Improved contractile performance of right ventricle in response to increased RV afterload in newborn lamb

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The purpose of the present study was to determine how the immature RV responds to an isolated, purely hemodynamic afterload increase. With the use of a combined pressure-conductance catheter, RV performance was quantified in newborn lambs by end-systolic pressure-volume relations, whereas RV afterload was increased by partial balloon occlusion of the pulmonary artery. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

PULMONARY DISEASES like infant respiratory distress syndrome are a common problem in neonatal critical care. Besides ventilatory problems, the existence of pulmonary hypertension in these pulmonary diseases can have fatal consequences for the infant. There are several factors such as hypoxemia, decreased preload for the left ventricle (LV), and increased afterload for the right ventricle (RV) that can affect the performance of the heart and might lead to heart failure. It is unclear whether the increased afterload for the RV is the factor responsible for cardiac dysfunction in these newborns. Guyton et al. (15) observed that when RV afterload is increased by pulmonary artery constriction in adult dogs, cardiac output (CO) increased slightly in the early phases of progressive pulmonary artery constriction. When constriction continued, systemic pressure dropped and heart failure occurred. Similarly, Vlahakes et al. (35) demonstrated that increasing RV afterload by constriction of the pulmonary artery did not affect CO at first, but when RV afterload increased further, CO and aortic pressure (Pao2) decreased and heart failure occurred as a result of myocardial ischemia. So far the effects of an isolated RV afterload increase, without decreased preload for the LV, have not been studied thoroughly. Furthermore, all studies investigating the effects of increased RV afterload have been performed in adult hearts. How the newborn heart will respond to an increased RV afterload has not previously been investigated. There are indications that the factors necessary for the adjustment to an increased demand are not yet available in the newborn heart (18, 21). Also, the newborn heart has little β-adrenergic contractile reserve to draw upon when afterload changes (2). On the other hand, it has been shown for the LV that the newborn heart showed a comparable adjustment to LV afterload increase as seen in the adult heart (19, 28).

The purpose of the present study was to determine how the immature RV responds to an isolated, purely hemodynamic afterload increase. With the use of a combined pressure-conductance catheter, RV performance was quantified in newborn lambs by end-systolic pressure-volume relations, whereas RV afterload was increased by partial balloon occlusion of the pulmonary artery.

MATERIALS AND METHODS

The surgical and experimental procedures were reviewed and approved by the animal research committee of the Leiden University Medical Center. The investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Animal preparation. Nine newborn lambs, age 9.0 ± 3.1 days and weighing 5.2 ± 1.2 kg, were studied. After premedication with ketamine hydrochloride (3 mg/kg body wt iv), general anesthesia was maintained using a continuous infusion of ketamine hydrochloride (8 to 30 mg·kg⁻¹·h⁻¹ iv)
supplemented with xylazine (3 mg/kg im). In addition, local anesthesia was applied with 1% lidocaine hydrochloride injected subcutaneously. During the study, the wounds were sprayed with lidocaine at regular intervals. The lambs were intubated and ventilated with an oxygen-air mixture, using a pressure-controlled ventilator (Babylog 8000, Dräger, Lübeck, Germany). Ventilation was adjusted to maintain arterial oxygen and carbon dioxide pressures within normal ranges throughout the study. Upon ventilation, pancuronium (0.2 mg/kg) was administered to achieve adequate muscle relaxation. An intravenous infusion of 5% dextrose in 0.5 N NaCl solution (15–20 ml·kg\(^{-1}\)·h\(^{-1}\)) was administered to maintain a normal base deficit (≤5 mmol/l).

