Cardiovascular Effects of Inhaled Nitric Oxide in a Canine Model of Cardiomyopathy

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Background. The inhalation of nitric oxide (NO) in patients with heart failure decreases pulmonary vascular resistance (PVR) and is associated with an increase in pulmonary artery wedge pressure (PAWP). The mechanism for this effect remains unclear.

Methods. In dogs rapid-paced for 8 weeks to induce cardiac dysfunction, we performed left ventricular pressure-volume analysis of unpaced hearts in situ to determine whether during NO inhalation (80 ppm), the mechanism for the rise in PAWP is due to: 1) primary pulmonary vasodilation; 2) a direct negative inotropic effect; or 3) impairment of ventricular relaxation.

Results. Inhalation of NO decreased PVR by 51% ± 3.8% (257 ± 25 vs 127 ± 18 dynes · sec · cm⁻⁵ [NO 80 ppm]; p < 0.001) and increased PAWP (15.4 ± 2.4 vs 18.1 ± 2.6 mm Hg [NO 80 ppm]; p < 0.001). Calculated systemic vascular resistance remained unchanged. Left ventricular (LV) end-diastolic pressure rose (16.4 ± 1.9 vs 19.1 ± 1.8 mm Hg [NO 80 ppm]; p < 0.001), as did LV end-diastolic volume (83.5 ± 4.0 vs 77.0 ± 3.4 mL [NO 80 ppm]; p = 0.006). LV peak +dP/dt was unchanged by NO (1,082 ± 105 vs 1,142 ± 111 mm Hg/sec [NO 80 ppm]; p = NS). There was a trend toward a stroke volume increase (17.4 ± 1.2 vs 18.8 ± 1.3 mL; p = NS), but the relaxation time constant and end-diastolic pressure-volume relation were both unchanged.

Conclusions. In this canine model of cardiomyopathy, inhaled NO decreases pulmonary vascular resistance. The associated increase in left ventricular filling pressure appears to be secondary to a primary pulmonary vasodilator effect of NO without primary effects on the contractile or relaxation properties of the left ventricle.


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quenty administered into a limited ventilation circuit, and all hemodynamic measurements were made after a 10-min period of equilibration.

All animals used in this study received care in compliance with “Guides for Care and Use of Laboratory Animals,” published by National Institutes of Health (NIH Publ. 86-23, rev. 1985). This investigation was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

**Anesthesia**

All animals were anesthetized with continuous intravenous hydromorphone (0.5 mg/kg/h) and diazepam (0.5 mg/kg/h). Autonomic blockade was established with continuous intravenous atropine (2 μg/kg/min) and esmolol (400 μg/kg/min). All baseline and subsequent inhalation hemodynamics were performed with the chest closed.

**Baseline Hemodynamics (pre-RVP)**

After induction of general anesthesia, a 7 Fr multi-electrode dual-field conductance catheter (Wester Labs, Baldwin Park, CA) and a 5 Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) were placed under fluoroscopic guidance along the long axis of the LV cavity via sterile cut-down of the carotid and right femoral arteries. Similarly, via the right jugular and right femoral veins, 20-mL occlusion catheters (Applied Vascular, Laguna Hills, CA) were placed at the superior and inferior vena cavae and right atrial junctions. A 7 Fr pulmonary artery flotation catheter (Arrow International, Inc, Reading, PA) was placed in the pulmonary artery via the left internal jugular vein. Volume measurements were obtained using a conductance catheter, which has previously been described [4–6]. All hemodynamic signals and the electrocardiogram were conditioned with electronic amplifiers (Gould Inc, Cleveland, OH), digitized at 250 Hz and saved to disk. Cardiac output was determined by thermodilution and by conductance catheter techniques. Steady-state values for heart rate, arterial pressure, pulmonary arterial pressure, pulmonary artery wedge pressure, LV systolic pressure, LV end-diastolic pressure, and LV peak +dP/dt was calculated by averaging at least 50 beats under experimental conditions.

All data were collected with the ventilator held at end-expiration. For determination of the end-systolic pressure-volume relation (ESPVR), end-diastolic pressure-volume relation (EDPVR) and stroke work–end diastolic volume relations, the 20-mL balloons in the vena cavae were temporarily and gradually inflated to decrease preload as described previously [5]. Data were collected from a steady-state period and each preload reduction period, a minimum of 3 times with at least 1 min of recovery between each sample collection.

