High-altitude chronic hypoxia during gestation and after birth modifies cardiovascular responses in newborn sheep

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Herrera EA, Pulgar VM, Riquelme RA, Sanhueza EM, Reyes RV, Ebensperger G, Parer JT, Valdéz EA, Giussani DA, Blanco CE, Hanson MA, Llanos AJ. High-altitude chronic hypoxia during gestation and after birth modifies cardiovascular responses in newborn sheep. *Am J Physiol Regul Integr Comp Physiol* 292: R2234–R2240, 2007. First published February 22, 2007; doi:10.1152/ajpregu.00909.2006.—Perinatal exposure to chronic hypoxia induces sustained pulmonary hypertension and structural and functional changes in both pulmonary and systemic vascular beds. The aim of this study was to analyze consequences of high-altitude chronic hypoxia during gestation and early after birth in pulmonary and femoral vascular responses in newborn sheep. Lowland (LLNB; 580 m) and highland (HLNB; 3,600 m) newborn lambs were catheterized under general anesthesia and submitted to acute sustained or stepwise hypoxic episodes. Contractile and dilator responses of isolated pulmonary and femoral small arteries were analyzed in a wire myograph. Under basal conditions, HLNB had a higher pulmonary arterial pressure (PAP; 20.2 ± 2.4 vs. 13.6 ± 0.5 mmHg, $P < 0.05$) and cardiac output (342 ± 23 vs. 279 ± 13 ml·min^{-1}·kg^{-1}, $P < 0.05$) compared with LLNB. In small pulmonary arteries, HLNB showed greater contractile capacity and higher sensitivity to nitric oxide. In small femoral arteries, HLNB had lower maximal contraction than LLNB with higher maximal response and sensitivity to noradrenaline and phenylephrine. In acute superimposed hypoxia, HLNB reached higher PAP and femoral vascular resistance than LLNB. Graded hypoxia showed that average PAP was always higher in HLNB compared with LLNB at any P_{O_2}. Newborn lambs from pregnancies at high altitude have stronger pulmonary vascular responses to acute hypoxia associated with higher arterial contractile status. In addition, systemic vascular response to acute hypoxia is increased in high-altitude newborns, associated with higher arterial adrenergic responses. These responses determined in intrauterine life and early after birth could be adaptive to chronic hypoxia in the Andean altiplano.

hypoxemia; pulmonary hypertension; vascular reactivity; neonatal lamb; highlands

EXPOSURE OF LOWLAND-ADAPTED animals to high altitude produces changes in the pulmonary and systemic circulations (19). In the pulmonary circulation, such hypoxia causes vasoconstriction resulting in pulmonary hypertension. Appropriate increases in pulmonary arterial vascular resistance are adaptive, matching pulmonary perfusion to the reduced oxygenation. However, excessive increases in pulmonary vascular resistance (PVR), if maintained over time, lead to structural changes in the pulmonary vasculature, such as an increase in vascular muscle cells and fibrosis in the adventitia of the vessel (14, 19, 35). Other pathologic conditions can manifest as high-altitude pulmonary hypertension and edema; in children this forms part of the subacute infantile mountain sickness (22, 34). Furthermore, persistent pulmonary hypertension in newborn infants and animals, and brisket disease in cattle are well-established health problems (1, 26). Moreover, chronic hypoxia can also produce changes in systemic vascular resistance (3, 27). These issues are important because currently nearly 140 million people reside at over 2,500 m above sea level, and are permanently exposed to chronic hypoxic conditions (20). In some of these populations, for example at La Paz, Bolivia (3,700 m) there is a high incidence of intrauterine growth restriction, preeclampsia, stillbirth, and respiratory distress in newborns, leading to an increased infant mortality rate (15).

In newborns, either at altitude or sea level, pulmonary hypertension is due to a failure to regulate PVR at birth, leading to hypoxemia and sustained pulmonary hypertension. One of the major factors associated with persistent pulmonary hypertension in the newborn is chronic hypoxia in utero (1). In humans, this syndrome occurs at a rate of 1 to 2 per 1,000 live births at low altitude, and is probably much higher at high altitude (15). As an example of how serious this condition can be, the pulmonary arterial pressure (PAP) of a group of newborns in Perú at 4,540 m above sea level was close to the systemic arterial pressure (SAP) (7).

