Pulmonary hemodynamics and vascular reactivity in asphyxiated term lambs resuscitated with 21 and 100% oxygen

Satyan Lakshminrusimha,1 Robin H. Steinhorn,2 Stephen Wedgwood,4 Fabio Savorgnan,3 Jayasree Nair,1 Bobby Mathew,1 Sylvia F. Gugino,1 James A. Russell,1,2 and Daniel D. Swartz1

1Department of Pediatrics and 2Department of Physiology and Biophysics University at Buffalo, Buffalo, New York; 3Department of Pediatrics, University of Tennessee, Memphis, Tennessee; and 4Department of Pediatrics, Northwestern University, Chicago, Illinois

Submitted 9 June 2011; accepted in final form 25 July 2011

Lakshminrusimha S, Steinhorn RH, Wedgwood S, Savorgnan F, Nair J, Mathew B, Gugino SF, Russell JA, Swartz DD. Pulmonary hemodynamics and vascular reactivity in asphyxiated term lambs resuscitated with 21 and 100% oxygen. J Appl Physiol 111: 1441–1447, 2011. First published July 28, 2011; doi:10.1152/japplphysiol.00711.2011.—An increase in oxygen tension is an important factor in decreasing pulmonary vascular resistance (PVR) at birth. Birth asphyxia results in acidosis and increased PVR. We determined the effect of resuscitation with 21 vs. 100% O2 on pulmonary hemodynamics, pulmonary arterial (PA) reactivity, and oxidant stress in a lamb model of in utero asphyxia. Term fetal lambs were acutely asphyxiated by intrauterine umbilical cord occlusion for 10 min resulting in acidosis (pH 6.96 ± 0.05 and Pco2 103 ± 5 Torr), bradycardia, systemic hypotension, and increased PVR. Lambs were treated with 30 min of resuscitation with 21% or 100% O2 (n = 6 each). PaO2 was significantly elevated with 100% O2 resuscitation compared with 21% O2 (430 ± 38 vs. 64 ± 8 Torr), but changes in pH and PaCO2 were similar. The 100% O2 induced greater increase in pulmonary blood flow and decrease in PVR at 1 min of life, but subsequent values were similar to 21% O2 group between 2 and 30 min of life. Oxygen uptake from the lung and systemic oxygen extraction was similar between the two groups. Pulmonary arteries showed increased staining for superoxide anions and increased contractility to norepinephrine following resuscitation with 100% O2. The increased PA contractility induced by 100% O2 was reversed by scavenging superoxide anions with superoxide dismutase and catalase. We conclude that resuscitation of asphyxiated lambs with 100% O2 increases PaO2, but does not improve lung oxygen uptake, decrease PVR at 30 min, or increase systemic oxygen extraction ratios. Furthermore, 100% O2 also induces oxidative stress and increases PA contractility. These findings support the new neonatal resuscitation guidelines recommending 21% O2 for initial resuscitation of asphyxiated neonates.

asphyxia; pulmonary vascular resistance; superoxide

The optimal concentration of oxygen during resuscitation of an asphyxiated newborn continues to be controversial. The fetal pulmonary vascular resistance (PVR) is high, and the rise in oxygen tension plays a crucial role in mediating the pulmonary vascular transition at birth (15). The new Neonatal Resuscitation Program guidelines recommend initial resuscitation of asphyxiated term newborn infants with 21% oxygen (9, 24). Resuscitation with 21% oxygen has been associated with reduced neonatal mortality (29, 36). However, during resuscitation of an asphyxiated acidotic newborn infant with potentially high PVR (17), there is a concern that room air resuscitation may result in inadequate pulmonary vasodilatation. It seems logical that resuscitating with 100% oxygen rapidly reverses hypoxemia and acidosis and dilates constricted pulmonary arteries more efficiently than room air (20).

