Inhaled nitric oxide and inhaled prostaglandin E₁: effect on left ventricular contractility when used for treatment of experimental pulmonary hypertension

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Received 23 March 1998; accepted 22 July 1998

Abstract

Objective: Pulmonary hypertension (PHT) is a life-threatening complication after isolated heart and lung transplantation. Recent work has shown that inhaled nitric oxide (NO) in combination with inhaled prostaglandin E₁ (PGE₁) reduce pulmonary hypertension but their influence on cardiac contractility is less well defined. Methods: This study investigated left ventricular contractility as measured by the ‘Preload Recruitable Stroke Work-Relation’ (PRSW) in 24 anesthetized open chest pigs, 12 receiving in random order NO (50 ppm), PGE₁ (20 mg/ml) and their combination compared to 12 controls. PHT was induced by embolization with glass beads (500 μm). Prior to induction of PHT, sonomicrometric crystals were placed on the heart to measure instantaneous cardiac dimensions. Instantaneous intraventricular pressure (micro-tip catheter) and intraventricular dimensions were recorded digitally, while intraventricular volumes were calculated from the intraventricular dimensions applying the cylindric ellipsoidal volume model for the left ventricle. PRSW was calculated from the instantaneous pressure and volume data during rapid vena caval occlusion by analysis of generated pressure-volume loops. All data were analyzed by MANOVA and corrected for heart rate (level of significance #: P < 0.05); PRSW-slope measures contractility, (PRSW-X-intercept did not change significantly). Results: PRSW-change ± SEM (in percent of initial PRSW after induction of PHT) was –14.6% ± 4.4% versus 1.6% ± 4.4% for NO versus Control (P = 0.004), –8.8% ± 4.6% versus 1% ± 3.3% (P = 0.18) for PGE₁ versus Control and –5.7% ± 4.4% versus 2.5% ± 4.2% for NO + PGE₁ versus Control (P = 0.33), respectively. In summary, application of NO 50 ppm significantly reduced left ventricular contractility while PGE₁ 20 mg/ml and the combination of NO and PGE₁ did not. Conclusion: If NO is not available, the sole application of nebulized PGE₁ (20 mg/ml) appears to be safe with respect to left ventricular contractility in the setting of PHT. The combination of NO and PGE₁ for the treatment of pulmonary hypertension should be considered for clinical application in situations where a combination of pulmonary hypertension and decreased left ventricular function is present. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Left ventricular function; Nitric oxide; Prostaglandin E₁; Inhalation of Drugs; Pulmonary hypertension

1. Introduction

Patients receiving an orthotopic thoracic organ transplantation show an increased susceptibility for PHT and/or right heart failure which often leads to disastrous complications [1,2]. Primary pathogenetic factors contributing to PHT in the case of heart transplantation are the effects of donor brain death, ischemia and concomitant reperfusion injury and air-embolism during reperfusion. These factors are thought to exert negative inotropic effects on the cardiomyocytes, leading to pulmonary vascular congestion and to PHT. In the case of lung transplantation ischemia and concomitant reperfusion injury, air-embolism during reperfusion, decreased endothelial function, increased leukocyte sequestration and formation of microthrombi, heparin/pro-
tamin infusion, atelectasis and hypoxia/hypercarbia are thought to contribute to the pathogenesis of PHT by a direct effect on the pulmonary circulation.

Currently available therapeutic measures include i.v. drug therapy, e.g. diuretics, digitalis, afterload-reducing therapy and prostaglandin, aside from mechanical support [3]. Recent work has shown that inhaled nitric oxide (NO) [4–5], inhaled prostaglandin E1 (PGE1) [4] and their combination [4] reduce pulmonary hypertension but their influence on cardiac contractility is less well defined. The intravenous infusion of PGE1 has been shown to improve the hemodynamic state in patients with end-stage chronic heart failure [6] but the cardiac effects of PGE1 application by inhalation have not been described previously. On the other hand, while NO has shown to reduce PHT in patients with congestive heart failure and in patients awaiting heart transplantation [7,8] the reduction in pulmonary vascular resistance (PVR) is usually accompanied by a rise in pulmonary capillary wedge pressure and reduction of cardiac index [7,8] which has even led to pulmonary edema [9]. Despite evidence of a negative inotropic effect of NO on humans [8,10] and animal preparations [11] its inotropic effect is a topic of ongoing debate [12–14].

