Basic Science Reports

Pharmacological Strategies for Improving Diastolic Dysfunction in the Setting of Chronic Pulmonary Hypertension

Edward P. Chen, MD; Damian M. Craig, MS; Hartmuth B. Bittner, MD, PhD; R. Duane Davis, MD; Peter Van Trigt, MD

Background—Right ventricular (RV) hypertrophy is an adaptive process that occurs in the setting of chronic pulmonary hypertension (CPH) and can lead to alterations in normal RV diastolic properties. This study was designed to investigate the effects of NO and milrinone on RV diastolic dysfunction in the setting of CPH and RV hypertrophy by use of a canine model of monocrotaline pyrrole (MCTP)–induced CPH.

Methods and Results—Sixteen mongrel dogs (22 to 24 kg) were used. Animals underwent percutaneous pulmonary artery (PA) catheterization to measure pulmonary hemodynamics before and 8 weeks after injection of 3 mg/kg MCTP (n=8) or placebo (control, n=8). Eight weeks after injection, all hearts were instrumented with a PA flow probe, sonomicrometric dimension transducers, and micromanometers. Data were collected at baseline and after both NO and milrinone administration. Diastolic properties were quantified by use of the end-diastolic pressure-volume relationship and the time constant of ventricular isovolumic relaxation. Eight weeks after injection, significant increases in the PA pressure and pulmonary vascular resistance were observed in MCTP dogs. Significant worsening of RV diastolic function occurred in association with significant increases in the ratio of RV dry weight to LV+septal dry weight. NO and milrinone administration both led to significant improvements in RV diastolic properties.

Conclusions—In the setting of MCTP-induced CPH, significant worsening of RV diastolic function was observed in association with significant increases in the ratio of RV dry weight to LV+septal dry weight, suggesting that these changes are partially due to RV hypertrophy. The significant improvement in RV diastolic properties after both NO and milrinone administration suggests that these agents may be effective forms of pharmacological therapy for improving RV diastolic dysfunction in the setting of CPH. (Circulation. 1998;97:1606-1612.)

Key Words: hypertension, pulmonary • diastole • ventricles • hypertrophy

The clinical management of diastolic dysfunction is challenging, and despite the existence of several therapeutic options, no single treatment is universally recommended. Contemporary clinical strategies for improving abnormal ventricular diastolic properties include the use of pharmacological agents that increase ventricular relaxation and ease cardiac loading conditions. These strategies, however, have been applied primarily toward treating LV diastolic dysfunction in the setting of systemic arterial hypertension and myocardial hypertrophy. Few reports describe the application of these measures with respect to improving RV diastolic dysfunction associated with cardiac hypertrophy in the setting of CPH.

Nitric oxide is not only a selective pulmonary vasodilator without significant hemodynamic effect on the systemic circulation but also an important mediator of cardiac diastolic function. Milrinone, conversely, is a phosphodiesterase inhibitor that possesses positive inotropic and selective pulmonary vasodilatory effects. In addition to these properties, milrinone is also a positive inotropic agent. Such properties suggest that both these agents may be potentially useful strategies for improving RV diastolic dysfunction in the setting of chronically elevated pulmonary vascular pressures. However, an appropriate large-animal model of CPH and RV hypertrophy has not previously been available for basic investigation of this problem. This study was therefore designed to investigate the effects of NO and milrinone on RV diastolic dysfunction in the setting of CPH and RV hypertrophy by use of a canine model of MCTP-induced CPH and functional assessment of RV chamber stiffness.

Methods

Hemodynamic Monitoring, Experimental Groups, and Drug Synthesis and Injection

The anesthetic regimens, mode of ventilatory support, and all invasive hemodynamic and metabolic monitoring used in this invest...
Experimental animals (MCTP, n=8, 22 to 24 kg) received an injection of 3 mg/kg MCTP dissolved in N,N-dimethyl formamide at a concentration of 40 mg/mL, and control animals (n=8, 22 to 24 kg) received dimethyl formamide as placebo. Consequently, the volume injected was dependent on the individual weight of each animal and varied from 1.65 to 1.8 mL. MCTP was synthesized by a previously well-established method.11

Institutional and Surgery

Eight weeks after MCTP injection, a standard median sternotomy and an anterior pericardiotomy were performed to expose the hearts of all animals. An ultrasonic flow probe (T208X, Transonic Systems Inc) was placed around the main pulmonary trunk to measure pulmonary blood flow. Micromanometers (MPC-500, Millar Instruments Inc) were placed into the RV and LV, right and left atrium, and pulmonary artery for continuous recording of intracavitary pressures. Hemispheric ultrasonic dimension transducers (1.5 mm OD, No. 1-1015-5A, Vernitron) were sewn to the epicardial surface of the heart across the base-apex major-axis and anteroposterior minor-axis diameters of the LV. Two additional transducers were also placed on the epicardial surfaces of the RV and LV free walls, and another was inserted into the interventricular septum to measure the septal-free wall minor-axis diameters of both the RV and LV.