**Pacemaker Insertion**

After a minimum recovery period of 3 days after baseline hemodynamic measurements, each dog underwent placement of a specially modified ventricular pacemaker (Model 8342; Medtronic Inc, Minneapolis, MN) designed to maintain prolonged pacing at over 200 bpm. The dogs were placed under general anesthesia and under sterile conditions, through a right anterior thoracotomy, a 2 × 3-cm section of apical pericardium was excised and a unipolar pace lead (Model 6917A; Medtronic Inc) was secured to the LV apex. All animals were allowed to recover for at least 7 days, after which the pacemakers were programmed to pace at a rate of 215 bpm for 8 weeks.

**Hemodynamic Assessment With and Without Inhaled NO**

After 8 weeks of RVP, animals were anesthetized and maintained with full autonomic blockade as previously described. No digitalis or diuretics were administered on the morning of catheterization. Instrumentation and placement of catheters in these animals followed the pre-RVP baseline hemodynamic protocol. All hemodynamic measurements were performed with the RVP suspended.

To establish baseline conditions, animals inhaled room air fractional inspired oxygen concentration (FIO2) (FIO2 = 19%; N2 = 81%) via the endotracheal system for 10 min before the hemodynamic measurements. Animals then inhaled NO (using methods previously described [1]) at 80 ppm for 10 min, and hemodynamic measurements were repeated with data analysis. Hemodynamic data were analyzed off-line using custom software. Analysis of steady-state signals provided heart rate (HR), stroke volume (SV), cardiac output (CO), stroke work (SW), LV peak and end-diastolic pressures (LVSP and LVEDP), maximum rates of pressure change (max +dP/dt), pulmonary artery pressure, and the time constant of isovolumic pressure decay (τ). Tau was calculated by fitting the data to a monoexponential decay as described by Raff and Glantz [7], which also fit the asymptotic pressure. Conductance catheter signals were calibrated to the estimates of LV volumes derived from echocardiograms obtained the prior day. Generation of ESPVR, EDPVR, and stroke work–end diastolic volume relation was derived from data obtained during the inflow inclusion periods. All data in which there was a change in HR > 5% were discarded to minimize the effects of cardiovascular reflexes.

The ESPVR data was fit using an iterative algorithm that finds the end-systolic point from each cardiac cycle that maximizes the slope of a line tangent to the cardiac pressure-volume loop, and using those points to regress a linear ESPVR. This relation is used to choose a new end-systolic point for a new linear regression, and the process is repeated until there is no further change [5]. The linear regression equation was used to predict end-systolic volumes at pressures over the range in which data had been collected. The values of these volumes were computed at 1-mm Hg intervals, and the data pooled from the pre-NO state and the NO state. From these data, the effect on the ESPVR by NO was determined. The stroke work–end-diastolic volume relation was determined by computing the work per cardiac cycle.
as the area enclosed by the traversed cardiac loop in the pressure-volume plane. The slope of this relation was computed using a linear regression of these stroke work values versus the corresponding EDV.

The EDPVR was fit using custom software based upon a nonlinear Levenberg-Marquardt regression technique to fit the data from the diastolic period from each loop [8]. Data from individual data sets obtained from venous balloon inflation was fit to the model: \( P(V) = P_0 + A(e^{B(P/V)} - 1) \), where \( P_0, A, \) and \( B \) are the curve fit parameters. This allowed the reconstruction of a model LVEDPVR for each animal without and with NO by varying EDV and computing the predicted EDP. Composite relations were created by pooling the predicted LVEDP from corresponding conditions at 1-mL increments over a physiologic volume range. For comparisons of relations without and with NO, the difference of LVEDP was predicted from the regressed values from the two conditions for each animal, and the \( t \) test used to compare pressures at 10-mL intervals.

Statistical Analysis

Statistical analyses were performed using a commercial statistical package (SigmaStat; Jandel Scientific, San Rafael, CA). All data were expressed as mean ± standard error of the mean, and the paired \( t \) test was used to test for differences between the groups. Differences were considered significant at the 0.05 probability level.