This study, therefore, investigated left ventricular contractility as measured by the load-independent ‘Preload Recruitable Stroke Work-relation’ (PRSW) in PHT treated with NO, PGE1, and the combination of both.

2. Material and methods

All pigs included in this study were held at the Central Animal Laboratory of the Hannover Medical School according to the German Animal Protection Law and received humane care in compliance with the European Convention on Animal Care. The study was approved by the local institutional ethics committee.

2.1. Experimental preparation

Under continuously administered general anesthesia with intravenous thiopental-sodium (5 mg/kg per h) and fentanyl (10 µg/kg per h) in combination with intermittent pancuronium relaxation (0.05 mg/kg) and while on continuous mechanical ventilation (Siemens-Elema AB, Solna, Sweden), the hearts of 24 healthy 7–8 months old pigs 39.1 ± 0.9 kg (mean ± SEM) were exposed through a median sternotomy. Mean arterial pressure, monitored with a micromanometer-tipped catheter (model MPC-500, Millar Instruments, Houston, TX) in the aortic root, was maintained at 55–75 mmHg, and blood temperature was kept at 38°C. Micromanometer-tipped catheters were placed in the pulmonary trunk, left atrium, left ventricle and right ventricle (Fig. 1).

A central venous catheter was placed through the right jugular vein. Using this instrumentation the pulmonary, left atrial, left ventricular, right ventricular and central venous pressures could be assessed. In each heart, pulse-transit ultrasonic dimension transducers were positioned across the major axis (base-apex) and minor axis (anteroposterior) diameters as well as across the septal-free wall minor axis diameter of the left ventricle [15,16].

The superior and inferior vena cava were prepared with circumferential occluders to enable variation of left and right ventricular filling pressures and volumes. The pericardium was left open. For induction of PHT, glass beads (0.5–1.0 g/kg) were applied into the pulmonary trunk.

After termination of each study the animal was sacrificed using 20 ml intracardial T61 ( Hoechst, Unterschleissheim/ Munich, Germany), a combination of embutramide, mebenzoinium-jodide and tetracaine-hydrochloride, 3 min after an intravenous bolus of fentanyl and thiopental had been given. Then the heart was excised and proper position of the transducers was confirmed. Left ventricular wall volume (Vwall) was measured by saline displacement after exciting the atria, right ventricular free wall, aortic and mitral valves, and chordae tendineae.

2.2. Experimental design and realization

The 24 pigs were randomly divided into two groups of 12 pigs each. One group received verum substances, the other control substances. After induction of PHT the application of verum substances: inhaled nitric oxide (NO) 50 ppm; inhaled nebulized prostaglandin E1 (PGE1) 20 µg/ml; and their combination and control substances: air; ethanole-saline (4 ml waterfree ethanole/100 ml saline, the solutant in the PGE1 solution); and their combination was performed according to the randomized study protocol. NO (1260 ppm) diluted in N2 (Linde AG, Höllriegelskreuth, Germany) was blended to the inspiratory limb of the respirator via its low pressure inlet. The proper inspiratory and expiratory NO concentration (50 ppm) were controlled by electrochemical NO-meters, PAC II NO (Draegerwerk AG, Lu beck, Germany) and their combination was performed for 15 min each before data-recording. The order of application was randomized according to a latin square.

2.3. Data acquisition

The ultrasonic dimension transducers, being coupled to a sonomicrometer (Triton Technology, San Diego, CA) and
the micromanometer-tipped catheters, which had been balanced and calibrated simultaneously to a mercury manometer immediately before each study, were connected to a multi-channel-12-bit A/D-converter (Scientific Solutions Inc., Solon, OH). Digitized data were recorded realtime on a 80386 40 MHz PC with 200 Hz sample rate to be analyzed later, offline.

For each intervention, physiologic data were digitized during vena caval occlusion for 15 s.

2.4. Data analysis

Left ventricular chamber volume \( V \) was calculated by fitting the epicardial base-to-apex \( (a) \), anteroposterior \( (b) \), and septal-free wall \( (c) \) left ventricular dimensions (Fig. 1) to a cylindric ellipsoidal volume model, representing the ‘epicardial’ ventricular surface, and subtracting ventricular wall volume \( V_{\text{wall}} \) [16] (Eq. (1)).

\[
V = \frac{\pi}{6} abc - V_{\text{wall}} \tag{1}
\]

Left ventricular transmural pressures \( (P) \) were measured simultaneously using the above mentioned intracavitary micromanometer, introduced through the left ventricular free wall.