Experimental Protocol During Pharmacological Studies

Eight weeks after MCTP injection, baseline functional and hemodynamic data were collected in every animal in the control and MCTP groups after the instrumentation procedure. Control animals did not receive any pharmacological treatment or undergo any further data collection. In MCTP animals, inhaled NO (NO, 777 ppm and NO2, <0.1 ppm, National Specialty Gases) was then administered into the ventilator at levels of 40 and 80 ppm, and data were again collected at each respective concentration. NO and NO2 levels were measured by continuous chemiluminescent analysis (model 42H, Thermo Environmental Instruments, Inc).

After each incremental change in the NO concentration, the animal's condition and hemodynamic parameters were allowed to equilibrate for 10 minutes before any further data collection. After data were obtained at the dose of 80 ppm, NO was stopped, and the level in the ventilator's inspiratory circuit was restored to 0 ppm. Pulmonary hemodynamic parameters were allowed to return to baseline, which usually occurred within 5 to 8 minutes of discontinuation.

Milrinone was then loaded as an initial dose of 50 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) given over 10 minutes and subsequently infused at rates of 0.5 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) and 1.0 \( \mu g \cdot kg^{-1} \cdot min^{-1} \). As with NO, after each incremental increase in the milrinone infusion rate, pulmonary hemodynamic parameters were permitted to stabilize for 10 minutes before additional data acquisition. The entire duration of anesthesia, including instrumentation, drug administration, and all data acquisition, was \( \approx 2.5 \) to \( 3 \) hours in each MCTP animal.

Raw data were digitized on-line, collected, and stored on a microprocessor (PDP 11/23, Digital Equipment Corp). All data were analyzed on a Dell Dimension XPS P90 computer (Dell Computer Corp) with software that has previously been well described.

Tissue Analysis

After collection of hemodynamic and functional data, all hearts were excised, and the atria were separated from the ventricles at the atroventricular groove. The RV free wall was separated from the LV and septum. The RV and LV from each animal were weighed, and wall volumes were then determined by saline displacement. To calculate percent water, all samples were placed in a 120°C oven and dried for 24 hours. The dry weight of each tissue sample, for both the RV and LV+septum, was measured, and these weight ratios were compared between experimental groups. The percentage of tissue water content was calculated from the following equation: (wet weight−dry weight ×100%)/wet weight.

Calculation of Ventricular Cavity Volumes

LV and RV cavity volumes were calculated by use of validated models.23 LV cavity volume was calculated by representing the epicardial surface of the LV as an ellipsoidal shell and subtracting ventricular wall volume according to the following equation: \( L V V = \pi b^2a/6 - V_{wall} \), where \( b \) is the minor-axis diameter, \( a \) is the major-axis diameter, and \( V_{wall} \) is LV wall volume.22 RV cavity volume was calculated according to the ellipsoidal shell subtraction method,13 which assumes an ellipsoidal shape for the LV as well as the total biventricular shell, by the following equation: \( R V V = BV V_{epi} - LV V_{epi} - FW \), where \( BV V_{epi} \) is the biventricular epicardial shell volume, \( LV V_{epi} \) is the LV epicardial shell volume, and FW is the volume of the RV free wall.13

Assessment of Biventricular Diastolic Properties

Chamber stiffness was quantified by fitting the exponential function \( FDP = AC \cdot e^{CPC} \) to EDPs and EDVs obtained during venal cavo occlusion for both the RV and LV by use of nonlinear least-squares regression. \( \alpha \) is defined as the change in the logarithmic function of ventricular pressure relative to the change in volume and represents an index of ventricular chamber stiffness, whereas \( A \) is a proportionality constant.