Results

Hemodynamics of Canine RVP-Cardiomyopathy Model

Baseline hemodynamic before and after 8 weeks of RVP, in these 7 dogs, revealed moderate LV systolic dysfunction, characterized by elevated LVEDP (4.1 ± 0.5 mm Hg [pre-RVP] vs 16.4 ± 1.9 mm Hg [post-RVP]), elevated PAWP (4.5 ± 0.4 mm Hg [pre-RVP] vs 15.4 ± 2.4 mm Hg [post-RVP]), decreased stroke volume (33.6 ± 2.6 mL [pre-RVP] vs 17.4 ± 1.2 mL [post-RVP]), and decreased cardiac output (4.0 ± 0.3 L/min [pre-RVP] vs 1.9 ± 0.1 L/min [post-RVP]), and a modest elevation of CVP (3.5 ± 1.5 vs 4.1 ± 2.2 mm Hg). Moderate pulmonary hypertension was present with a mean PVR of 257 ± 25 dyne·sec·cm\(^{-5}\). LV peak +dP/dt fell from 1,654 to 1,081 mm Hg/sec, and end-systolic elastance (Ees) from 2.6 to 1.5 mm Hg/mL with 8 weeks of pacing. The stroke work–EDV relation had a decreased slope (53.2 ± 3.5 vs 32.5 ± 2.9 mm Hg; \( p = 0.001 \)) after RVP. Lusitropy was also delayed, as demonstrated by the prolonged \( \tau \) (29.5 ± 2.4 vs 63.0 ± 2.8 msec). Pressure-volume analysis in these animals revealed significant LV systolic dysfunction, as demonstrated by the rightward displaced ESPVR and the decreased slope of the ESPVR (\( p < 0.001 \); Fig 1).

Hemodynamic Effects of Inhaled NO

Inhalation of NO caused no change in heart rate, mean systemic arterial pressure, systemic vascular resistance, or pulmonary artery (systolic, diastolic, or mean), but caused a 16% ± 7% increase in the mean PAWP (Table 1; 15.4 ± 2.4 mm Hg [post-RVP baseline without NO] vs 18.1 ± 2.6 mm Hg [NO 80 ppm]; \( p < 0.001 \)). There was an increase in CVP (Table 1; 4.1 ± 1.4 mm Hg [post-RVP baseline without NO] vs 5.1 ± 0.9 mm Hg [NO 80 ppm];

**Table 1. Canine Cardiomyopathy Hemodynamics**

<table>
<thead>
<tr>
<th>Hemodynamic Parameter</th>
<th>Pre-RVP Baseline</th>
<th>Post-RVP Baseline</th>
<th>Post-RVP NO 80 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>120.1 ± 5.5</td>
<td>104.6 ± 5.2</td>
<td>105.9 ± 4.3</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>76.3 ± 4.7</td>
<td>73.8 ± 4.5</td>
<td>76.4 ± 4.7</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>3.5 ± 1.5</td>
<td>4.1 ± 1.2</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>10.3 ± 0.8</td>
<td>22.3 ± 2.4</td>
<td>22.4 ± 2.5</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.1 ± 0.5</td>
<td>16.4 ± 1.9</td>
<td>19.1 ± 1.8</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>4.5 ± 0.4</td>
<td>15.4 ± 2.4</td>
<td>18.1 ± 2.6</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>4.0 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>PVR (dyne·sec·cm(^{-5}))</td>
<td>117 ± 12</td>
<td>257 ± 25(^{b})</td>
<td>127 ± 18(^{a})</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>33.6 ± 2.6</td>
<td>17.4 ± 1.2</td>
<td>18.8 ± 1.3</td>
</tr>
<tr>
<td>EDV (mL)</td>
<td>49.1 ± 2.5</td>
<td>77.0 ± 3.4</td>
<td>83.5 ± 4.0(^{d})</td>
</tr>
<tr>
<td>+dP/dt (max mm Hg/sec)</td>
<td>1,654 ± 141</td>
<td>1,081 ± 103(^{p})</td>
<td>1,142 ± 111</td>
</tr>
<tr>
<td>Ees (mm Hg/mL)</td>
<td>2.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>( \tau ) (ms)</td>
<td>29.5 ± 2.4</td>
<td>63.0 ± 2.8</td>
<td>63.1 ± 4.1</td>
</tr>
<tr>
<td>PRSW (mm Hg)</td>
<td>53.2 ± 3.5</td>
<td>32.5 ± 2.9</td>
<td>32.9 ± 3.2</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \); pre-RVP vs post-RVP baseline. \( ^{b} p < 0.01 \); pre-RVP vs post-RVP baseline. \( ^{c} p < 0.001 \); pre-RVP vs post-RVP baseline. \( ^{d} p < 0.05 \); post-RVP baseline vs post-RVP NO 80 ppm. \( ^{e} p < 0.01 \); post-RVP baseline vs post-RVP NO 80 ppm. \( ^{f} p < 0.001 \); pre-RVP vs post-RVP NO 80 ppm.

