume analysis. Induction and basic surgical preparation were performed as described above. The upper abdomen was opened by a small incision in the linea alba to provide access to the inferior vena cava between the liver and diaphragm for occlusion experiments (see below). A catheter was inserted in the bladder via a suprapubic approach. The right common carotid artery was isolated, and a 2-Fr pressure conductance catheter (SPR-838, Millar Instruments) was inserted. The conductance catheter was advanced retrograde into the LV. Pressure-volume signals were registered continuously with Chart software (AD Instruments) at a sample rate of 1,000 samples/s. Adequate placement of the catheter was verified by the pressure-volume (P-V) loop signals. After a stabilization period of at least 15 min, baseline hemodynamic experiments were performed as described above. These were followed by inferior vena cava occlusion (IVCO) experiments during a short period of apnea and continuous positive airway pressure ventilation.

Measurements were recorded on Chart software and analyzed offline with PVAN software (version 3.6, Millar Instruments). Volume calibration of the conductance values was done by plotting the mean conductance catheter values for maximal and minimal volumes of the baseline loops preceding the IVCO experiment against the end-diastolic volume (EDV) and end-systolic volume (ESV) determined by echocardiography (see above.) This yielded an equation with values for the slope and y-intercept that were entered in the PVAN program.

The following parameters calculated by the PVAN program were used to assess changes in contractility and elastance: end-systolic elastance (Ees; in mmHg/μl), which was calculated from the linear end-systolic pressure-volume relationship (ESPVR) and linear volume intercept (V0; in μl); maximal elastance, which was calculated from quadratic curve fit of the ESPVR (E′ max; in mmHg/μl) and its volume intercept (in μl); the slope of the relationship between EDV and dP/dt max (in mmHg·s⁻¹·μl⁻¹) and its volume intercept (in μl); and the slope of the end-diastolic pressure-volume relationship (EDPVR; in mmHg/μl).

End-organ damage and histology. After exsanguination through the abdominal aorta under anesthesia, organs were harvested and weighed. Glomerulosclerosis and tubulointerstitial damage were scored on periodic acid-Schiff (PAS)-stained kidney sections (8). Cardiomyocyte circumference was measured on PAS-stained myocardial slices in sections with transversely cut myocardial fibers and was traced on the cellular border on photomicrographs of at least 50 different cardiomyocytes with a computer-assisted image-analysis system (OptiMas, Houston, TX). Digital photomicrographs of transverse sections of the heart stained with Sirius red were taken to measure the collagen content of the heart using ImageJ software (35). The percentage of the collagen area was calculated by dividing the Sirius red-stained area by the total LV tissue area (8).

Quantitative PCR. The expression of atrial natriuretic peptide (ANP; Rn00561661), neuronal NOS (nNOS; Rn05837937), inducible NOS (iNOS; Rn00561646), and endothelial NOS (eNOS; Rn02132634) in cardiac apical tissue was assessed by quantitative PCR as previously described (44). Cycle time (Ct) values were normalized to mean Ct values of calnexin (Rn00596877) and β-actin (Rn00667869), which we previously determined to be the two most stable housekeeping genes across all groups using the geNorm program (http://medgen.ugent.be/~vdjvdesomp/genorm/). Statistical analysis was performed on ΔΔCt values, and results were graphed as fold changes (2ΔΔCt).

1 Supplemental Material for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.
Western blot analysis for 3-nitrotyrosine. Frozen heart (LV), left kidney, and liver tissue samples from 4 rats/group were homogenized in a lysis buffer [20 mM Tris (pH 7.4), 10% glycerol, 0.1% SDS, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 2 mM Na3VO4, 0.150 M NaCl, 0.5% sodium deoxycholate, and 5% protease inhibitor cocktail; 1 ml]. Protein content was measured with a DC protein assay (500-0113, 500-0114, and 500-0115, Bio-Rad). Then, 10 µg of protein were loaded for LV and liver samples and 25 µg of protein for left kidney samples on a 8.5% Tris-glycine gel and blotted on nitrocellulose membranes. Blots were stained with antibodies to 3-nitrotyrosine (3-NT; SC-55256, Santa Cruz Biotechnology) and β-actin (A5441, Sigma). Bands were visualized using chemiluminescence blotting substrate (POD, Roche), and membranes were stripped with Re-Blot Plus Strong (no. 2504, Millipore).