A parameter of myocardial stiffness was quantified according to the following equation: myocardial stiffness = \( \alpha V_{es} \), where \( \alpha \) is chamber stiffness and \( V_{es} \) is ventricular wall mass.16

To assess ventricular isovolumic pressure decay, the period of isovolumic relaxation was defined from the nadir of the ventricular pressure derivative (dP/dtmin) to the point at which ventricular pressure decreased to the level of atrial pressure.12 The time constant of isovolumic pressure decay (\( \tau \)) was then calculated from the following monoeponential model: \( P_{iv} = P_{iv0} e^{-t/\tau} + P_s \), where \( P_s \) is the initial pressure above \( P_e \) during isovolumic relaxation, \( \tau \) is time, \( P_s \) is the asymptote to which pressure decays, and \( \tau \) is the time required for pressure at any point on the isovolumic curve to fall to 1/e of its original value.19 RV/LV model parameters were estimated by nonlinear least-squares regression.

Assessment of Biventricular Systolic Function

RV and LV systolic function were assessed with load-insensitive means. The highly linear relationship between stroke work and end-diastolic chamber volume was calculated from data acquired during vena cava occlusion by use of least-squares linear regression. The slope of these linear regressions is known as the PRSW and represents a load-independent index of systolic function and myocardial contractility.18

Experimental Approval and Animal Rights

The experimental setup and procedures conformed to the guidelines established by the American Physiological Society and the National Institutes of Health publication 86–23, revised 1985. The experiments described in this report were approved by the Duke University Institutional Animal Care and Use Committee (DUA-ACUC Assigned Registry A621–95–9R1).
**Pharmacological Strategies for Diastolic Dysfunction**

**TABLE 1. Hemodynamic Measurements Before and 8 Weeks After MCTP or Placebo Injection**

<table>
<thead>
<tr>
<th></th>
<th>HR, bpm</th>
<th>RAP, mm Hg</th>
<th>sRVP, mm Hg</th>
<th>dRVP, mm Hg</th>
<th>mPAP, mm Hg</th>
<th>PCWP, mm Hg</th>
<th>CO, mL/min</th>
<th>PVR, dyne · s · cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>91 (5)</td>
<td>3.4 (0.4)</td>
<td>24.9 (0.7)</td>
<td>0.6 (0.2)</td>
<td>10.1 (0.6)</td>
<td>3.6 (0.3)</td>
<td>1278 (64)</td>
<td>407 (54)</td>
</tr>
<tr>
<td>MCTP (n=8)</td>
<td>85 (5)</td>
<td>3.1 (0.3)</td>
<td>25.0 (1.3)</td>
<td>0.8 (0.2)</td>
<td>9.7 (0.6)</td>
<td>4.1 (0.4)</td>
<td>1197 (73)</td>
<td>379 (65)</td>
</tr>
<tr>
<td><strong>8 weeks after injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>94 (5)</td>
<td>3.4 (0.4)</td>
<td>24.0 (0.4)</td>
<td>0.8 (0.2)</td>
<td>10.0 (0.5)</td>
<td>3.7 (0.3)</td>
<td>1344 (76)</td>
<td>421 (37)</td>
</tr>
<tr>
<td>MCTP (n=8)</td>
<td>89 (4)</td>
<td>5.6† (0.3)</td>
<td>33.3† (1.5)</td>
<td>4.1† (0.3)</td>
<td>20.2† (1.0)</td>
<td>5.7† (0.8)</td>
<td>1256 (69)</td>
<td>988† (72)</td>
</tr>
</tbody>
</table>

HR indicates heart rate; RAP, right atrial pressure; sRVP, right ventricular systolic pressure; dRVP, right ventricular end-diastolic pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; and PVR, pulmonary vascular resistance. Values are mean±SEM (in parentheses).

†P<.0001 vs control.