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The mechanisms resulting in pulmonary hypertension at high altitude have not yet been fully elucidated. However, it has been suggested that during chronic hypoxia in newborns, production of vasocontrictor factors within the lung is enhanced, while synthesis of vasodilators may be reduced (1, 6, 31).

We hypothesized that newborn sheep gestated and born at high altitude would have a higher basal PAP and an increased pulmonary vascular response to a superimposed episode of acute hypoxemia. In addition, these animals would show an enhanced systemic vascular response to acute hypoxia relative to lowland born species.

To test the hypothesis, we investigated basal pulmonary and systemic cardiorespiratory function and the response to a period of superimposed hypoxia in newborn lambs gestated and born either at sea level or at altitude (3,600 m). To determine the mechanisms underlying changes in cardiorespiratory physiology, we assessed the sensitivity of the pulmonary vasculature to several degrees of oxygenation and determined vasoconstrictor and vasodilator responses in isolated small pulmonary and femoral arteries.

METHODS

Animals

All experimental protocols were reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Chile. Animal care, maintenance, procedures, and experimentation were performed in accordance with the *American Physiological Society Guiding Principles for Research Involving Animals and Human Beings* (2).

Fourteen newborn sheep born and raised at the University of Chile farm, Santiago, 580 m above sea level [Lowland newborn (LLNB), mean weight of 7.0 ± 0.4 kg; 7–12 days of age] and 10 newborn sheep, born and raised at Putre Research Station, International Center for Andean Studies (INCAS), University of Chile, 3,600 m above sea level [Highland newborn (HLNB), mean weight of 5.6 ± 0.4 kg; $P < 0.05$, compared with LLNB; 8–12 days of age] were studied. The highland sheep had been at altitude for at least 50 generations. The newborns and ewes were housed in an open yard with access to food and water ad libitum. Similar but uninstrumented animals (LLNB: $n = 6$, HLNB: $n = 7$) were used for the collection of small arteries for ex vivo experiments.

Surgical Preparation

The lambs were premedicated with atropine (0.04 mg/kg im; Atropina Sulfato; Laboratorio Chile, Santiago, Chile). All surgical procedures were performed under general anesthesia with ketamine, 10 mg/kg im (Ketostop; Drag Pharma-Invectec, Santiago, Chile) and diazepam 0.1–0.5 mg/kg im (Laboratorio Biosano, Santiago, Chile) with additional local infiltration of 2% lidocaine (Dimecaína; Laboratorio Beta, Santiago, Chile). Polyvinyl catheters (1.2 mm ID) were placed in the descending aorta and inferior vena cava via a hindlimb artery and vein. In the contralateral femoral artery, a Transonic blood flow transducer (Transonic Systems, Ithaca, NY) was installed. The polyvinyl catheters and the flow probe cable were exteriorized percutaneously through the animal's flank and kept in a pouch sewn onto the skin. In addition, an Edwards Swan-Ganz catheter (5 French; Baxter Healthcare, Irvine, CA) was inserted into the pulmonary artery via an external jugular vein, exteriorized, and placed in a pouch around the neck of the animal. All vascular catheters were filled with a heparinized solution of 0.9% NaCl (500 IU heparin/ml 0.9% NaCl) and plugged with a copper pin. Ampicillin 10 mg/kg iv (Ampicilina, Laboratorio Best-Pharma, Santiago, Chile) and gentamicin 4 mg/kg iv (Gentamicina Sulfato, Laboratorio Biosano), were administered every 12 h while the animals were instrumented. The experiments commenced 3 days after surgery.

Experimental Protocols

In vivo experiments. ACUTE HYPOXIA. All experiments were based on a 3-h protocol divided into three periods: 60 min of basal (breathing room air), 60 min of hypoxemia, and 60 min of recovery. To induce hypoxemia, a transparent loosely tied polyethylene bag was placed over the animal's head into which a controlled mixture of air, N_2, and CO_2 (~10% O_2 and 2–3% CO_2 in N_2) was passed at ~20 l/min. The gas mixture reduced arterial P_{O_2} to ~30 mmHg. After the 60 min of hypoxemia, the animal was returned to breathing air for a further 60 min (recovery).