Statistics. Data are presented as means ± SE. Some of the data from 5 control rats and 12 SNX + L-NNa + Veh-treated rats have been described in a previous study (8). Data were analyzed and graphed using SigmaPlot 11.0 (Systat Software, San Jose, CA). One-way ANOVA with the Holm-Sidak post hoc test was done per time point across all groups, with the SNX group or the Veh-treated group. Accordingly, at the start of treatment, levels of plasma urea were not significantly different between rats allocated to Veh (13.3 ± 1.1 mmol/l) or Mols (14.2 ± 1.5 mmol/l) treatment groups. Plasma urea was not affected by Mols therapy (see Supplemental Material). Calculated creatinine clearance was also similar on week 11 (Veh: 31.0 ± 0.02 ml·min⁻¹·100 g⁻¹ vs. Mols: 29.0 ± 0.04 ml·min⁻¹·100 g⁻¹). On week 13, creatinine clearance was still comparable between these groups (data not shown). On week 15, creatinine clearance was higher in the Mols-treated group (0.42 ± 0.04 ml·min⁻¹·100 g⁻¹) versus the Veh-treated group (0.30 ± 0.03 ml·min⁻¹·100 g⁻¹, P = 0.014; Fig. 3A).

Treatments with Mols did not influence the levels of proteinuria (see Supplemental Material). Urinary excretion of stable NO metabolites over 24 h, obtained under fasting conditions, was greatly enhanced by Mols treatment, confirming the enhanced production of NO (Fig. 3B).

Echocardiographic structure and function. Cardiac volumes and systolic function, as measured on B-mode cine loops obtained with 15-MHz cardiac ultrasound, are shown in Fig. 4 and Table 1. Three weeks after L-NNa was stopped (week 11), rats were stratified and allocated to either the Mols-treated group or the Veh-treated group. Accordingly, at the start of treatment, levels of LV FAC were similar in both groups (31.6 ± 0.9% in the Veh-treated group vs. 31.2 ± 2.1% in the Mols-treated group, Fig. 4). Mols treatment significantly improved LV FAC to 41 ± 2% during the 4-wk treatment period, whereas there were no significant changes over time in untreated rats and control rats by two-way repeated-measures ANOVA.

There were no significant differences in other cardiac variables on week 11 before the start of treatment (Table 1). By week 15, EDV was lower in Mols-treated rats compared with Veh-treated rats, and ESV was even more so (Table 1). This resulted in a higher ejection fraction (EF) and stroke volume. HR was slightly lower in rats with Mols treatment (not significant), resulting in a trend toward higher cardiac output (P =

RESULTS

Survival and clinical state. During the treatment period, no further mortality was observed in the Mols-treated group, whereas five animals died in the Veh-treated group (Fig. 1). Overall differences in survival curves were statistically significant by Kaplan-Meier log-rank testing (P = 0.045), but with the post hoc Holm-Sidak test for multiple comparisons there were no significant differences between individual groups. Rats receiving Mols treatment also exhibited less morbidity, generally fared better, and were more active.

Tail-cuff SBP. As previously described (8), rats with SNX + L-NNa treatment became hypertensive with a SBP of 202 ± 3 mmHg by week 8 (Fig. 2). After the withdrawal of L-NNa, SBP dropped gradually in Veh-treated rats, which was significant over time. Treatment with Mols mildly reduced blood pressure compared with Veh-treated rats, which was only significant on week 15 (151 ± 5 vs. 165 ± 4 mmHg, P < 0.05).

Renal function and urinary NO metabolite excretion. At the time of stratification, levels of plasma urea were not significantly different between rats allocated to Veh (13.3 ± 1.1 mmol/l) or Mols (14.2 ± 1.5 mmol/l) treatment groups. Plasma urea was not affected by Mols therapy (see Supplemental Material). Calculated creatinine clearance was also similar on week 11 (Veh: 31.0 ± 0.02 ml·min⁻¹·100 g⁻¹ vs. Mols: 29.0 ± 0.04 ml·min⁻¹·100 g⁻¹). On week 13, creatinine clearance was still comparable between these groups (data not shown). On week 15, creatinine clearance was higher in the Mols-treated group (0.42 ± 0.04 ml·min⁻¹·100 g⁻¹) versus the Veh-treated group (0.30 ± 0.03 ml·min⁻¹·100 g⁻¹, P = 0.014; Fig. 3A).