<table>
<thead>
<tr>
<th></th>
<th>NO, ppm</th>
<th>RAP, mm Hg</th>
<th>sRVP, mm Hg</th>
<th>dRVP, mm Hg</th>
<th>mPAP, mm Hg</th>
<th>PCWP, mm Hg</th>
<th>CO, mL/min</th>
<th>PVR, dyne · s · cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>5.6 (0.3)</td>
<td>33.3 (1.5)</td>
<td>4.1 (0.3)</td>
<td>20.2 (1.0)</td>
<td>5.7 (0.8)</td>
<td>1256 (69)</td>
<td>988 (72)</td>
</tr>
<tr>
<td>40</td>
<td>4.6† (0.2)</td>
<td>29.3† (2.2)</td>
<td>3.4 (0.4)</td>
<td>15.9† (0.9)</td>
<td>7.0 (0.4)</td>
<td>1465† (130)</td>
<td>551† (83)</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>4.5† (0.2)</td>
<td>31.1† (1.5)</td>
<td>3.5 (0.4)</td>
<td>15.7† (0.8)</td>
<td>7.5† (0.4)</td>
<td>1481† (102)</td>
<td>515† (60)</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis was performed with commercially available software (SigmaStat Version 2.0, Jandel Corp). Hemodynamic data obtained before and after injection of MCTP within the MCTP group were analyzed with standard two-tailed paired Student's t tests. Unpaired Student's t tests were used to compare all data between MCTP and control.

To test for significant changes in the parameters of diastolic function as well as systolic function after NO inhalation within the MCTP group, a one-way ANOVA for repeated measurements was used. Follow-up comparison of each index of ventricular diastolic properties at the individual levels of NO were done with paired Student's t tests. Comparison of changes in RV diastolic function occurring after milrinone infusion was performed in a similar fashion. The results are expressed as mean±SEM. A difference was considered statistically significant at P<.05.

**Results**

There was no significant difference in the baseline pulmonary hemodynamic indices between control and MCTP dogs; however, 8 weeks after MCTP injection, significant differences in these same indices were observed between the two groups (Table 1).

**Diastolic Properties in MCTP-Induced CPH**

The exponential EDP-EDV model accurately represented (r²>0.993) all pressure and volume data during transient vena caval occlusion in both groups. No significant differences in LV α were observed between control (.021±.005) and MCTP (.024±.005). There was, however, a significant increase in the RV α of MCTP (.070±.006, P<.05) compared with control (.046±.008). RV αVw was significantly greater in MCTP (.724±.066) than in control (.383±.068, P<.005), while no significant difference was observed in LV αVw between the two groups (.560±.108 versus .495±.105).

The monoexponential model of isovolumic pressure decay accurately predicted (r²>0.993) the fall of ventricular pressure during isovolumic relaxation for the RV and LV. There was no significant difference in LV τ (41±3 versus 42±3 ms) between the two experimental groups. RV τ was significantly longer, however, in MCTP (84±15 ms, P<.05) than in control (35±3 ms).

**Systolic Function in MCTP-Induced CPH**

Highly linear relationships (r²>0.95) were obtained between calculated RV and LV EDV and stroke work during vena caval occlusion in both control and MCTP 8 weeks after MCTP injection. There was no significant difference in LV PRSW between MCTP (94.65±6.26X10⁻³ erg/mL) and control (87.98±7.23X10⁻³ erg/mL). However, significant increase in RV PRSW were observed in MCTP (46.90±3.16X10⁻³ erg/mL, P<.0005) compared with the control values (24.56±3.12X10⁻³ erg/mL).

**Effect of NO on Diastolic Properties and Systolic Function**

NO led to significant improvements in pulmonary hemodynamic indices (Table 2). In addition, significant decreases occurred in RV α as well as RV αVw, whereas no significant improvements in these same parameters were observed in the LV (Table 3). RV and LV τ were significantly shorter after NO inhalation; however, RV/LV PRSW was not significantly different (Table 3). Although NO did not lead to significant changes in the LV α of MCTP dogs, a substantial downward shift was observed in the LV EDP-EDV relationship of these animals (Fig 1). The changes occurring in the EDP-EDV relationship as a result of NO inhalation for both the LV and RV are illustrated in Figs 1 and 2, respectively.

**TABLE 2. Effects of NO on Hemodynamic Measurements in MCTP-Induced Pulmonary Hypertension**

<table>
<thead>
<tr>
<th>NO, ppm</th>
<th>RAP, mm Hg</th>
<th>sRVP, mm Hg</th>
<th>dRVP, mm Hg</th>
<th>mPAP, mm Hg</th>
<th>PCWP, mm Hg</th>
<th>CO, mL/min</th>
<th>PVR, dyne · s · cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.6 (0.3)</td>
<td>33.3 (1.5)</td>
<td>4.1 (0.3)</td>
<td>20.2 (1.0)</td>
<td>5.7 (0.8)</td>
<td>1256 (69)</td>
<td>988 (72)</td>
</tr>
<tr>
<td>40</td>
<td>4.6† (0.2)</td>
<td>29.3† (2.2)</td>
<td>3.4 (0.4)</td>
<td>15.9† (0.9)</td>
<td>7.0 (0.4)</td>
<td>1465† (130)</td>
<td>551† (83)</td>
</tr>
<tr>
<td>80</td>
<td>4.5† (0.2)</td>
<td>31.1† (1.5)</td>
<td>3.5 (0.4)</td>
<td>15.7† (0.8)</td>
<td>7.5† (0.4)</td>
<td>1481† (102)</td>
<td>515† (60)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. Values are mean±SEM (in parentheses).