Arterial blood samples (0.3 ml) were taken in heparinized syringes at 15 and 45 min of normoxemia, each 15 min during the hypoxemic hour, and after 15 and 45 min of recovery. Arterial pH, P_{O_2}, P_{CO_2} (model ABL 555 blood gas monitor; Radiometer, Copenhagen, Denmark; measurements corrected to 39°C), hemoglobin concentration, percentage saturation of hemoglobin (Sa_{O_2}), and oxygen content using an animal (ovine) program option (model OSM3 hemoximeter; Radiometer) were calculated. SAP, PAP, and right atrial pressure were measured continuously using pressure transducers and recorded by a data acquisition system (Powerlab/8SP System and Chart v4.1.2 Software; ADInstruments, New South Wales, Australia) connected to a personal computer. Heart rate and mean systemic arterial blood pressure (MAP) were obtained from this record. In addition, femoral blood flow (FBF) was also measured continuously and recorded by the data acquisition system connected to a personal computer.

Cardiac output was determined just after the blood sampling by the thermodilution method as the average of three determinations after injection of 3 ml of chilled (0°C) NaCl 0.9% into the pulmonary artery (model COM-2 cardiac output computer, Baxter, Irvine, CA).

Systemic vascular resistance (SVR), PVR, total oxygen consumption ($\dot{V}_{O_2}$), and total oxygen extraction were calculated by using the following equations

$$\text{SVR} = \frac{\text{MAP} - \text{right atrial pressure (mmHg)}}{\text{Cardiac output (ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1})}$$

$$\text{PVR} = \frac{\text{PAP} - \text{pulmonary wedge pressure (mmHg)}}{\text{Cardiac output (ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1})}$$

$$\dot{V}_{O_2} = \frac{O_2 \text{ content (ascending aorta} - \text{pulmonary artery)} \times 100}{\text{Cardiac output (ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1})}$$

$$O_2 \text{ extraction} = \frac{O_2 \text{ content (ascending aorta} - \text{pulmonary artery)} \times 100}{O_2 \text{ content of ascending aorta}}$$

GRADED HYPOXIA. To evaluate the P_{O_2} sensitivity of the pulmonary vascular bed, a graded hypoxia protocol was performed, during which the aortic P_{O_2} (Pa_{O_2}) was reduced in steps of 10 mmHg (Pa_{O_2}) in lowland newborns. In highland newborns, an oxygenated mixture was given to increase the Pa_{O_2} in 10 mmHg steps, until reaching values equivalent to those at sea level. In each experimental step, pH and blood gases, PAP, and SAP were registered.

Ex vivo experiments. SMALL ARTERY ISOLATION. Newborn sheep were euthanized with an overdose of sodium thiopenthone 100 mg/kg iv. (Tiopental; Laboratorio Biosano, Santiago, Chile). The left lung and a hindlimb were removed by dissection and immediately immersed in cold saline. Fourth generation pulmonary arteries (counting from the pulmonary artery trunk, internal diameter: LLNB, 410 ± 20 μm; HLNB, 454 ± 20 μm) and third generation femoral arteries (counting from the main femoral artery, internal diameter: LLNB, 406 ± 31 μm; HLNB, 388 ± 15 μm) were dissected from each vascular bed.

MEASUREMENT OF ARTERIAL REACTIVITY. Isolated arteries were mounted between an isometric force transducer (model DSC 6; Kistler

Table 1. *Cardiorespiratory variables in lowland (LLNB) and highland (HLNB) newborn sheep during a superimposed episode of acute hypoxemia*