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![Fig. 1. Survival curves from the start of treatment on week 11 up to week 15. Group size, before within-group mortality, is also shown. Con, control; Mols, molsidomine; SNX, subtotal (5/6th) nephrectomy; Veh, vehicle; L-NNa, N-nitro-L-arginine.](http://ajpheart.physiology.org/)
The slope of ESPVR was not significantly altered by Mols treatment. Mols treatment decreased arterial elastance (Ea) by 10.2 ± 0.33 mmHg/m allowing a leftward shift of the P-V loop and ESPVR in the Mols-treated group with an increase in SV despite a minor decrease in afterload.

Organ weights, histological damage, and cardiac gene expression. Rats with Mols treatment had significantly lower LV masses than Veh-treated rats (Table 3), and the left kidney remnant mass was also numerically lower (not significant), but the significance disappeared when organ weights were corrected for body weight. However, we also measured tibia length in six rats from the Mols-treated group and five rats from the Veh-treated group and found these to be similar (4.25 ± 0.04 vs. 4.25 ± 0.02 cm, respectively, not significant).

Right ventricular weight and wet lung weight were not affected by Mols therapy (see Supplemental Material). Cardiomyocyte area was slightly larger with Mols versus Veh treatment, but this was also not significant (Table 3). Furthermore, the collagen area fraction in the heart, as assessed using Sirius red-stained transverse sections, was not affected by Mols treatment. In the kidney, Mols treatment decreased the amount of tubulointerstitial injury (P < 0.0045) but did not influence glomerulosclerosis.

Cardiac expression of ANP and the NOS isoforms was determined by quantitative PCR in apical tissue. SNX (+ l-NNA) + Veh rats (n = 12) showed grossly increased expression of ANP versus control rats (n = 9), with a fold change of 15 ± 1 versus 1.1 ± 0.2 (P < 0.001), respectively; Mols treatment (n = 8) decreased ANP expression (11 ± 1, P < 0.05). Fold change expression of NOS isoforms is shown in Fig. 6. Compared with control rats, the expression of nNOS
was significantly higher in SNX (+ L-NNA) + Veh-treated rats. The expression of iNOS was also numerically higher (not significant) in the SNX (+ L-NNA) + Veh-treated group versus in the control group but was significantly reduced in the Mols-treated group. The expression of eNOS was not different versus in the control group but was significantly reduced in the Veh-treated group.

**DISCUSSION**

The results confirmed our hypothesis that the persistent LVSD in SNX (+ L-NNA) + Veh-treated rats is linked to the low systemic NO availability. NO appears to be a critical modulator of cardiac function in the setting of reduced kidney function. In this rat model of combined CKD and cardiac dysfunction, supplementing NO with Mols significantly improved cardiac systolic function, reduced LV mass, improved creatinine clearance, reduced tubulointerstitial injury, and abrogated further mortality. The positive effects were sustained over a 4-wk treatment period.

**Effect of Mols on renal function and structure.** Although it is well known that NOS inhibition can induce and worsen renal injury, data on the effects of NO supplementation on existing renal injury are scarce. Benigni et al. (5) reported that Mols treatment, starting 3 wk after SNX, significantly reduced SBP with only minor effects on serum creatinine and proteinuria. In rats with dietary hypercholesterolemia, Mols treatment prevented proteinuria and both glomerular and tubulointerstitial injury (1). In our study, plasma urea and proteinuria were unaltered by Mols therapy. This may have been related to the time point at which treatment was started. Nevertheless, creatinine clearance increased at the end of the 4-wk treatment period. This could be due to pregglomerular vasodilatation, an increase in cardiac output (and hence renal perfusion), or a combination of these. Because blood pressure was reduced, this mechanism was not dampened by renal autoregulation (i.e., afferent arteriolar constriction), insofar as this was still operating in the remnant kidney (6). However, Mols treatment may also have reduced venous congestion, thus decreasing renal “afterload” (14). Finally, the reduction in tubulointerstitial injury in Mols-treated rats may have reduced hydrostatic pressures in the renal interstitium.