CO = cardiac output; +dP/dt = maximum derivative of left ventricular pressure; EDV = end-diastolic volume; Ees = end-systolic elastance; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; MAP = mean arterial pressure; PAP = mean pulmonary arterial pressure; PAWP = mean pulmonary arterial wedge pressure; PRSW = slope of stroke work–end-diastolic volume relation; PVR = mean pulmonary vascular resistance; SV = stroke volume; \( \tau \) = time constant of isovolumic left ventricular pressure decay.

Fig 1. LV pressure-volume loops and regressed ESPVR from 1 animal before rapid ventricular pacing at baseline (left) and after rapid ventricular pacing with heart failure before inhalation of NO (right). Heart failure in these animals was accompanied by a rightward shift in LV volume relations and a decrease in the ESPVR slope (Ees).
There was also an increase in stroke volume, which did not reach statistical significance (17.4 ± 1.2 vs 18.8 ± 1.3 mL; p = NS). Cardiac output by thermodilution remained unchanged at 1.9 L/min. The mean PVR decreased by 51% ± 10% (Table 1; 257 ± 25 vs 127 ± 8 dynes · sec · cm⁻² [NO 80 ppm]; p < 0.001).

**Effects of Inhaled NO on LV Function**

Heart rate was not affected by either preload reduction or inhaled NO, demonstrating effective autonomic blockade (data not shown). The LV peak +dP/dt was unchanged by NO (1,082 ± 105 mm Hg/sec [post-RVP baseline without NO] vs 1,142 ± 111 mm Hg/sec [NO 80 ppm]; p = NS), despite a significant increase in LV end-diastolic pressure (16.4 ± 1.9 mm Hg [post-RVP baseline without NO] vs 19.1 ± 1.8 mm Hg [NO 80 ppm]; p < 0.001). Isovolumic pressure decay time constant τ was similarly unaffected by NO (63.0 ± 2.8 msec [post-RVP baseline without NO] vs 63.1 ± 4.1 ms [NO 80 ppm]; p = NS). A representative set of LV loops and the resultant ESPVRs from one animal are shown in Figure 2. No significant shift of the ESPVR by NO was observed. The results for all animals studied are shown in Figure 3, which demonstrates that the slope of the ESPVR (Ees) did not change after inhalation of NO (Fig 2; 1.56 ± 0.14 mm Hg/mL for both conditions). The right portion of Figure 3 shows the composite ESPVRs, which appear parallel, but rightward shifted with NO. The line at the left shows the volume shift with the administration of NO, and it shows less than a 2-mL shift. At no pressure was the rightward shift of the ESPVR significant. There was also no significant change of the slope of the stroke work–end-diastolic volume relation (preload recruitable stroke work [PRSW]; 32.5 ± 2.9 mm Hg [post-RVP baseline without NO] vs 32.9 ± 3.2 mm Hg [NO 80 ppm]; p = NS). Finally, LV end-diastolic volume increased after inhalation of NO, as measured directly by the conductance catheter (Table 1; 77.0 ± 3.4 mL [post-RVP baseline without NO] vs 83.4 ± 4.0 mL [NO 80 ppm]; p = 0.006). The increased LV end-diastolic volume led to a stroke volume increase, but statistical significance was not reached.

The effects of inhaled NO on the EDPVR are shown in Figure 4. The curves represent the composite EDPVR predicted from the curve fit relation from all 7 animals before NO (top), and that from data collected during the administration of NO (bottom). It shows that there is a small downward shift of the EDPVR with NO. The difference of predicted pressure from the curve fit relations was calculated, and the standard deviation of the difference computed. The error bars shown on the baseline curve are those standard deviations of the difference, shown at 10-mL increments. There was a slight depression of the EDPVR by NO, but at no volume was the pressure difference significant.

**Comment**

In our previous study in patients with advanced heart failure, we observed that inhalation of NO was not associated with a decrease in pulmonary artery pressures. Rather, the decrease in PVR reflected a decrease in the transpulmonary pressure gradient due entirely to an increase in PAWP. Semigran and associates [9] also reported that inhaled NO resulted in an increase in PAWP in patients with heart failure. The absence of either an increase in PAWP or a decrease in PA pressures in a subset of heart failure patients with hemodynamically “compensated” heart failure in our previous study suggests that a mechanism other than primary vasodila-
tion could be responsible for the observed PVR-lowering effects of inhaled NO.