*P<.05 vs 0 ppm NO.
†P<.01 vs 0 ppm NO.
TABLE 3. Effects of NO on Biventricular Diastolic Properties and Systolic Function

<table>
<thead>
<tr>
<th></th>
<th>RV α</th>
<th>LV α</th>
<th>RV αVw</th>
<th>LV αVw</th>
<th>RV τ, ms</th>
<th>LV τ, ms</th>
<th>RV PRSW, erg x 10^6/mL</th>
<th>LV PRSW, erg x 10^6/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm NO</td>
<td>0.070 (0.006)</td>
<td>0.024 (0.005)</td>
<td>0.724 (0.066)</td>
<td>0.560 (0.108)</td>
<td>84 (15)</td>
<td>41 (3)</td>
<td>46.9 (3.2)</td>
<td>94.7 (6.3)</td>
</tr>
<tr>
<td>40 ppm NO</td>
<td>0.039* (0.008)</td>
<td>0.019 (0.004)</td>
<td>0.406* (0.086)</td>
<td>0.454 (0.093)</td>
<td>47* (5)</td>
<td>35* (1)</td>
<td>45.8 (2.4)</td>
<td>99.0 (7.0)</td>
</tr>
<tr>
<td>80 ppm NO</td>
<td>0.035f (0.005)</td>
<td>0.020 (0.004)</td>
<td>0.365f (0.052)</td>
<td>0.459 (0.096)</td>
<td>44* (5)</td>
<td>34* (2)</td>
<td>45.7 (2.3)</td>
<td>106.6 (6.0)</td>
</tr>
</tbody>
</table>

α indicates chamber stiffness; αVw, myocardial stiffness; and τ, time constant of isovolumic relaxation. Values are mean±SEM (in parentheses).

*P<.05 vs 0 ppm NO.
†P<.005 vs 0 ppm NO.

Effect of Milrinone on Diastolic Properties and Systolic Function

Milrinone infusion caused significant decreases in the pulmonary vascular resistance, which were associated with significant improvements in pulmonary blood flow (Table 4). Although there were decreases in the mean pulmonary artery pressure at 0.5 and 1.0 μg · kg⁻¹ · min⁻¹, these differences were not statistically significant.

Milrinone infusion also led to significant improvements in RV α, RV αVw, RV/LV τ, and RV/LV PRSW (Table 5). There was no significant change in LV α after milrinone infusion; however, as with NO, a substantial downshifting of the EDP-EDV relationship was also noted (Fig 3). The changes occurring in the EDP-EDV relationship after milrinone infusion for both the LV and RV are illustrated in Figs 3 and 4, respectively.

Tissue Analysis in the Setting of MCTP-Induced CPH

There was a significant increase in the RV dry weight of MCTP dogs (10.29±0.55 g, P<.05) compared with control animals (8.40±0.51 g). Both the ratio of RV dry weight to LV+septal dry weight (0.45±0.02 versus 0.37±0.01, P<.05) and the ratio of RV dry weight to body weight (0.43±0.02 versus 0.36±0.02 g RV/kg body wt, P<.05) were also significantly elevated in MCTP animals compared with the control group. No significant differences were observed in the LV dry weights (23.3±1.4 versus 23.3±1.3 g) or in the RV (79.6±0.4% versus 78.6±0.4%) and LV (79.0±0.4% versus 78.4±0.3%) water contents between the two experimental groups.

Discussion

CPH represents an important clinical sequela that commonly occurs as a result of chronic obstructive pulmonary disease. It is also associated with a number of other pathological processes, including long-standing congestive heart failure, mitral valve disease, congenital heart defects, and chronic pulmonary embolism; alternatively, CPH may exist as a primary process. This state of chronically elevated pulmonary vascular pressures can lead to adaptive mechanisms in the RV and eventually result in altered systolic and diastolic mechanics.