Left ventricular stroke work \( (SW) \) was calculated as the integral of left ventricular transmural pressure \( (P) \) with respect to the calculated chamber volume \( (V) \) over each cardiac cycle (Eq. (2)).

\[
SW = \int P \, dV \tag{2}
\]

The relation between left ventricular stroke work and end-
significant changes in the slope together with no increase of the effect. If changes are mixed, decrease in slope combined with a decrease of the effect, interpretation of results is more complex.)

Comparison of cardiac contractility as measured by the PRSW-relation and expressed by changes in slope (with constant x-intercept) were made between verum and control groups for each verum-/control-substance. To control for inter-individual differences, e.g. in transducer placement, first changes of parameters within each of the 24 hearts between initially induced PHT and after application of the verum-/control-substances were computed. These differences were analyzed by MANOVA (slope and x-intercept being the dependant variables with the null hypothesis that the slope and x-intercept means are the same for the two verum- and control-group categories) using SPSS software (SPSS, Chicago, IL) and corrected for heart-rate by including heart-rate as the covariate. The level at which statistical significance was accepted was P < 0.05. Unless otherwise stated, data are expressed as mean ± SEM.

3. Results

Pulmonary arterial pressure was increased significantly after the application of glass beads (all numbers mean ± SEM; verum-group: from 16.1 ± 1.0 mmHg to 26.8 ± 1.0 mmHg; control-group: from 15.8 ± 1.2 mmHg to 26 ± 1.1 mmHg) as well as reduced significantly after the application of all three therapeutic regimen (inhaled NO: 20 ± 0.6 mmHg, i.e. 25% reduction; inhaled PGE1: 22 ± 0.5 mmHg, i.e. 16% reduction; combination: 20 ± 0.6 mmHg, i.e. 25% reduction while in the control-group the pressures were 25.1 ± 1.1 mmHg, 25.1 ± 0.9 and 24.1 ± 0.7 mmHg, respectively after application of control substances).

Concerning left ventricular contractility and compar-

### Table 1

<table>
<thead>
<tr>
<th>PRSW mean ± SEM (slope:erg *10^(-3)/ml, x-intercept:ml)</th>
<th>Baseline</th>
<th>NO</th>
<th>PGE1</th>
<th>NO + PGE1</th>
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<tr>
<td>Verum PRSW slope</td>
<td>74.2 ± 4.0</td>
<td>63.4 ± 4.7</td>
<td>67.7 ± 4.8</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Verum PRSW x-intercept</td>
<td>14.8 ± 2.5</td>
<td>14.4 ± 2.3</td>
<td>14.0 ± 2.5</td>
<td>15.0 ± 2.7</td>
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<tr>
<td>Control PRSW slope</td>
<td>68.8 ± 3.6</td>
<td>70.9 ± 4.1</td>
<td>70.5 ± 4</td>
<td>71.51 ± 2.8</td>
</tr>
<tr>
<td>Control PRSW x-intercept</td>
<td>8.6 ± 1.4</td>
<td>9.0 ± 1.7</td>
<td>8.9 ± 1.4</td>
<td>9.1 ± 1.5</td>
</tr>
<tr>
<td>PRSW r^2 ± SEM^1</td>
<td>Baseline</td>
<td>NO</td>
<td>PGE1</td>
<td>NO + PGE1</td>
</tr>
<tr>
<td>Verum</td>
<td>0.95 ± 0.01</td>
<td>0.93 ± 0.03</td>
<td>0.95 ± 0.01</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>0.94 ± 0.02</td>
<td>0.94 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>PRSW mean ± SEM (%-change)^2</td>
<td>NO vs. baseline</td>
<td>PGE1 vs. baseline</td>
<td>NO + PGE1 vs. baseline</td>
<td></td>
</tr>
<tr>
<td>Verum PRSW slope (%)</td>
<td>-14.6 ± 4.4</td>
<td>-8.8 ± 4.6</td>
<td>-5.7 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Control PRSW slope (%)</td>
<td>1.6 ± 4.4</td>
<td>1 ± 3.3</td>
<td>2.5 ± 4.2</td>
<td></td>
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<tr>
<td>P-value^3</td>
<td>0.004</td>
<td>0.18</td>
<td>0.33</td>
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<td>Multivariate</td>
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<tr>
<td>Univariate PRSW x-intercept</td>
<td></td>
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</tbody>
</table>

^1PRSW (slope and x-intercept) mean values in the verum- and control group at baseline after induction of PHT with glass beads (baseline), glass beads plus nitric oxide (NO), glass beads plus prostaglandin E1 (PGE1) and glass beads plus the combination of nitric oxide and prostaglandin E1 (NO + PGE1).