### Table 2. LV hemodynamic variables and pressure-volume relationships on week 16

<table>
<thead>
<tr>
<th></th>
<th>SNX (+ L-NNA) + Veh</th>
<th>SNX (+ L-NNA) + Mols</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>End-systolic pressure, mmHg</td>
<td>170 ± 9</td>
<td>159 ± 8</td>
<td>130 ± 5†</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>163.5 ± 3.5</td>
<td>8.5 ± 1.1*</td>
<td>7.2 ± 0.6*</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>9,112 ± 655</td>
<td>9,415 ± 676</td>
<td>7,622 ± 565</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>−6,592 ± 370</td>
<td>−8,201 ± 687</td>
<td>−9,081 ± 791†</td>
</tr>
<tr>
<td>Time constant of relaxation, ms</td>
<td>21.9 ± 1.8*</td>
<td>21.0 ± 1.3†</td>
<td>15.0 ± 1.3†</td>
</tr>
<tr>
<td>Arterial elastance, mmHg/µL</td>
<td>0.61 ± 0.05</td>
<td>0.48 ± 0.04*</td>
<td>0.40 ± 0.04†</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>151 ± 9</td>
<td>129 ± 9</td>
<td>103 ± 8*</td>
</tr>
<tr>
<td>Systemic vascular resistance index, mmHg·mL⁻¹·min⁻¹·100 g⁻¹</td>
<td>6 ± 0</td>
<td>4.2 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

**Pressure-volume relationships**

<table>
<thead>
<tr>
<th></th>
<th>SNX (+ L-NNA) + Veh</th>
<th>SNX (+ L-NNA) + Mols</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>End-systolic elastance, mmHg/µL</td>
<td>0.54 ± 0.08</td>
<td>0.43 ± 0.08</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Volume intercept, µL</td>
<td>91 ± 45</td>
<td>−149 ± 104*</td>
<td>−265 ± 43†</td>
</tr>
<tr>
<td>Maximal elastance, mmHg/µL</td>
<td>2.03 ± 0.50</td>
<td>1.66 ± 0.46</td>
<td>1.30 ± 0.30</td>
</tr>
<tr>
<td>Volume intercept calculated from quadratic fit of ESPVR, µL</td>
<td>225 ± 43</td>
<td>47 ± 75*</td>
<td>−20 ± 34†</td>
</tr>
<tr>
<td>dP/dt-EDV, mmHg·s⁻¹·µL⁻¹</td>
<td>19 ± 3</td>
<td>12 ± 1</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>dP/dt-EDV intercept, µL</td>
<td>185 ± 37</td>
<td>−191 ± 109†</td>
<td>−207 ± 122‡</td>
</tr>
<tr>
<td>ESPVR slope, µL</td>
<td>0.017 ± 0.003</td>
<td>0.021 ± 0.003</td>
<td>0.013 ± 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats/group. ESPVR and EDVPR, end-systolic and end-diastolic pressure-volume relationship, respectively. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. the SNX (+ L-NNA) + Veh-treated group.
However, afterload was only mildly affected, which can explain why EF was still somewhat lower and ESP and ESV still somewhat higher than in control rats.

Mols was found to have strong antihypertensive effects in rats with SNX (5) and when administered together with NOS inhibition (19) but not in normal or spontaneously hypertensive rats (1, 26). This suggests that Mols mainly affects blood pressure in disease states where NO production is lowered. In our model, the resistance vessels may have been less responsive to exogenous NO as tail-cuff SBP and carotid artery MAP were only mildly lowered by Mols treatment. Thus, the changes in calculated $E_a$ and SVRI appeared to be mainly mediated by the improved systolic function, which was able to overcome the increased afterload.

Mols, like other NO donors, can function as a venodilator and thereby decreases cardiac preload and wall stress. The decrease in preload has no negative consequences as output is almost normalised at significantly lower filling pressures, and the decrease in EDV is likely secondary to the increase in SV. Analysis of P-V relationships showed that Mols treatment induced a leftward shift of the linear ESPVR without a change in slope ($E_a$). Similar changes were seen in the dP/dr-EDV relationship. This is indicative of increased contractility, as similar ESPs could be generated at a lower volume (Fig. 5). Because in the rat ESPVR shows contractility-dependent curvilinearity (10), we also determined $E_{max}$ and $V_o$ derived from a quadratic curve fit. This yielded similar results to the linear ESPVR, with a significant left shift of ESPVR in Mols-treated rats.