Current evidence from animal studies demonstrates the following changes in pulmonary hemodynamics with varying oxygen concentration during resuscitation in term newborns. Medbo et al. (21) studied pulmonary hemodynamics in response to 21 vs. 100% oxygen in 1- to 3-day-old asphyxiated piglets. Resuscitation with 21 or 100% oxygen resulted in similar changes in pulmonary arterial pressures. However, this study was done in postnatal piglets when PVR had already decreased from high fetal levels to low postnatal levels (21). We (13) have previously shown that resuscitation of normal term lambs with 100% oxygen at birth produced a more rapid initial drop in PVR compared with resuscitation with 21% oxygen but impaired subsequent pulmonary vasodilator response to inhaled nitric oxide and acetylcholine. Resuscitation of near-term lambs with chronic intrauterine pulmonary hypertension with 100 or 21% oxygen resulted in a similar decrease in PVR (16). However, these fetal lambs were not acidic or hypercarbic at birth, as seen in asphyxiated neonates before resuscitation.

Oclusion of the umbilical cord is a commonly used model of acute asphyxia in lambs (4, 19). Cord occlusion in term fetal lambs resulted in an initial increase in PVR followed by a decrease to control fetal levels by 4–5 min (4). Resuscitation with “oxygen-enriched air” (exact oxygen concentration was not reported) resulted in a fall in PVR (4). There have not been any studies reporting the effect of inspired oxygen concentration in resuscitative gas on pulmonary hemodynamics after asphyxiation at birth before decrease in fetal PVR.

The main purpose of providing supplemental oxygen during resuscitation is to increase oxygen uptake from the lungs and improve its delivery to the tissues. Oxygen uptake from the lung (ml·min⁻¹·kg⁻¹) and systemic oxygen extraction ratio (OER) have not been evaluated in asphyxiated animals during resuscitation with varying levels of oxygen.

We (12, 23) have previously reported that ventilation of nonasphyxiated term and preterm lambs with 100% oxygen at birth results in increased contractility of pulmonary arterial rings at 24 h of age. Whether 100% oxygen alters pulmonary arterial contractility following resuscitation of asphyxiated animals at birth is not known.

The objective of our study was to evaluate changes in pulmonary hemodynamics following acute asphyxia with hypercarbia and acidosis by umbilical cord occlusion and resuscitation with 21 and 100% oxygen concentrations at birth for

Address for reprint requests and other correspondence: S. Lakshminrusimha, Division of Neonatology, Women and Children’s Hospital of Buffalo, 219 Bryant St., Buffalo, NY 14222 (e-mail: slakshmi@buffalo.edu).

http://www.jap.org
8750-7587/11 Copyright © 2011 the American Physiological Society

isolated pulmonary arterial rings were pretreated with propranolol (10^{-6} M) to block β-adrenergic receptors and constricted with increasing doses of norepinephrine (10^{-8} M to 10^{-6} M). Some arteries were pretreated with the antioxidants polyethylene glycol-conjugated superoxide dismutase (75 U/ml bath concentration) and polyethylene glycol-conjugated catalase (1,200 U/ml) for 30 min before constriction with norepinephrine as previously described (22). Both enzymes were added together to avoid hydrogen peroxide-induced changes in vascular reactivity (37). Polyethylene glycol improves cell membrane permeability. Constriction response was measured as force generated in grams and normalized to tissue weight.

In situ analysis of superoxide generation. Frozen lung sections were exposed to 5 μM dihydroethidium (DHE; Molecular Probes/Invitrogen) in PBS as previously described (7). Slides were incubated in a light-protected humidified chamber at 37°C for 30 min. Ethidium-stained slices were observed by fluorescence microscopy with excitation at 518 nm and emission at 605 nm. Fluorescent images were captured using a CoolSnap digital camera with Metamorph imaging software (Molecular Devices, Sunnyvale, CA). Tissue sections were processed and imaged in parallel.

Statistical analysis. Sample size measurements were based on prior published data in nonasphyxiated lambs (13). With the use of PVR values at 30 min of life, to detect a 20% difference in PVR, with a power of 80% and a type I error probability of 0.05, a total of six lambs per group were needed. Comparison between 21 and 100% oxygen groups was performed by ANOVA repeated measures using Statview 4.0 (Abacus Concepts). Isolated vessel data were analyzed by ANOVA repeated measures. Data are presented as means ± SE. Significance was accepted at P < 0.05.