^2The changes in percent of baseline-values are given concerning PRSW slope. PRSW x-intercept did not change significantly in any case.

^3The multivariate P-value for the null hypothesis (slope and x-intercept means are the same for the two verum- and control-group categories) are demonstrated. the univariate P-value for slope and x-intercept is noted in the case of a significant multivariate P-value, i.e. for NO versus baseline.
ing the verum- and the control-group (Table 1) baseline PRSW-values showed no statistically significant differences ($P = 0.51$). PRSW-slope (measuring contractility, PRSW-$x$-intercept did not change significantly) showed a significant reduction in the verum group under sole application of NO 50 ppm by $14.6 \pm 4.4\%$ (Figs. 2 and 3). There were no significant differences in PRSW between verum and control group under sole application of PGE$_1$ 20 $\mu$g/ml nor under the combination of NO and PGE$_1$. (PRSW-change $\pm$ SEM in percent of initial PRSW after induction of PHT was $-14.6\% \pm 4.4\%$ versus $1.6\% \pm 4.4\%$ for NO versus Control ($P = 0.004$), $-8.8\% \pm 4.6\%$ versus $1\% \pm 3.3\%$ (not significant, $P = 0.18$) for PGE$_1$ versus Control and $-5.7\% \pm 4.4\%$ versus $2.5\% \pm 4.2\%$ for NO + PGE$_1$ versus Control (not significant, $P = 0.33$, respectively).

In summary, application of NO significantly reduced left ventricular contractility while PGE$_1$ and the combination of NO and PGE$_1$ did not.

4. Discussion

The efficacy of all three therapeutic regimens (inhaled NO, inhaled PGE$_1$ and the combination) to effectively lower pulmonary pressure in PHT has been shown previously in the setting of experimental hypoxic PHT [4]. Here we present the effects of those substances in a setting of experimental PHT which was induced by pulmonary embolization with glass beads. This study put the focus on the effect of NO and PGE$_1$ on left ventricular contractility. Therefore, an experimental model of PHT without inherent negative inotropic effect had to be used (i.e. application of glass beads) as opposed to hypoxic PHT with the concomitant hypoxic ventricular effects.

Concerning the reduction of pulmonary pressure in hypoxic PHT, NO seems to be more potent than PGE$_1$, but the combination of both did not reduce the potential of NO to lower PHT [4]. The effects in this study with embolic PHT were similar to those with hypoxic PHT. Again NO as well as NO plus PGE$_1$ had the same potential to reduce the experimental embolic PHT (by 25%). Sole application of PGE$_1$ had a slightly less effect in reducing embolic PHT, but still reduced it significantly by 16%.

Concerning left ventricular contractility sole application of NO 50 ppm showed a significant reduction, while the combined application of NO and PGE$_1$ as well as sole application of PGE$_1$ did not.

In clinical studies a negative inotropic effect of biconormonary infusion of substance P (which releases NO from the endothelium) [17] and an improvement of the positive inotropic response to the $\beta$-adrenergic stimulation with dopamine of NG-monomethyl-L-arginine (a NO synthase inhibitor) [10] are discussed. Further evidence of a negative inotropic effect of NO is demonstrated by a marked increase in ventricular preload with little benefit to pulmonary pressures in patients with impaired cardiac reserve receiving inhaled NO [8] as well as in animal preparations [11].

Other studies could not find a significant reduction of LV contractility following inhalation of NO [13,14,18]. Inhalation of 20 ppm NO delivered for 10 min to humans with normal LV function showed no significant inotropic effect [13].

A second study evaluated left and right ventricular contractility in dogs with monocrotaline induced pulmonary hypertension after experimental orthotopic cardiac transplantation. Starting sequential administration of NO (40 ppm for 10 min and then 80 ppm for 10 min) 60 min after weaning from cardiopulmonary bypass did not show significant changes [14].