Variable	Group	Basal	Hypoxemia	Recovery
pH	LLNB	7.410±0.007	7.391±0.007	7.423±0.007
	HLNB	7.453±0.006‡	7.433±0.008‡	7.414±0.008*
P_{CO_2}, mmHg	LLNB	36.8±0.4	35.8±0.5	32.4±0.4†
	HLNB	32.1±0.4‡	31.8±0.5‡	30.9±0.5
P_{O_2}, mmHg	LLNB	79.0±1.9	30.9±0.6†	82.4±2.0
	HLNB	40.6±2.4‡	31.3±0.5†	43.7±2.4‡
Hb, g/dL	LLNB	10.9±0.5	11.3±0.4†	10.3±0.4
	HLNB	11.9±0.7	12.4±0.7†	11.8±0.7
Sa_{O_2}, %	LLNB	94.4±0.7	52.7±1.7†	96.0±0.7
	HLNB	66.1±1.9‡	50.3±2.0†	66.6±2.0‡
O_2 cont, $mlO_2 \cdot dl^{-1}$	LLNB	13.9±0.2	8.0±0.3†	13.3±0.2
	HLNB	10.9±0.3‡	8.7±0.3†	10.9±0.3‡
$\dot{V}O_2$, $mlO_2 \cdot min^{-1} \cdot kg^{-1}$	LLNB	16.4±0.9	17.6±0.8	16.0±1.0
	HLNB	18.8±0.8‡	19.2±1.2	21.3±1.2‡
O_2 Extraction, %	LLNB	41.3±1.5	60.1±2.0†	40.3±2.0
	HLNB	51.0±2.5‡	61.7±2.5*	55.3±2.5‡

Values are shown as means ± SE. Significant differences $P < 0.05$: *vs. basal; †vs. all; ‡vs. LLNB.

Morce, Seattle, WA) and a displacement device in a myograph (dual-wire myograph; Danish Myo Technologies, Aarhus, Denmark) using two stainless steel wires (diameter: 40 μm). During mounting and experimentation, the myograph organ bath was filled with 10 ml Krebs-Henseleit buffer maintained at 39°C and aerated with 95% $O_2$5% CO_2. Each artery was stretched to its individual optimal lumen diameter, i.e., the diameter at which it developed the strongest contractile response to 125 mM K^+, using a diameter-tension protocol as previously described for pulmonary and femoral arteries (28, 36, 37).

Contractile agonists were evaluated under basal tone. A concentration-response curve was constructed for potassium chloride (KCl) by exposing the arteries to 11 different concentrations of KCl (4.75–125 mM) with each dose maintained for 2 min, and the segment washed with Krebs-Henseleit buffer before the next concentration was introduced. Cumulative concentration-response curve for noradrenaline (10^{-10}–10^{-3} M) and phenylephrine (10^{-10}–10^{-3} M) were constructed by increasing the organ chamber concentration of the drug incrementally after a steady-state response had been measured. Concentration-response curves for the nitric oxide donor sodium nitroprusside (10^{-8} M–10^{-4} M) were constructed during contraction induced by 125 mM K^+.

SOLUTIONS AND DRUGS. Krebs-Henseleit buffer contained (in mM) 118.5 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, and 5.5 glucose with a pH of 7.4. In 125 mM K^+ buffer, all of the NaCl was replaced by an equimolar amount of KCl. Noradrenaline and phenylephrine were obtained from Sigma (St Louis, MO) and sodium nitroprusside from Prolabo (Paris, France).

Data Analysis

For in vivo experiments all values were expressed as means ± SE. Statistical analysis was performed using a two-way analysis of variance. Differences between means were assessed using the Newman-Keuls test. Significance was accepted when $P < 0.05$ (12).

For ex vivo experiments dose-response curves were analyzed in terms of sensitivity and maximal response (E_{max}) by fitting experimental data to a sigmoid equation (Origin version 5.0; MicroCal Software, MA). Contractile responses were expressed in terms of tension [force in miliNewton divided by length of the arterial segment (in mN/mm)] or as percentage of maximal response to KCl (%E_{max}). Relaxant responses were expressed as a percentage of reduction of 125 mM K^+-induced contraction. Sensitivity was calculated as pD_2,