Instrumentation. To facilitate the insertion of catheters, 6-Fr sheaths were placed in the right and left femoral vein, the left femoral artery, the right jugular vein, and in the right carotid artery. To measure RV pressure and volume, a 5-Fr combined conductance-pressure catheter with 10 electrodes, 7-mm spacing (Millar Instruments, Houston, TX) was introduced into the RV through the right jugular vein. The conductance catheter was connected to a Leycom Sigma-5 signal conditioner processor (CardioDynamics, Zoetermeer, The Netherlands) to obtain an instantaneous RV volume signal. A 5-Fr thermodilution catheter was placed in the pulmonary artery through the right femoral vein to measure CO. The proximal lumen of the same catheter was used for hypertonic saline injections in the vena cava inferior to determine RV parallel conductance (see below). The thermodilution catheter was also used for partial occlusion of the pulmonary artery, as described in the study protocol, using a 1-ml balloon on the tip of this catheter.

To assess pressure-volume relations, a 4-ml latex balloon catheter was placed through the left femoral vein in the vena cava inferior to reduce inflow to the RV (see below). All catheters were positioned under fluoroscopic guidance. \( P_{a_{\text{ao}}} \) was measured from the fluid-filled sideport of the sheath through the carotid artery in the aortic arch. Blood samples for measurement of arterial blood gasses and pH were drawn from the sheath in the femoral artery.

Study protocol. To determine the effects of an increased RV afterload on the contractile performance of the RV, we examined hemodynamics and RV contractile performance before and during 4 h of partial balloon occlusion of the pulmonary artery and again after the occlusion was released. After instrumentation was completed, a 15-min period was allowed for the lambs to obtain hemodynamic stability. When hemodynamic stability was reached, baseline measurements were performed. Subsequently, the RV afterload was increased by inflating the balloon in the pulmonary artery. Measurements were performed at 15 and 30 min and 1, 2, 3, and 4 h of increased RV afterload. The pulmonary artery balloon was then deflated, and after stabilizing for 5 min the final measurements were performed. At each condition, a set of measurements was performed to calibrate the conductance catheter method and to assess hemodynamics and contractile performance. This measurement set consisted of assessing blood resistivity, injecting 0.6 ml of NaCl 10% iv to measure parallel conductance, measuring CO with the thermodilution method, and acquiring pressure-volume loops during inflow reduction. Inflow reduction and saline injections were performed at end expiration with the ventilator turned off. From the measurements the following variables were determined: contractile performance of the RV, using the end-systolic pressure-volume relation (slope and volume intercept), end-systolic pressure \( P_{es} \), end-diastolic volume \( V_{ed} \), and stroke volume \( SV \). In addition, standard hemodynamic variables were determined: heart rate \( HR \), CO, mean \( P_{a_{\text{ao}}} \), arterial blood gasses \( (P_{O_2} \text{ and } P_{CO_2}) \), and pH.

Conductance catheter. The application of the conductance catheter for measuring ventricular volume has been described and validated extensively for the LV (3, 5). More recently, the same method has been shown to be applicable for measuring RV volume as well (11, 31, 32). Briefly, electrical conductance was measured at three levels in the RV. To obtain absolute volume, the conductance signals \( G(t) \) were converted to volume signals \( V(t) \) as \( V(t) = (\alpha \cdot [L^2 \cdot \rho \cdot G(t) - V]) \). Here, \( \alpha \) is a dimensionless slope factor, \( L \) is the distance between the sensing electrodes, \( \rho \) is the resistivity of the blood, and \( V_c \) is the correction volume to account for the conductance of surrounding tissue (commonly referred to as parallel conductance). The \( V_c \) was measured with the hypertonic saline method (5). The \( \alpha \) was assessed by comparing the uncalibrated conductance catheter CO with CO obtained by the thermal dilution method.