RESULTS

Fourteen lambs were instrumented and underwent asphyxia by cord occlusion. Two lambs were excluded from analysis: one lamb became asystolic during the cord occlusion period and could not be resuscitated; another lamb required chest compressions and epinephrine during resuscitation resulting in significant increases in heart rate, SBP, and pulmonary arterial pressure. Birth weight and hemoglobin levels were similar between the groups (Table 1).

Cord occlusion resulted in a significant drop in heart rate from 151 ± 10 to 61 ± 5/min (Fig. 1). Bradycardia was accompanied by a significant drop in SBP (Fig. 2). Resuscitation increased heart rate significantly within 2 min of ventilation in both groups (Fig. 1). There was no difference in heart rate between 21 and 100% resuscitation groups. Mean SBP increased with resuscitation. Although there was trend towards higher SBP in 21% oxygen group, this difference did not reach statistical significance (Fig. 2).

There was no change in mean pulmonary arterial pressure (Fig. 3), but left pulmonary arterial flow markedly dropped following cord occlusion (Fig. 4). The decrease in left pulmonary arterial blood flow could be attributed to the decrease in heart rate during cord occlusion, as we observed no change in left pulmonary arterial blood flow when corrected for heart rate (0.12 ± 0.004 at baseline and 0.12 ± 0.03 ml·kg^{-1}·heartbeat^{-1} after 10 min of cord occlusion). Calculated PVR increased significantly with cord occlusion, but this change was primarily related to the decrease in pulmonary blood flow due to bradycardia and not due to increase in pulmonary arterial pressure (Figs. 3, 4, and 5).

Pulmonary arterial pressure increased initially with the onset of resuscitation and then decreased without any significant difference between the 21 and 100% oxygen groups (Fig. 3).
Left pulmonary arterial blood flow markedly improved with the onset of resuscitation in both the groups (Fig. 4). Left pulmonary arterial blood flow was significantly higher at 1 min of age with 100% oxygen resuscitation compared with 21% oxygen resuscitation, but all subsequent measurements were similar between the two groups. PVR markedly decreased with resuscitation in both groups (Fig. 6). There was no difference in PVR between the two groups over the 30-min resuscitation period. A significantly greater decrease in PVR was observed in the 100% oxygen resuscitation group at 1 min (1.7 ± 0.2 with 21% oxygen and 0.9 ± 0.3 with 100% oxygen; \( P < 0.05 \) by ANOVA), but there was no significant difference in PVR between these two groups at any other time point.

Cord occlusion for 10 min resulted in similar levels of fetal hypercarbia and acidosis in both the 21 and 100% oxygen groups (Fig. 6). Resuscitation resulted in a significant decrease in \( \text{PaCO}_2 \), and increase in \( \text{pH} \) and \( \text{PaO}_2 \) within 5 min in both groups. There was no significant difference in \( \text{pH} \) or \( \text{PaCO}_2 \) levels between 21 and 100% oxygen groups, although a tendency towards lower \( \text{pH} \) was observed in the 100% oxygen group at 30 min of age (\( P = 0.08 \); Fig. 6C). Resuscitation with 100% oxygen ventilation produced significantly greater increases in \( \text{PaO}_2 \) (Fig. 6A and Table 1), as well as higher \( \text{PaO}_2 \) levels in pulmonary arterial gases (Table 1).

**Oxygen uptake from the lung.** Simultaneous blood gases from pulmonary artery and the left atrium were drawn at 5 and 30 min after the onset of resuscitation. Left atrial and pulmonary arterial \( \text{PO}_2 \) and oxygen saturations were significantly higher following 100% oxygen resuscitation at 5 and 30 min (Table 1). There was no significant difference in oxygen content between left atrial and pulmonary arterial blood (A-V \( \text{DO}_2 \)) between the two groups. The oxygen uptake from the left lung (obtained by multiplying left pulmonary blood flow with A-V \( \text{DO}_2 \)) was not increased by 100% oxygen ventilation (Table 1).