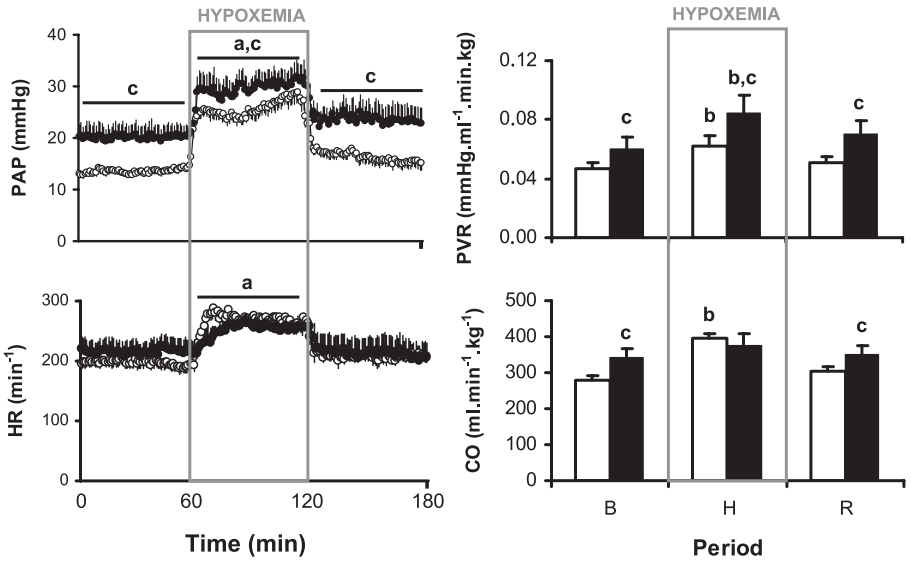

Fig. 1. Pulmonary arterial pressure (PAP), heart rate (HR), pulmonary vascular resistance (PVR), and cardiac output (CO) in lowland newborn sheep (LLNB; white circles and bars) and highland newborn sheep (HLNB; black circles and bars). B, basal; H, hypoxemia; R, rest. Values expressed as means ± SE. Significant differences $P < 0.05$: a, vs. basal; b, vs. all in same altitude group; c, vs. LLNB.

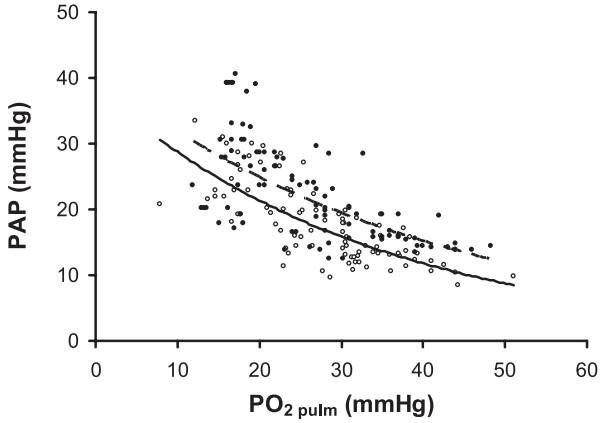

Fig. 2. Correlation between PAP and pulmonary P_{O_2} in LLNB (open circles, solid line) and HLNB (solid circles, broken line). LLNB, $r = 0.77$, $P < 0.001$; HLNB, $r = 0.70$, $P < 0.001$.

where $pD_2 = -\log [EC_{50}]$, EC_{50} being the concentration at which 50% of the maximal response was obtained. Data are shown as means ± SE. Differences between mean values were assessed by Student's *t*-test and were considered significant if $P < 0.05$.

RESULTS

Blood Gases and Acid-Base Status

During the basal period Pa_{O_2}, Pa_{CO_2}, Sa_{O_2}, and O_2 content were lower in HLNB than in the LLNB, but pH was higher. Moreover, $\dot{V}_{O_2}$ and extraction were also augmented in HLNB (Table 1). During acute hypoxia, significant falls in Pa_{O_2}, Sa_{O_2}, and O_2 content occurred in all animals. In addition, the Pa_{CO_2} was maintained at similar values in both groups (isocapnic hypoxia). $\dot{V}_{O_2}$ was also maintained, associated in both groups with increased oxygen extraction (Table 1). During recovery, the altered variables returned to basal values in both groups, except for the Pa_{CO_2} in LLNB and pH in HLNB, which remained decreased in this period (Table 1).

For the graded hypoxia study, the Pa_{O_2}, Sa_{O_2} and O_2 content changed during each step, with no changes in pH or in Pa_{CO_2} (data not shown).