The reduction in $\tau$, a relatively load-independent measure of relaxation (42), however, indicates that active diastolic relaxation (lusitropy) was also more efficient in the Mols-treated group. Many of the same mechanisms that influence lusitropy also affect contractility, by improving excitation-contraction coupling. Mols-derived NO may have enhanced excitation-contraction coupling by increasing Ca$^{2+}$ reuptake by the sarcoplasmic reticulum or by altering the $\delta$-nitrosylation of Ca$^{2+}$ channels (39). Furthermore, in isolated rat hearts, 4 wk after SNX, the group of Raine et al. found a reduced responsiveness to increasing Ca$^{2+}$ concentrations (34) and abnormal Ca$^{2+}$ cycling together with reduced contraction and relaxation

Table 3. Cardiorenal histological damage on week 16

<table>
<thead>
<tr>
<th>Organ weights</th>
<th>SNX (+ L-NNA) + Veh</th>
<th>SNX (+ L-NNA) + Mols</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, g</td>
<td>1.23 ± 0.02</td>
<td>1.12 ± 0.03†</td>
<td>0.83 ± 0.02‡</td>
</tr>
<tr>
<td>LV mass/100 g body wt, g/g</td>
<td>0.33 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.19 ± 0.00‡</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>1.49 ± 0.05</td>
<td>1.31 ± 0.08</td>
<td>1.47 ± 0.05</td>
</tr>
<tr>
<td>Left kidney weight/100 g body wt, g/g</td>
<td>0.40 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td>0.34 ± 0.01†</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cardiomyocyte area, $\mu m^2$</td>
<td>600 ± 31</td>
<td>662 ± 33</td>
<td>396 ± 18‡</td>
</tr>
<tr>
<td>Collagen area fraction, %</td>
<td>4.04 ± 0.38</td>
<td>5.75 ± 1.02</td>
<td>0.24 ± 0.09‡</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Normal glomeruli (score: 0)</td>
<td>40 ± 3</td>
<td>48 ± 7</td>
<td>70 ± 2‡</td>
</tr>
<tr>
<td>Partial glomerulosclerosis (score: 1–2)</td>
<td>32 ± 4</td>
<td>23 ± 3</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Severe glomerulosclerosis (score: 3–4)</td>
<td>28 ± 5</td>
<td>39 ± 8</td>
<td>37 ± 1‡</td>
</tr>
<tr>
<td>Tubulointerstitial score</td>
<td>6.37 ± 0.63</td>
<td>4.96 ± 0.52*</td>
<td>0.81 ± 0.15‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats/group. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. the SNX (+ L-NNA) + Veh-treated group.
velocities in single cardiomyocytes (31). Thus, reduced sensitivity of cardiac myofilaments to Ca^{2+} may also play a role in the cardiac dysfunction observed in our severe SNX model, and this may have been restored by NO supplementation. A positive effect of Mols on coronary perfusion, either directly or secondary to decreased wall stress, may also have been present (2).

Wilson et al. (45) recently investigated the effect of concomitant hydralazine and isosorbide dinitrate (H-ISDN) on cardiac function in aldosterone-infused mice. Untreated mice exhibited diastolic dysfunction but no decrease in EF, and H-ISDN improved the (Doppler-derived) diastolic indexes. Positive effects of NO donors have also been described on cardiac diastolic function in humans with normal hearts (33) and with pressure overload hypertrophy (30). Furthermore, a beneficial effect on cardiac function, remodelling, and mortality in patients with heart failure was documented for long-term treatment with H-ISDN (12, 13). With respect to Mols, several

![Graph A: Expression of neuronal NOS (nNOS)](image)

![Graph B: Expression of inducible NOS (iNOS)](image)

![Graph C: Expression of endothelial NOS (eNOS)](image)

**Fig. 6.** Levels of cardiac expression of the following NO synthase (NOS) isoforms: neuronal NOS (nNOS; A), inducible NOS (iNOS; B), and endothelial NOS (eNOS; C). Values are means ± SE. *P < 0.05 vs. the SNX (+L-NNA) + Veh-treated group.

![Graph A: 3-Nitrotyrosine (3-NT) content in heart] (image)

![Graph B: 3-Nitrotyrosine (3-NT) content in kidney] (image)

![Graph C: 3-Nitrotyrosine (3-NT) content in liver] (image)

**Fig. 7.** 3-Nitrotyrosine (3-NT) content in heart (A), kidney (B), and liver (C) tissue samples (n = 4 samples/group). Levels of 3-NT were not significantly different across groups in any of the organs. Values are means ± SE.
studies (27, 29, 40) have shown a beneficial hemodynamic profile of this compound compared with organic nitrates in patients with angina pectoris, ischemic heart disease, and heart failure, even when tolerance to organic nitrates was present. In patients with heart failure, an infusion of Mols for 24 h induced an increase in cardiac output that was not seen with a similar infusion of organic nitrates (27).