**OER.** At 30 min of age, the systemic arterial oxygen content was 14.5 ± 0.7 ml/dl in the 21% oxygen group and 17.5 ± 0.4 ml in the 100% oxygen group (\( P < 0.01 \)). The mixed venous oxygen content was 12.1 ± 0.9 ml/dl in the 21% oxygen group and 14.5 ± 0.2 ml/dl in the 100% oxygen group (\( P < 0.05 \)). There was no significant difference in arteriovenous difference in oxygen content between the two groups. Systemic OER was similar in 21 and 100% oxygen resuscitation groups despite marked differences in \( \text{PaO}_2 \) levels (Table 1).

---

**Table 1. Birth weight, hemoglobin level at birth, and oxygen extraction from the left lung in lambs at 5 and 30 min after birth following resuscitation with 21 and 100% oxygen**

<table>
<thead>
<tr>
<th></th>
<th>21% Oxygen (n = 6)</th>
<th>100% Oxygen (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g.</td>
<td>3,140 ± 292</td>
<td>3,216 ± 445</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.1 ± 0.4</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>Pulmonary arterial ( \text{PO}_2 ), Torr</td>
<td>34 ± 5</td>
<td>72 ± 8*</td>
</tr>
<tr>
<td>Pulmonary arterial oxygen saturation, %</td>
<td>80 ± 3</td>
<td>92 ± 2*</td>
</tr>
<tr>
<td>Left atrial ( \text{PO}_2 ), Torr</td>
<td>68 ± 15</td>
<td>454 ± 33*</td>
</tr>
<tr>
<td>Left atrial oxygen saturation</td>
<td>92 ± 3</td>
<td>998 ± 0.2*</td>
</tr>
<tr>
<td>Pulmonary artery to left atrial oxygen content difference, ml/dl</td>
<td>1.8 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Oxygen uptake by left lung, ml·kg(^{-1})·min(^{-1})</td>
<td>1.8 ± 0.4</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Systemic oxygen extraction ratio</td>
<td>0.12 ± 0.07</td>
<td>0.08 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; 5 and 30 min are the times after onset of resuscitation. *\( P < 0.05 \), compared with 21% oxygen at the corresponding time point.

---

**Fig. 1.** Changes in heart rate following asphyxia by cord occlusion and resuscitation with 21% oxygen or 100% oxygen. Birth refers to the onset of ventilation. *\( P < 0.05 \), data at birth compared with baseline; †first time point when \( P < 0.05 \), compared with birth by ANOVA. There was no difference in the heart rate response between the 2 groups.

**Fig. 2.** Changes in mean systemic blood pressure following asphyxia by cord occlusion and resuscitation with 21% oxygen or 100% oxygen. Birth refers to the onset of ventilation. *\( P < 0.05 \) at birth compared with baseline; †first time point when \( P < 0.05 \), compared with birth by ANOVA. There was no difference in the systemic blood pressure response between the 2 groups.
The fluorescence intensity with DHE in the pulmonary arteries was 2.24 ± 0.46-fold higher following resuscitation with 100% oxygen compared with 21% oxygen (P < 0.05; Fig. 7, A and B).

Isolated pulmonary arterial ring contraction. Pulmonary arterial rings contracted in a concentration-dependent manner to norepinephrine. Resuscitation with 100% oxygen for 30 min markedly increased contraction to norepinephrine compared with 21% oxygen (Fig. 7C). Pretreatment with superoxide dismutase and catalase reversed the increased contraction induced by 100% oxygen resuscitation but did not alter contraction response in the 21% oxygen resuscitation group.

**DISCUSSION**

Birth asphyxia accounts for ~0.7 to 1.2 million annual deaths in the world (18). Appropriate resuscitative measures during the first few minutes of life can result in substantial reduction in mortality and morbidity from birth asphyxia (34). A recent meta-analysis demonstrates that resuscitation of asphyxiated infants with 21% oxygen reduces neonatal mortality (29).