Pulmonary Cardiovascular Variables

During the basal period, PAP, cardiac output, PVR, and heart rate were significantly higher in the HLNB than in the LLNB (Fig. 1). During acute hypoxemia, there was a brisk and maintained increase of PAP, reaching higher values in HLNB compared with the LLNB (Fig. 1). Cardiac output did not change significantly in hypoxemia, although PVR increased in both groups of animals, but PVR reached higher values in HLNB than LLNB (Fig. 1). Heart rate increased only significantly in LLNB during hypoxia (Fig. 1).

The PAP and P_{O_2} values during the graded hypoxia studies were plotted on a correlation graph (Fig. 2), where at any specific P_{O_2} value, the average mean PAP was higher in the HLNB.

Systemic Cardiovascular Variables

The SAP was similar in LLNB and HLNB. As noted above, cardiac output was higher in the HLNB, and the SVR was also lower in HLNB (Fig. 3).

At rest, the FBF and femoral vascular resistance (FVR) were similar in both groups of lambs. During superimposed hypoxemia, FBF declined and FVR increased in both groups, but the changes in the HLNB group occurred much faster and to a greater magnitude (Fig. 3).

During recovery, all the cardiovascular variables returned to normoxemic levels in both groups (Figs. 1 and 3). The SAP and SVR values did not change during the graded hypoxia (data not shown).

Contractile Responses of Small Pulmonary Arteries

Response to potassium chloride. The contractile responses of small pulmonary arteries to potassium chloride from HLNB showed a higher maximal response than LLNB ($P <$

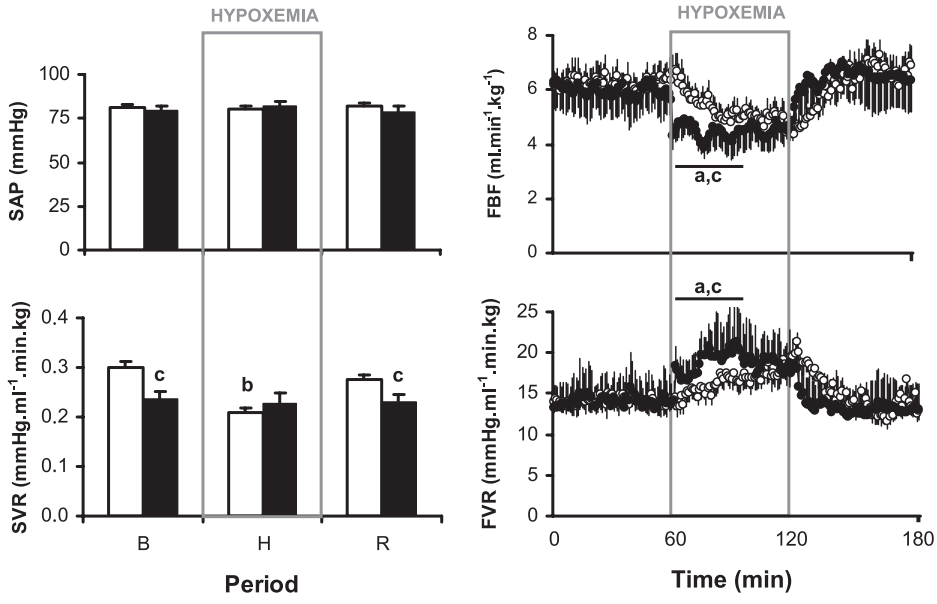

Fig. 3. Systemic arterial pressure (SAP), systemic vascular resistance (SVR), femoral blood flow (FBF), and femoral vascular resistance (FVR) in LLNB (white circles and bars) and HLNB (black circles and bars). Values are expressed as means ± SE. Significant differences $P < 0.05$: a, vs. basal; b, vs. all in same altitude group; c, vs. LLNB.