Measurements. To obtain pressure-volume relations, RV pressure and volume signals were recorded during gradual inflation of the inferior vena cava balloon to reduce inflow to the heart. In each beat during this inflow reduction, end systole was defined as the point in the cardiac cycle of maximal elastance. Elastance is defined as \( P(t)(V(t) - V_0) \), where \( P(t) \) is the instantaneous RV pressure, \( V(t) \) is instantaneous RV volume, and \( V_0 \) is the theoretical RV volume at zero RV pressure. \( V_0 \) was determined by an iterative algorithm, as described and validated extensively for the LV (3, 5).
described by Kono et al. (20). The end-systolic pressure-volume relation (ESPVR) was determined by fitting a straight line through the end-systolic pressure-volume points (23). Even if these points showed some nonlinearity (see Fig. 3a), the ESPVR was calculated by linear regression. But to avoid the problem of linear extrapolation to zero pressure, we used a volume intercept at a fixed pressure of 25 mmHg within the pressure range encountered (V25) to quantify the position of the ESPVR, as previously applied to nonlinear ESPVRs of the LV (34). The slope of the ESPVR (Ees) and its volume position (V25) represent relatively load-independent measures of contractile performance. An increase in Ees (17, 23) as well as a decrease of volume intercept (6, 16), or both (11), represent an improved contractile performance.

General hemodynamic quantities (Pes, VEd, and SV) were determined from the steady-state beats just before each inflow reduction. All calculations were performed using custom-made software. HR and Pao were measured continuously, using a Hewlett-Packard monitoring system, and information was imported in a personal computer simultaneously with the conductance catheter acquisitions.

Statistical analysis. The effect of increased RV afterload on contractile performance of the RV was analyzed using a multiple linear regression implementation of repeated measures analysis of variance (13). In this model dummy variables were used to code the different conditions (C1-C8: baseline 15 and 30 min, 1, 2, 3, and 4 h of occlusion, and after occlusion) and animals (L1-L9: lambs 1–9). The regression equation was

\[ y = a^0 + \sum a_i^1 \cdot L_i + \sum a_i^2 \cdot C_i \]

where y represents the dependent variable of interest (Pes, Ees, V25, PEd, SV, CO, or HR). For the animals, effects coding and for the conditions, reference coding was used (13). Consequently, the intercept of the regression equation, a0 yields the mean value of the dependent variable in the control condition (baseline), and each coefficient represents the difference between the dependent variable at that condition and baseline. A P value of <0.05 was considered statistically significant. Data are presented as means ± SD.

RESULTS

Figure 1 shows how an almost twofold increase of Pes of the RV, as a measure of afterload for the RV, was applied during 4 h. During these 4 h of increased afterload, CO and SV were maintained, whereas VEd did not change significantly. The contractile performance of

![Graphs showing ESPVR](http://ajpheart.physiology.org/)

Fig. 2. Mean values (±SD) of volume intercept of end-systolic pressure-volume relation (ESPVR), quantified at pressure 25 mmHg (V25), and of slope (Ees) of ESPVR at baseline, at 15 and 30 min, and 1, 2, 3, and 4 h of partial occlusion of pulmonary artery, and after pulmonary artery occlusion was released. *P < 0.05 vs. baseline.

Fig. 3. A: typical example in one animal of RV pressure-volume loops during inflow reduction at baseline (black loop) and at 30 min pulmonary artery occlusion (gray loop). At 30 min of occlusion ESPVR has shifted leftward compared with baseline, indicating an improved contractile performance. Note slight nonlinearity of end-systolic points in occluded condition as well as definition of the volume intercept V25 (see text). B: typical example in same animal of steady-state beats to compare pressure-volume loop at baseline (black loop) with pressure-volume loop at 30 min of occlusion (gray loop). During occlusion systolic pressure and stroke work (area in loop) are increased, whereas end-diastolic volume, end-systolic volume, and stroke volume remain unchanged.
the RV improved (Fig. 2) as indicated by a significant decrease of the V25 of the ESPVR. In addition the Ees of the ESPVR tended to increase, although this increase was only statistically significant at 2 h of occlusion. These two findings represent a leftward shift of the ESPVR, varying between 70% at 15 min and 30% at 2 h, which indicates an improvement of the contractile performance. Figure 3A shows a typical example of pressure-volume loops during inflow reduction at baseline and at 30 min of occlusion in one of nine animals. It illustrates how the ESPVR becomes somewhat steeper and the volume intercept decreases in response to pulmonary artery occlusion. Figure 3B shows two steady-state beats at baseline and at 30 min of occlusion in the same animal. It illustrates how the RV is able to maintain its stroke volume against an increased afterload without changing its end-diastolic and end-systolic volume (see DISCUSSION). In addition, Fig. 3B afterload without changing its end-diastolic and end-systolic volume indicates an improvement of the contractile performance. Figure 3B shows two steady-state beats at baseline and at 30 min of occlusion in one of nine animals. It illustrates how the ESPVR becomes somewhat steeper and the volume intercept decreases in response to pulmonary artery occlusion. Figure 3B shows two steady-state beats at baseline and at 30 min of occlusion in the same animal. It illustrates how the RV is able to maintain its stroke volume against an increased afterload without changing its end-diastolic and end-systolic volume (see DISCUSSION). In addition, Fig. 3B demonstrates how, at any volume during ejection, a higher pressure is reached, which is an unmistakable hallmark of enhanced holosystolic contractile performance. This behavior can be explained only by an increased force generated by the constituting myocardial cells at unchanged cell length (6). Table 1 gives HR, Pao, arterial blood gasses, and pH at all conditions. The HR showed an increase in time, which was significant at 4 h of occlusion and remained significant after the pulmonary artery balloon was released. Pao showed a gradual decrease during the experiment. Blood gasses and pH all remained within the normal range.