A limitation of our study is that we did not assess the effects of Mols in control rats. Lusitropic effects of NO have been documented in normal hearts (21, 33). However, cardiac and renal function are, by definition, optimal in control rats, and it is unlikely that Mols treatment would have led to a further significant increase in cardiac function and that the observed effects of Mols treatment in rats with cardiorenal failure are unspecific. To our knowledge, we are the first to study the role of reduced NO availability and NO supplementation on in vivo cardiac function in CKD in a model of cardiorenal failure. Mols, or other tolerance-free nitrates, may be a future therapeutic option for patients with cardiorenal disease.

Effects of Mols on cardiac histology and mRNA expression. Longitudinal assessment of calculated LV mass from cardiac sonography showed that in Veh-treated rats LV hypertrophy progressed, while this appeared to be halted in Mols-treated rats. These differences were confirmed by crude terminal organ weights. Although these differences disappeared when values were corrected for body weight, we found that tibia length was similar in Veh- and Mols-treated groups, indicating that growth was not affected and the differences in body weight were likely related to changes in body composition.

Cardiomyocyte-restricted overexpression of eNOS was found to attenuate LV hypertrophy and cardiomyocyte hypertrophy in mice with pressure overload (11), and treatment of spontaneously hypertensive rats with Mols significantly reduced heart weight (26). In a model of aortic constriction in rats, the NO donor LA-419 reduced cardiomyocyte hypertrophy but also lessened cardiac patchy and perivascular fibrosis (37). Conversely, H-ISDN treatment did not prevent the development of cardiac fibrosis in aldosterone-infused mice compared with untreated aldosterone-infused mice (45). In our study, the extent of cardiac collagen deposition was also not different in hearts of Mols-treated rats versus Veh-treated rats. Both the unchanged fibrosis and EDPVIR suggest that passive diastolic properties of the heart were not significantly affected by Mols treatment. The slightly lower cardiac expression of ANP in the Mols-treated group is in line with the reduced loading conditions observed with echocardiography and hemodynamic assessment.

Expression in cardiac tissue of constitutive NOS isoforms (eNOS and nNOS) was not decreased in either the Veh- or Mols-treated groups compared with the control group, suggesting that at least reduced cardiac expression did not play a significant role in the persistent LVSD. The expression of nNOS was even significantly higher than the control group in the SNX (+ l-NNA) + Veh-treated group, which has also been documented in rats with heart failure (4). On the other hand, Mols treatment reduced iNOS expression, which is associated with worsened damage and cardiac function (39).

3-NT protein assay. We measured 3-NT protein levels in different tissues (heart, liver, and kidney) to assess the amount of nitrosative stress due to peroxynitrite formation occurring in each study group and found these to be similar across groups in each organ, even in the liver, where SIN-1, the active metabolite of Mols, is generated. Peroxynitrite has been implicated in the pathophysiology of cardiac dysfunction (32) and may have been increased in our model. Although SIN-1 can generate peroxynitrite in vitro, it appears to function solely as an NO donor at in vivo O2 concentrations (17, 38). We also found no increase in 3-NT formation, whereas levels of NO metabolite excretion (and hence production) were greatly increased by Mols treatment (Fig. 3).

Conclusions. Supplementation of NO with the tolerance-free NO donor Mols improved cardiac systolic and diastolic function and improved survival in a rat model of CKD, LVSD, and low NO availability. It also ameliorated LV hypertrophy, reduced tubulointerstitial injury in the kidney, and improved creatinine clearance. Our study suggests that in CKD, cardiac function is strongly modulated by NO availability through actions on active diastolic relaxation and systolic ejection with minor effects on afterload. Although a reduction of NO availability is widely regarded as one of the hallmarks of CKD (3), the effect of direct supplementation has not yet been investigated in detail. The absence of significant effects on systemic arterial pressure and the resistance to tolerance make Mols an attractive adjuvant therapeutic option, for example, in hemodialysis patients, in whom abnormalities of cardiac function are often seen and associated with an adverse outcome (47). Supplementation of NO with Mols might thus help support cardiac function in patients with advanced CKD and LVSD or heart failure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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