In a third study applying 20, 40 and 80 ppm of inhaled NO in a porcine model of PHT (induced by a intravenous...
thromboxane A\textsubscript{2} analog) no statistically significant changes on LV contractile function were detected [18]. While all three studies also used load-independent parameters of contractility, it is important to note, that in the first [13] hearts with normal LV function were investigated. In the second study [14] increasing doses of NO were sequentially applied without randomization to a heart that was only 60 min after weaning from cardiopulmonary bypass after cardioplegic arrest. A negative inotropic effect of the increasing doses of inhaled NO might therefore be masked by a positive inotropic effect of recovery from cardioplegic arrest. To overcome these problems in our study the substances were applied in randomized order and further the comparison was made between a verum- and a control-group.

In the third study [18] an intravenous thromboxane A\textsubscript{2} analog was applied for induction of PHT and the conductance catheter technique was used to assess left ventricular function. On one hand methodological differences in measuring left ventricular contractility might have contributed to the different results. While the conductance catheter technique yielded SEMs of the left ventricular volume of around 9 ml, our SEMs of left ventricular volume were around 2 ml (with about equal sample sizes: 10 pigs vs. 12 pigs). Therefore, a component of measuring left ventricular volume less precise with the conductance catheter technique can not be excluded. On the other hand the combined application of NO and thromboxane A\textsubscript{2} may well have led to a zero net effect on the myocardial contractility, similar to the antagonistic effects on the pulmonary circulation [18]. In our study no antagonists of NO were used. Rather, PHT was induced mechanically in an equal fashion in the verum- and control-group.

An effect of ventricular interaction in our study secondary to a different reduction of PHT (25% by NO and the combination of NO and PGE\textsubscript{1} vs. 16% by sole PGE\textsubscript{1}) is unlikely due to the fact that NO and the combination reduced PHT to equal pulmonary pressures and only the case of sole NO application led to a significant reduction of contractility. Furthermore, performing the same MANOVA for the changes of the septal-free-wall dimension (c in Fig. 1) in steady state conditions (i.e. without vena caval occlusions) shows no significant difference between verum- and control-group for changes of the end-diastolic length under each of the three conditions (NO, PGE\textsubscript{1} and combination). If different outflow impedances of the right ventricle and concomitant higher right ventricular end-diastolic volumes had caused an end-diastolic septal shift towards the left ventricle, differences in the end-diastolic septal-free-wall dimension should have occurred. Therefore, a diastolic ventricular interaction can be excluded. Analyzing steady state conditions for end-systolic changes of the septal-free-wall dimension, a significant difference between the verum- and the control-group can be demonstrated under sole NO and the combination (more increase in verum than in control; an increase in the end-systolic septal-free-wall distance demonstrates less contraction in this dimension). A contribution of systolic ventricular interaction under steady state conditions can not be excluded in these cases and could be explained by the better reduction of PHT compared to sole application of PGE\textsubscript{1} (no significant difference to control with less reduction of PHT by PGE\textsubscript{1}; note that in control also no reduction of PHT occurred). Nevertheless, this effect under steady state conditions has to be viewed separately and is likely to be excluded by the applied methodological approach of measuring contractility. One of the advantages of the vena caval occlusion technique as a method for assessing left ventricular performance is that this technique produces a rapid reduction in right ventricular volume and pressure, which precedes the reduction in left ventricular volume and pressure, thereby reducing the interactive contribution of the right ventricle to left ventricular function [16]. If the demonstrated negative inotropic effect of NO was due to ventricular interaction, the combination of NO and PGE\textsubscript{1} with the same reduction of PHT would have shown a significant negative inotropic effect, as well.

NO has shown to reduce PHT in patients with congestive heart failure and in candidates awaiting heart transplantation [7,8]. The reduction in pulmonary vascular resistance (PVR) is usually accompanied by a rise in pulmonary capillary wedge pressure and reduction of cardiac index [7,8]. In some instances it has even lead to pulmonary edema in stable severe heart failure [9].

As opposed to the normal LV a variety of molecular alterations with subsequent functional consequences occur in heart failure and cardiac hypertrophy [19–21]. Of these the following may be interpreted to be adaptive in character [19]:

The molecular changes of the contractile proteins and their regulatory proteins with fundamental alterations of the cross-bridge mechanics. The downregulation of beta-adrenoceptors with subsequent functional reduction of the contractile reserve.

On the other hand, the reduction of the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase with a related reversed of the force-frequency relationship may be causally related to the heart failure syndrome [19].