**DISCUSSION**

The present study shows that in the newborn heart the RV improves its contractile performance in response to an increased RV afterload (Figs. 2 and 3). Clearly, rather than being independent determinants of cardiac performance, afterload and contractile performance interact: The increased slope, and more pronouncedly the decreased volume intercept, reflects the increased contractile state in response to a rise in afterload, as previously shown for the LV as well (4, 19, 24, 34). Apparently, in this newborn model, maintenance of the CO during an increased afterload is not obtained by an increased VED (Frank-Starling mechanism). Instead, the RV maintains its output by improving its contractile performance through a mechanism known as homeometric autoregulation (27, 28) or Anrep effect (36); end-diastolic volume and end-systolic volume of the RV are almost unchanged, despite a substantial increase in systolic RV pressure (Fig. 1B).

Earlier studies of our group have demonstrated that the LV of the newborn lamb shows homeometric autoregulation with an improved contractile performance in response to an increased afterload of the LV (19). Our present results demonstrate that the RV of newborn lambs shows the same phenomenon of homeometric autoregulation in response to an increased RV afterload. These findings are consistent with those in a recent study of Szabo et al. (33). Investigating the effects of pulmonary artery constriction in dogs, they found indirect evidence for homeometric autoregulation in the RV. However, their conclusions were based on measurements of segmental wall dimensions as a reflection of ventricular volume changes, and thus they could not demonstrate the classical picture of constant SV at a constant VED. By plotting pressure against segmental wall dimension, these investigators found that the pressure-dimension loops displayed an almost vertical elongation, while end-diastolic dimension increased little. Also using dimensional measurements, but converting them to approximate RV volume, Karunanithi et al. (17) studied pressure-volume relations in response to pulmonary artery constriction in adult dogs. Although homeometric autoregulation is not mentioned, these investigators show an improved contractile performance of the RV, based on a 45% increase of the slope of the ESPVR, in response to an increased afterload of the RV.

It has been suggested that homeometric autoregulation as seen in the LV is explained by an increased coronary perfusion secondary to the increased Pao (14). Indeed, several investigators demonstrated that increased coronary perfusion results in an improvement of the contractile performance of the LV (1), and also, though to a lesser extent, of the RV (12). However, in the case of an isolated increase of the RV afterload, without an increase in Pao (Table 1), the RV homeometric autoregulation obviously cannot be explained by an increased coronary perfusion. The mechanism for homeometric autoregulation, at least for the RV, must be sought to lie elsewhere. Studies in isolated cat papillary muscles have shown that homeometric autoregulation also occurs without changing the coronary perfusion (26, 27).