Myocardial hypertrophy is a compensatory process of the heart that can develop in the setting of a sustained pressure overload, such as CPH, and is perhaps one of the most important causes of diastolic dysfunction.4 Hypertrophied myocardium is particularly susceptible to diastolic dysfunction as a result of structural changes (increased LV mass) and impaired ventricular relaxation.6 In a previous investigation, a significant correlation was noted between myocardial hypertrophy and diastolic dysfunction, which later improved after regression of the hypertrophy.17

Approximately 10 years ago, NO was identified as an important endogenous biological mediator of vascular tone.8,19 Since that initial discovery, clinical and experimental investigations have shown that NO is a selective pulmonary vasodilator without significant effect on the systemic circulation. Recent reports have also suggested that NO is an important regulator of cardiac diastolic function.5,6 Milrinone is a phosphodiesterase inhibitor that possesses positive inotropic, selective pulmonary vasodilator, and positive lusitropic effects.8 In a canine model of embolism-induced pulmonary hypertension using autologous muscle, milrinone increased cardiac index while improving mean pulmonary artery pressure and pulmonary vascular resistance.30 Clinically, in the setting of congestive heart failure, significant improvements have been observed in the indexes of LV diastolic performance as well as overall global im-

**Figure 1.** Effects of NO on the LV EDP-EDV relationship, as plotted from average values of chamber stiffness, in setting of MCTP-induced CPH. There were no significant changes in LV chamber stiffness after NO inhalation at either 40 or 80 ppm compared with 0 ppm, although a substantial downward shift was observed in this relationship.

**Figure 2.** Effects of NO on the RV EDP-EDV relationship, as plotted from average values of chamber stiffness, in setting of MCTP-induced CPH. Significant improvements in RV chamber stiffness were observed after NO inhalation at both 40 and 80 ppm compared with 0 ppm. There was no further significant decrease in RV chamber stiffness at 80 vs 40 ppm. *P<.05 vs 0 ppm.
TABLE 4. Effects of Milrinone on Hemodynamic Measurements in MCTP-Induced Pulmonary Hypertension

<table>
<thead>
<tr>
<th>Milrinone, μg · kg⁻¹ · min⁻¹</th>
<th>RAP, mm Hg</th>
<th>sRVP, mm Hg</th>
<th>dRVP, mm Hg</th>
<th>mPAP, mm Hg</th>
<th>PCWP, mm Hg</th>
<th>CO, mL/min</th>
<th>PVR, dynes · s · cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.6 (0.3)</td>
<td>33.3 (1.5)</td>
<td>4.1 (0.3)</td>
<td>20.2 (1.0)</td>
<td>5.7 (0.8)</td>
<td>1256 (69)</td>
<td>988 (72)</td>
</tr>
<tr>
<td>0.5</td>
<td>5.0 (0.2)</td>
<td>36.4 (2.6)</td>
<td>3.8 (0.3)</td>
<td>18.2 (1.3)</td>
<td>7.1 (0.4)</td>
<td>1549 (72)</td>
<td>560 (95)</td>
</tr>
<tr>
<td>1.0</td>
<td>5.5 (0.3)</td>
<td>39.8 (2.8)</td>
<td>3.8 (0.3)</td>
<td>18.7 (1.9)</td>
<td>8.0 (0.5)</td>
<td>1631 (42)</td>
<td>555 (73)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. Values are mean±SEM (in parentheses).
*P<.05 vs 0 μg · kg⁻¹ · min⁻¹ milrinone.
†P<.01 vs 0 μg · kg⁻¹ · min⁻¹ milrinone.

Evolution of diastolic function after administration of milrinone.  

Thus, NO and milrinone may both be potentially useful pharmacological strategies for improving altered RV diastolic properties in the setting of CPH. However, experience with these two agents in treating RV diastolic dysfunction is currently limited. Experimental investigation of this problem has not previously been possible because of lack of an appropriate large-animal model of CPH.