Recent studies show a cGMP-induced reduction in myofilament response to Ca\textsuperscript{2+} by NO [22,23] which may add to the above mentioned reduction of the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase in the failing heart and thereby potentiate the negative inotropic effect of NO. This may explain why the negative inotropic effects are mainly observed in the failing heart and not in the heart with normal function.

On the other hand myocardial relaxation is enhanced by the cGMP-induced reduction in myofilament response to Ca\textsuperscript{2+} by NO [23]. This support in myocardial relaxation seems to be helpful in conditions with primarily diastolic dysfunction such as (brief) hypoxia/re-oxygenation states [23]. This effect may well have been operative in the above mentioned study of experimental orthotopic cardiac transplantation with monocrotaline induced pulmonary
mitochondrial respiration. Here a loss of the regulatory failing heart there seems to be a defect in the ability of derived NO can attenuate mitochondrial respiration. In the normal myocardial muscle endogenous endothelium the NO molecule, enhancing its physiologic effects [25]. In S-nitrosohemoglobin, which acts as a souped-up version of NO (between 0.1 and 5 s). The active agent may still reach concentrations. Detected better in the upper range of clinically used concentrations.

A number of different concentrations of NO (generally up to 80 ppm) had been administered clinically in the past with a tendency to lower concentrations (10 ppm or less), recently [5,7,8]. For our study a concentration of 50 ppm was used for the assessment of the inotropic effects of inhaled aerosolized PGEl.

Intravenous application of PGEl has been demonstrated to exert no or a small species-dependent positive inotropic effect on cardiac contractility [6]. To our knowledge, no data concerning LV function were available previously for intravenously administered PGEl. In this study, sole application of PGEl did not result in a statistically significant change of LV-contractility in the pig. Furthermore, an increase in cardiac output (however, at least in part induced by a reduction of outflow impedance) was found in humans when administering PGEl intravenously [6]. Therefore, inhaled application of PGEl in humans is not expected to have negative inotropic side effects when used therapeutically.

Interestingly, a reversal of the negative inotropic effect of NO could be demonstrated by combining NO with PGEl, but the molecular mechanism of interaction remains to be revealed.

A number of different concentrations of NO (generally up to 80 ppm) had been administered clinically in the past with a tendency to lower concentrations (10 ppm or less), recently [5,7,8]. For our study a concentration of 50 ppm was chosen, because an inotropic effect was expected to be detected better in the upper range of clinically used concentrations.

The 14.6% reduction of LV contractility by inhaled NO may be explained despite the short half-life of the molecule NO (between 0.1 and 5 s). The active agent may still reach the endocardial and endothelial coronary sites in the form of S-nitrosohemoglobin, which acts as a souped-up version of the NO molecule, enhancing its physiologic effects [25]. In normal myocardial muscle endogenous endothelium-derived NO can attenuate mitochondrial respiration. In the failing heart there seems to be a defect in the ability of coronary blood vessels to produce NO and to attenuate mitochondrial respiration. Here a loss of the regulatory role of NO in optimizing the O2 utilization and a 54%-higher basal O2 consumption rate than that of normal muscle were found [12]. Furthermore, recent studies suggest that the same conformational change that, upon reaching oxygen-poor tissues, reduces the affinity of hemoglobin for oxygen also releases S-nitrosothiol (SNO) [25]. Therefore the increased mitochondrial respiration in the failing heart may trigger an increased release of SNO which may then exert its negative inotropic effects via the above mentioned mechanisms (i.e. cGMP-induced reduction in myofilament response to Ca++ by nitric oxide [22,23] which may add to the reduction of the sarcoplasmic reticulum Ca++-ATPase in the failing heart). Again, this may explain why the negative inotropic effects are mainly observed in the failing heart and less in the heart with normal function.

Lacking more detailed information concerning the interaction of NO and PGEl, at present only a descriptive recommendation can be given from the present study:

If NO is not available, the sole application of nebulized PGEl 20 μg/ml appears to be safe with respect to left ventricular contractility in the setting of PHT. Combined application of NO 50 ppm and PGEl 20 μg/ml is favorable as opposed to sole application of NO 50 ppm with respect to LV contractility for the treatment of PHT. The combination of NO and PGEl for the treatment of pulmonary hypertension should be considered for clinical application in situations where a combination of pulmonary hypertension and decreased left ventricular function is present.

Inhaled PGEl-application should be used for short-term treatment only until a risk of toxicity of the ethanol-saline preparation of PGEl can be excluded for higher-dose and/or long-term inhalation.