Successful transition at birth is dependent on establishment of lungs as the organ of gas exchange. This process requires a dramatic reduction in PVR to allow for an 8- to 10-fold increase in pulmonary blood flow at birth (15). Birth asphyxia results in hypercarbia, acidosis, and hypoxemia; factors that may prevent reduction in PVR at birth resulting in pulmonary hypertension (17). Resuscitation with 21% oxygen (as opposed to 100% oxygen) may theoretically be insufficient to decrease PVR at birth. However, resuscitation with 100% oxygen can produce extremely high alveolar and arterial PO2 levels and may lead to oxygen toxicity (12, 22, 33). Previous studies have looked at the effect of 21 and 100% oxygen on pulmonary hemodynamics in asphyxiated postnatal piglets where PVR has already decreased from high fetal values. To our knowledge, this is the first study to address the effect of 21 and 100% oxygen resuscitation at birth on pulmonary hemodynamics, lung oxygen uptake, and systemic OER in an animal model with prenatal asphyxia.

We induced asphyxia by using a well-established model of intrauterine umbilical cord occlusion (1, 4, 19) and determined changes in pulmonary hemodynamics during occlusion and subsequent resuscitation. Following occlusion, we observed a significant drop in heart rate (Fig. 1), SBP (Fig. 2), and pulmonary blood flow (Fig. 4) but found no significant change in pulmonary arterial pressure (Fig. 3). PVR acutely increased following cord occlusion (Fig. 5). This increase in PVR was mainly secondary to decreased pulmonary blood flow and not secondary to an increase in pulmonary arterial pressure. It was interesting to note that the decrease in pulmonary blood flow per minute was mainly due to a decrease in heart rate and not due to change in stroke volume (Fig. 4). During the fetal and immediate newborn period, the main determinant of cardiac...
output is heart rate (2, 5, 8). These results emphasize the importance of heart rate during asphyxia and neonatal resuscitation as stressed in the new neonatal resuscitation guidelines (9, 24).

Despite the presence of significant acidosis following cord occlusion (Fig. 6C), resuscitation with either 21 or 100% oxygen significantly decreased Pao2, and significantly increased pH and PaO2, similar to previous studies in this model (19). As expected, 100% oxygen produced greater increases in PO2 and oxygen saturation (SO2) levels in both systemic and pulmonary arteries at 5 and 30 min (Table 1). However, oxygen uptake from the left lung was similar following 100 vs. 21% oxygen resuscitation despite exposure to higher alveolar PO2 levels (table 1). Since pulmonary arterial SO2 is an accurate reflection of mixed venous SO2 (26), our findings further suggest that birth asphyxia leads to impaired systemic oxygen extraction, similar to that reported following cardiac arrest in adult humans (27). Calculated systemic OER were lower in asphyxiated lambs compared with values described in normal newborn infants (25, 30) and those observed in normal lambs without asphyxiation (0.2 ± 0.1 with 21% oxygen and 0.14 ± 0.04 with 100% oxygen at 30 min of life, unpublished data from our laboratory, n = 6 lambs each). There was no significant difference in systemic OER between 21 and 100% oxygen resuscitation groups (Table 1).

Resuscitation resulted in a rapid increase in heart rate, SBP, and pulmonary blood flow (Figs. 1, 2, and 4). Pulmonary arterial pressure increased initially with resuscitation, probably secondary to increased blood flow (Fig. 3). A similar increase in pulmonary arterial pressure with the onset of ventilation following asphyxia has been reported in fetal lambs (4). Despite marked acidosis and hypercarbia, ventilation with either 21 or 100% oxygen produced rapid and sustained decrease in PVR (Fig. 5). Resuscitation with 100% oxygen resulted in a greater decrease in PVR by 1 min, a finding that was similar to our previous results in nonasphyxiated lambs (13). However, this difference is probably of no clinical significance, as we observed no difference in PVR between the two groups during the remaining period of resuscitation. We have previously shown that in healthy newborn lambs studied just after birth, PVR increases when Pao2 falls below 52.5 ± 1.7 Torr. However, when supplemental oxygen is used to increase the Pao2 above this value, PVR does not further decrease (16). Rudolph and Yuan have shown a similar relationship between Pao2 and PVR in newborn calves (28). The present study indicates that this relationship holds true in lambs exposed to acute asphyxia at birth. As Pao2 levels were higher than this “change point” level in lambs resuscitated with 21% oxygen, further increases in alveolar and arterial oxygen tension produced by 100% oxygen resuscitation did not further decrease PVR. Rudolph and Yuan (28) have also shown that hypoxemia (Pao2 <40 Torr) in the presence of acidosis results in marked increase in PVR. In the current study, resuscitation in 21% oxygen resulted in Pao2 >60 Torr soon after birth (Fig. 6A) leading to a decrease in PVR. In models associated with parenchymal lung disease [respiratory distress syndrome in preterm lambs (10) or meconium aspiration in term lambs (31)], 21% oxygen may not be adequate to achieve Pao2 >40 Torr and supplemental oxygen may be necessary during resuscitation at birth.