A potential explanation for the improved contractile performance of the RV could come from the consider-

Table 1. Hemodynamic variables and blood gas values throughout study

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>Pao, mmHg</th>
<th>PO2, kPa</th>
<th>PCO2, kPa</th>
<th>pH</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>164 ± 5</td>
<td>93 ± 3</td>
<td>141 ± 2.1</td>
<td>5.1 ± 0.3</td>
<td>7.36 ± 0.02</td>
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<tr>
<td>15 min</td>
<td>166 ± 10</td>
<td>86 ± 5</td>
<td>9.5 ± 3.7</td>
<td>6.3 ± 0.6*</td>
<td>7.27 ± 0.04*</td>
</tr>
<tr>
<td>30 min</td>
<td>169 ± 10</td>
<td>86 ± 5</td>
<td>21.6 ± 4.0*</td>
<td>5.2 ± 0.6</td>
<td>7.29 ± 0.04*</td>
</tr>
<tr>
<td>1 h</td>
<td>169 ± 9</td>
<td>83 ± 5*</td>
<td>15.5 ± 3.6</td>
<td>6.2 ± 0.6*</td>
<td>7.27 ± 0.04*</td>
</tr>
<tr>
<td>2 h</td>
<td>166 ± 9</td>
<td>79 ± 5*</td>
<td>16.8 ± 3.6</td>
<td>4.8 ± 0.6</td>
<td>7.38 ± 0.04</td>
</tr>
<tr>
<td>3 h</td>
<td>175 ± 9</td>
<td>78 ± 5*</td>
<td>14.5 ± 3.6</td>
<td>4.7 ± 0.6</td>
<td>7.34 ± 0.04</td>
</tr>
<tr>
<td>4 h</td>
<td>185 ± 9*</td>
<td>72 ± 5*</td>
<td>12.0 ± 3.7</td>
<td>4.9 ± 0.6</td>
<td>7.36 ± 0.04</td>
</tr>
<tr>
<td>After</td>
<td>183 ± 9*</td>
<td>73 ± 5*</td>
<td>13.3 ± 4.2</td>
<td>4.4 ± 0.6</td>
<td>7.41 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; Pao, arterial pressure. *P < 0.05, compared with baseline.
Our findings show that, not only was the RV able to maintain CO in response to an increased afterload, but that the heart even tended to increase CO when occlusion was continued, an increase that becomes significant at 4 h of pulmonary artery occlusion. It is not logical that this increased CO is caused by the increased afterload per se, because CO remained high after the pulmonary artery balloon was deflated. Looking at the mean HR of the nine lambs in this study, the same pattern is seen. So the late increase of CO can be explained mainly by the increase of HR. This increased HR is probably a sympathetic reflex to the gradual decrease of $P_{ao}$ during the experiments, which indicates systemic vasodilatation. The decreased LV afterload might also participate directly in the increase of CO. Anesthesia effects may well be the explanation for this gradually increased CO toward the end of the experiment (9).

In this study model we only investigated the acute effects of an increased RV afterload. The clinical situation of an increased afterload usually continues for much longer than 4 h. How the RV responds to chronically increased afterload cannot simply be extrapolated from the results of our study. Furthermore, the clinical situation of increased RV afterload is often accompanied by other factors that may influence the contractile performance of the heart. Severe respiratory diseases of the newborn often induce hypoxic pulmonary vasoconstriction and pulmonary arterial hypertension, causing increased afterload of the RV. In these situations the complex pulmonary conditions may overshadow the effect of an isolated pulmonary artery constriction. Besides effects of increased RV afterload, ventilation-related increase of intrathoracic pressure may influence biventricular performance. However, at this stage we specifically aimed to study the effects of an isolated purely hemodynamic RV afterload increase. Along the same lines, we did not study the effects of a severe pulmonary artery constriction because this would inevitably have led to a decrease in CO with its consequences for reduction in filling of the LV and a subsequent substantial decrease in $P_{ao}$. Indeed, as shown by Vlahakes et al. (35), this leads to reduction of coronary perfusion and to cardiac failure as shown by Guyton et al. (15). The findings from our model, however, may help to put the findings in the much more complex clinical situation into perspective.

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