References

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Appendix A. Conference discussion

Dr F. Ciulli (Sheffield, UK): May I ask where you sampled the nitric oxide concentration from?

Dr Krieg: We took those measurements in the inhaled as well as in the exhaled air. We were first looking for any differences. We could not detect any significant differences between inhaled and exhaled air. But these are the points where we did those measurements.

Dr Ciulli: Was it a pulse delivery system or was it continuous?

Dr Krieg: Yes, it was a pulse delivery system.

Dr M. Hein (Kiel, Germany): Did you compare PGE1 infusion versus no inhalation, and did you test different dosages of NO concentrations, because for the therapy of pulmonary hypertension in the clinic concentrations between 5 and, at maximum, NO 20 ppm were used.

Dr Krieg: We didn’t do a i.v. infusion with PGE1, because of the known side effects of increasing V/Q mismatch. Therefore we were concentrating on the inhaled application. Regarding your second question, we did look at different nitric oxide concentrations. Basically we were first looking at a dose response curve but finally decided to do the study with an NO concentration of 50 ppm because at the time of the study it wasn’t that clear that lower doses of inhaled nitric oxide would be as effective as the higher ones.

Dr Hein: Ten ppm NO might not have a negative inotropic effect?

Dr Krieg: It may not. We don’t know. At least we didn’t measure it.

Dr G. Szabo (Heidelberg, Germany): The first question is do you measure nitric oxide in blood? It would be very important because we know that nitric oxide has a very short half-time in blood and it is questionable if it is inhaled that it has a significant effect on the left ventricle. Second, you do not show any parameters of the changes of afterload/preload after your intervention. You showed only the preload recruitable stroke work as a single index. Is it possible that nitric oxide and prostaglandin have different effects in amount on the changes of afterload and preload and your results may be explained in the different quantitative effects on nitric oxide and prostaglandin on afterload and preload conditions?

Dr Krieg: First, we did not measure nitric oxide in the blood since the half-time is just a few seconds and it is expected to be bound to hemoglobin very quickly. So basically all you can measure are nitrates and nitrates, but nitric oxide itself is very difficult to measure in the blood, so we didn’t do that. Your second question concerning differences in preload, we chose the preload recruitable stroke work relation as a parameter of contractility because it is preload-independent. That is, because it is measured over a range of different preload levels, so to speak, and only by measuring the stroke work at different preload levels can you generate this parameter. Therefore it is preload-independent, which is why we did it, and it has been shown that it is afterload-insensitive.

Dr Szabo: I ask this question because it’s true that under certain circumstances preload recruitable stroke work is load-independent, but it is also known that the intrinsic myocardial contractility may be changed due to altered loading conditions during a longer period (within 10 min). It means that if you apply your therapy, you can change preload conditions in the different groups and the hearts may adapt with a change of intrinsic contractility. In this case your change of contractility reflects not to the applied drug but to the differently altered loads.

Dr Krieg: Yes, that is true, but to take care of that problem we did our study in a randomized fashion: all the pigs received the same treatment, getting nitric oxide, prostaglandin, the combination, and the control substances, respectively. This was switched according to a Latin square randomization schedule. So the time effect was taken out by this randomization process.

Dr C.M. Peniston (Toronto, Canada): First of all, did your treatments actually reduce the pressure in the pulmonary arteries? Secondly, do you think that somehow there could be an interaction between the effects of your drugs on the right ventricle which might affect the contractility of the left ventricle?

Dr Krieg: First, yes, pulmonary artery pressure was reduced by all three measurements, nitric oxide, prostaglandin, and the combination reduced it. The microsphere beads induced pulmonary hypertension. After the injection of microspheres, pulmonary pressure rose to about 26 from 15 mmHg, while after giving those substances we had a markedly reduced pulmonary pressure, a drop for about 5 to 10 points, almost back to normal. Regarding
your second question concerning the right ventricular contractility. In a different experiment we also measured right ventricular contractility and could not detect any changes in contractility on the right side, which is a little bit confusing at first, but after thinking it over for a while, we hypothesized that maybe the process of how the contractility is decreased works over the endocardium. If NO would exert its negative inotropic effect through the coronaries, you would expect that both sides, left and right, would be depressed in their contractility. It may be the case that the way from the lungs to the coronaries is too long but the way from the lungs to the endocardium is still short enough for nitric oxide to have some effect on contractility.