Pulmonary arterial rings isolated from lambs resuscitated with 100% oxygen showed increased contractility similar to our previous observations in term and preterm nonasphyxiated lambs (11, 12, 23). In the current study, we evaluated these vessels after only 30 min of exposure to hyperoxia, further indicating that hyperoxia rapidly induces vascular dysfunction. We further found evidence for increased superoxide anion formation in pulmonary arteries on frozen lung sections (Fig.
To further establish the role of reactive oxygen species in mediating increased pulmonary arterial contractility, we scavenged superoxide and hydrogen peroxide by incubating the arterial rings with superoxide dismutase and catalase and noted a complete reversal of the increased contractility induced by resuscitation with 100% oxygen. PA, pulmonary artery; AW, airway. C: force of contraction of 5th generation pulmonary arterial rings to norepinephrine ($10^{-8}$ to $10^{-6}$ M). Contraction responses following incubation with polyethylene glycol-conjugated superoxide dismutase (SOD) and catalase (CAT) are shown as dashed lines. $\ddagger P < 0.05$, compared with 21% Res by ANOVA repeated measures.

There are several limitations to this study. Cord occlusion represents acute total asphyxia. Response to resuscitation following chronic partial asphyxia or hypovolemic or hemorrhagic asphyxia may be different from the results reported in this study. The PVR in this study was calculated and may not represent true resistance in the pulmonary vascular circuit. Ventilation through endotracheal tube was immediately initiated at the beginning of resuscitation, unlike a typical clinical scenario where intubation and ventilation may not occur until 1–2 min after birth. Lastly, we did not measure systemic blood flow, as placing a flow probe around the aorta is technically difficult and prolongs the instrumentation time in fetal lambs. We acknowledge that significant differences in systemic flow may have influenced the interpretation of differences observed in pulmonary hemodynamics.

To conclude, we have shown that acute asphyxia by cord occlusion results in hypercarbia, acidosis, and increased PVR secondary to decreased pulmonary blood flow. This decrease in pulmonary blood flow is predominantly due to decreased heart rate. Resuscitation with 21 and 100% oxygen result in similar increases in heart rate, systemic blood pressure, pulmonary blood flow, and PVR. Although 100% oxygen resuscitation produces greater increases in systemic arterial and pulmonary arterial oxygen tension, this is not due to higher oxygen uptake by the lung and does not lead to improved systemic oxygen extraction. We demonstrate that brief resuscitation with 100% oxygen for 30 min increases superoxide anion formation in the pulmonary arteries, increases contractility of isolated pulmonary arteries, and does not offer any advantage in correcting pulmonary hemodynamics compared with 21% oxygen.
findings indicate that the new neonatal resuscitation guidelines recommending the use of 21% oxygen for the initial resuscitation of asphyxiated term infants will promote a similar degree of pulmonary vasodilation and oxygen delivery and will avoid oxygen toxicity as opposed to 100% oxygen.

GRANTS

Support for this work was provided by National Institute of Child Health and Human Development Grant 1RO3-HD-060138-01 (to S. Lakshminrusimha) and National Heart, Lung, and Blood Institute Grant 2RO1-HL-054705-14 (to R. H. Steinhorn).

DISCLOSURES

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

REFERENCES