The decrease in the transpulmonary artery pressure gradient remains most consistent with a pulmonary vasodilator effect of inhaled NO. The mechanism responsible for the pulmonary vasodilator effect of inhaled NO appears to be via diffusion of NO gas to the vascular smooth muscle of the pulmonary resistance vessels, thereby causing a direct vasodilator effect. The lack of a change in pulmonary artery pressure with NO must be the result of shifts of blood volume from the pulmonary arterial vasculature to the pulmonary venous circuit. The rise in LVEDP is probably the result of a small increase in pulmonary flow (though not statistically significant) and the minimal reserve of the failing LV to increase stroke volume with elevations of EDP when filling is already high. Hence, the administration of NO resulted in increased LV end-diastolic volume and pressure. In this regard, it is reasonable to propose that pulmonary vasodilation with inhaled NO increases transpulmonary venous return to the failing left ventricle, and results in LV distention, increased PAWP, and increased LVEDV and LVEDP.

A recent study examining the hemodynamic effects of inhaled NO, by Hare and associates [10], in patients with advanced heart failure, supported with a left ventricular assist device (LVAD), demonstrated results that support the primary pulmonary vasodilator effects of inhaled NO in humans. In their patients, when the LVAD stroke volume output was kept fixed, inhaled NO resulted in a rise in left atrial pressure (LAP). When the LVAD stroke volume output was allowed to vary dynamically in response to changes in venous return, the pump stroke volume output rose after inhalation of NO, without measurable changes in LAP. This result does not rule out a direct myocardial depression as a possibility. However, in the patients studied who were completely reliant on the LVAD for cardiac output, a rise in PAWP was observed after inhalation of NO, similar to the effects observed in patients with advanced heart failure [9] and in the canine cardiomyopathy model that we report here.

Our results are consistent with the recent report by Argenziano and associates [2] in normal pigs. In their experiments, NO was administered to open-chest pigs with normal cardiac function. A thromboxane A2 analog was infused systemically to transiently induce pulmonary hypertension. Before the administration of the thromboxane analog, NO at 20, 40, and 80 ppm failed to significantly alter LV function. This is surprising because it has been reported that a NO antagonist augments β-receptor responsiveness [3]. After the infusion of the thromboxane A2 analog with resultant elevation of pulmonary artery pressures, NO appeared to improve LV function. This change was too little to be statistically significant. However, the curvilinearity of their ESPVR precludes acceptable comparisons simply based on statistical comparisons of the regressed ESPVR slope.

An alternative explanation for the vasodilator effect of inhaled NO is that the decrease in transpulmonary gradient results from passive recruitment of precapillary vessels because of increased pulmonary venous pressure that is caused by a primary effect of inhaled NO to increase LV filling pressure. Passive pulmonary vasodilation has been demonstrated in animal experiments [11], but has not been described in humans. This possibility would require that inhaled NO exert a direct effect on myocardial or cardiac function, resulting in an increase in LV filling pressure. The increase in PAWP after inhalation of NO does not appear to be secondary to a significant negative inotropic effect on LV function. This conclusion is supported by the lack of decrease in LV end-systolic elastance, slope of the stroke work–LV end-diastolic volume relationship, and LV peak +dP/dt. There were, in fact, increases in all of the parameters. The lack of change in $\tau$, in the setting of an unchanged LV peak +dP/dt, further suggests that inhaled NO does not exert a significant negative lusotropic effect on the myocardium. No detectable change was found in the LV end-diastolic pressure–volume relationship either. When considering only the steady-state data in Table 1, it is seen that the mean LV end diastolic volume increased 6.4 mL, but the end-diastolic pressure increased only 2.8 mL with NO. The slope of the EDPVR at this portion of the curve is steeper from each of the curves shown in Figure 3 than these data indicate. Therefore, these data are also consistent with there being a small rightward shift of the EDPVR with inhaled NO, but less than is detectable by the curve-fitting technique used to determine the LVEDPVR. An explanation for this is that with an elevated right ventricular afterload, small decreases in that afterload lower the passive (diastolic) LV elastance [12], presumably through ventricular interdependence.

In this canine RVP model of cardiomyopathy, inhalation of NO: 1) decreased PVR; 2) had no hemodynamically significant effects on the contractile or relaxation...
properties of the LV; and 3) resulted in a significant increase in LV end-diastolic pressure and volume. The decrease in transpulmonary gradient with no change in the contractile or relaxation properties of the LV is most consistent with a primary and selective pulmonary vasodilator effect of inhaled NO. These observations suggest that NO-dependent vasodilation is inadequate in heart failure relative to the activity of vasoconstrictor factors present.

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