In this investigation, the effects of NO and milrinone on RV diastolic and systolic mechanics were assessed in a recently established canine model of MCTP-induced CPH. MCTP-induced CPH is characterized by a proliferative vasculitis. Pulmonary vascular injury, after the initial injection, affects the medium and small pulmonary arterioles. Subsequent vascular remodeling leads to endothelial degeneration and hyperplasia, smooth muscle hypertrophy and medial thickening, and perivascular connective tissue proliferation. The development of these microscopic changes along with the ensuing rise in pulmonary vascular pressures is gradual. In addition, the characteristic parenchymal features of this model are often observed in the pulmonary vasculature of patients with pulmonary hypertension of differing etiologies, including end-stage cardiac disease as well as a subtype of primary pulmonary hypertension known as plexogenic pulmonary arteriopathy.

MCTP-induced CPH has a significant impact on RV diastolic properties and an insignificant effect on LV diastolic function. RV α and RV αVw were significantly elevated in animals with MCTP-induced CPH, whereas no significant differences were observed in LV α or αVw. The significant increase in RV α should be interpreted with caution, because it may have been due to collagen recruitment. It is unlikely, however, that these changes were the result of MCTP-induced myocardial damage, because previous morphological analysis using light and electron microscopy suggests that monocrotaline itself has no direct toxic effect on myocardial tissue.

Ventricular chamber stiffness, as estimated by the EDP-EDV relationship, represents a useful means for quantifying diastolic function in an intact heart. Pressure-volume relationships provide important information regarding diastolic function and have been applied in clinical and experimental investigations. Another technique that is often used for assessing diastolic properties involves assessment of myocardial stiffness with parameters of stress and strain. In a recent study comparing patients with normal and hypertrophied hearts, however, myocardial diastolic stiffness, as defined by use of stress-strain relations, remained relatively unchanged despite dramatic shifts in the passive pressure-volume relations. Measurement of pressure-volume relations may therefore be a more sensitive means to assess myocardial diastolic properties than estimation of stress-strain relations.

TABLE 5. Effects of Milrinone on Biventricular Diastolic Properties and Systolic Function

<table>
<thead>
<tr>
<th>Milrinone, μg · kg⁻¹ · min⁻¹</th>
<th>RV α, mm Hg</th>
<th>LV α, mm Hg</th>
<th>RV αVw, mL</th>
<th>LV αVw, mL</th>
<th>RV τ, ms</th>
<th>LV τ, ms</th>
<th>RV PRSW, dynes · cm⁻³ · mL⁻¹</th>
<th>LV PRSW, dynes · cm⁻³ · mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.070 (0.006)</td>
<td>0.024 (0.005)</td>
<td>0.724 (0.066)</td>
<td>0.560 (0.108)</td>
<td>84 (15)</td>
<td>41 (3)</td>
<td>46.9 (3.2)</td>
<td>94.7 (6.3)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.045* (0.007)</td>
<td>0.024 (0.005)</td>
<td>0.463* (0.069)</td>
<td>0.573 (0.120)</td>
<td>32* (6)</td>
<td>23* (2)</td>
<td>54.7 (4.1)</td>
<td>114.5* (5.8)</td>
</tr>
<tr>
<td>1.0</td>
<td>0.033† (0.004)</td>
<td>0.019 (0.004)</td>
<td>0.342† (0.041)</td>
<td>0.443 (0.095)</td>
<td>36† (5)</td>
<td>23† (2)</td>
<td>63.6* (6.5)</td>
<td>134.3* (9.5)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 3. Values are mean±SEM (in parentheses).
*P<.05 vs 0 μg · kg⁻¹ · min⁻¹ milrinone.
†P<.005 vs 0 μg · kg⁻¹ · min⁻¹ milrinone.
due to hypertrophy. The significant augmentation of RV systolic function associated with the significant worsening of RV diastolic properties may provide insights into the mechanisms of myocardial dysfunction in the setting of CPH.

NO-mediated improvement of ventricular diastolic properties can occur indirectly through easing of RV loading conditions or via direct myocardial relaxation. It has previously been shown that endogenous NO released from coronary endothelial cells plays an important role in the modulation of myocardial relaxation activity. In other investigations using sodium nitroprusside as an exogenous donor, NO was shown to exert a direct myocardial relaxant effect in isolated ejecting hearts, independent of any changes in cardiac loading, as well as in isolated papillary muscles. Similar results were observed in the clinical setting of Paulus and associates, where significantly improved LV diastolic properties were observed after global intracoronary infusion of sodium nitroprusside.

In this report, however, the beneficial effects of NO appear to be related to its well-established vasodilator activity. By improving pulmonary hemodynamic indices, NO led to a significant easing of RV loading conditions, which were observed in association with significant decreases in RV diastolic properties and LV $\tau$. Although the use of sodium nitroprusside as an exogenous NO donor might be equally effective in decreasing RV $\alpha$ and RV $\tau$ in this model, such conclusions are not possible on the basis of the data presented in this study. Future studies should examine whether sodium nitroprusside would be as effective as inhaled NO for improving RV diastolic properties in MCTP-induced CPH.

Although the lusitropic effects of milrinone have been clearly demonstrated in this study in association with significant improvements in pulmonary blood flow, the vasodilatory properties of this agent can potentially play a key role in its overall effects and may partially account for the observed improvements in RV $\alpha$, RV $\tau$, and overall hemodynamic status in this model. In a previous report by Monrad and associates, the relative ratio of the lusitropic and vasodilator actions of milrinone was examined in patients with advanced congestive heart failure. In that investigation, significant improvement of LV diastolic function was observed in association with relatively small decreases in mean arterial pressure, suggesting that the influence of milrinone on cardiac relaxation at the level of the individual myocyte plays a more fundamental role in its overall effects than its vasodilator actions. In the present report, significant improvements in RV diastolic properties as well as LV $\tau$ occurred in association with slight decreases in mean pulmonary artery pressure after milrinone infusion, which would appear to be consistent with the findings of Monrad and associates. Further investigation should attempt to elucidate the relative contributions of the lusitropic and vasodilatory effects of milrinone in MCTP-induced CPH.

Evaluation of diastolic function with in vivo models necessitates careful measurement of the dynamic changes occurring in ventricular pressure and volume within an intact heart. With respect to EDP, use of transmural pressures is required for an accurate assessment of diastolic properties. Estimates of $\alpha$ from EDP-EDV relationships, however, generally ignore any possible restrictive effect of the pericardium and assume that no external forces are imposed on the heart when intraventricular pressure is elevated. When the pericardium is left intact, the possibility exists that pericardial pressures might exceed zero and violate these assumptions. Previous investigations have demonstrated that in the setting of pressure or volume overload, an intact pericardium contributes significantly to increases in EDP and may affect the EDP-EDV relationship independently of changes in ventricular diastolic properties. Admittedly, use of an open-chest, open-pericardium model could potentially introduce a degree of experimental artifact and might be considered a study limitation. It should be noted, however, that this type of experimental preparation allows one to obtain true transmural pressures, which, as previously discussed, are requisite for accurate assessment of ventricular diastolic properties.

It is acknowledged that the absolute level of pulmonary hypertension occurring after MCTP injection may not accurately reflect the degree of CPH observed clinically. Consequently, the amount of hypertrophy induced in this model was much less than what one would expect in the clinical setting. It is important to point out, however, that the baseline pulmonary vascular pressures observed in the dogs before drug injection were significantly lower than normal levels found in human beings and that a twofold increase in pulmonary hemodynamic indices was achieved. Even at this level of pulmonary hypertension, significant alterations in RV diastolic properties were observed, which improved significantly after pharmacological therapy. A potential way to achieve a higher degree of pulmonary hypertension would be...
to perform multiple injections of MCTP at regular intervals to theoretically cause repeated injury to the pulmonary vascular bed and produce greater scarring of the pulmonary circulation. Higher pulmonary hemodynamic indices might, in turn, lead to more severe hypertrophy. When such a model of CPH is used for basic investigation, however, it is reasonable to state that the resultant pulmonary hypertension should not be so severe that clinical right-sided heart failure would ensue and result in a highly unstable situation.

In summary, a canine model of MCTP-induced CPH provides a useful means to evaluate various pharmacological strategies for improving RV diastolic dysfunction in the setting of CPH. There was a significant worsening of RV diastolic properties, which was significantly improved after NO and milrinone administration. These data suggest that NO and milrinone may both be effective pharmacological strategies in the treatment of diastolic dysfunction in the setting of CPH.

Acknowledgment
Dr Chen is a recipient of a National Research Service Award, fellowship HL-09489.

References
Pharmacological Strategies for Improving Diastolic Dysfunction in the Setting of Chronic Pulmonary Hypertension
Edward P. Chen, Damian M. Craig, Hartmuth B. Bittner, R. Duane Davis and Peter Van Trigt

Circulation. 1998;97:1606-1612
doi: 10.1161/01.CIR.97.16.1606

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/97/16/1606

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