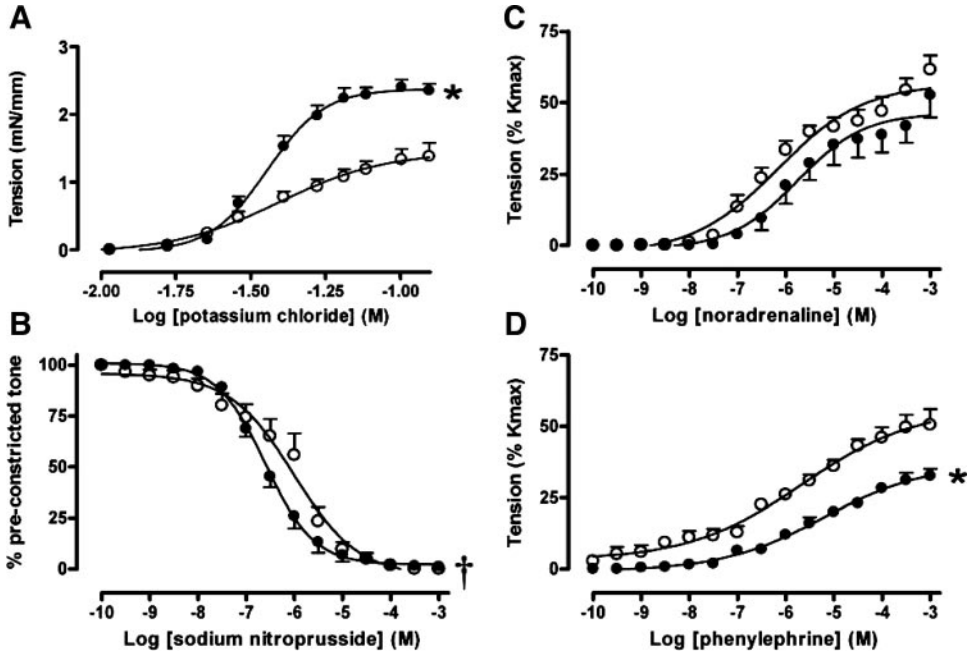

Fig. 4. Responses to potassium chloride (*A*), sodium nitroprusside (*B*), noradrenaline (*C*), and phenylephrine (*D*) in pulmonary small arteries from LLNB (white circles) and HLNB (black circles). Values are shown as means ± SE. Significant differences $*P < 0.05$ for maximal response; $\dagger P < 0.05$ for sensitivity tested by two-sample *t*-test HLNB vs. LLNB.

0.05) with similar sensitivity between both groups (Fig. 4*A*, Table 2).

Response to sodium nitroprusside, a nitric oxide donor. Exposure of maximally K^+-precontracted small pulmonary arteries to the nitric oxide donor sodium nitroprusside induced relaxation, with no changes in the maximal relaxation but a higher sensitivity in the HLNB compared with the LLNB group (Fig. 4*B*, Table 2).

Adrenergic response. The contractile responses of small pulmonary arteries to noradrenaline showed no significant differences between the groups in maximal response or sensitivity (Fig. 4*C*, Table 2). However, the response to phenylephrine showed a lower maximal response in HLNB with similar sensitivity compared with LLNB (Fig. 4*D*, Table 2).

Contractile Responses of Small Femoral Arteries

Response to potassium chloride. Isolated small femoral arteries showed a lower maximal response in HLNB than in LLNB, but a similar sensitivity (Fig. 5*A*, Table 2).

Response to sodium nitroprusside. Exposure of maximally K^+-precontracted arteries to the nitric oxide donor sodium nitroprusside induced a higher sensitivity in HLNB compared with LLNB (Fig. 5*B*, Table 2).

Adrenergic response. The response to noradrenaline showed a significant increase in maximal response and sensitivity in HLNB compared with LLNB. In addition, the response to phenylephrine in small femoral vessels also showed a higher maximal response and sensitivity in HLNB compared with LLNB (Fig. 5, *C–D*, Table 2).

DISCUSSION

Our results support the hypothesis that newborn lambs born and raised at high altitude enhances the vascular contractile responses in the pulmonary and femoral beds, during basal conditions and during a superimposed hypoxic challenge compared with newborn lambs born and raised at low altitude. The HLNB have a greater arterial pressure and contractile activity in the pulmonary bed with a lower contractile capacity but increased adrenergic responses in a systemic arterial vasculature. This is consistent with hypoxic-induced changes in the pulmonary and systemic vascular beds, which appears to operate during fetal and neonatal life in the HLNB (13, 32).

Highland lambs are lighter compared with lowlanders at the same age, showing intrauterine growth restriction (11). This intrauterine growth reduction may also be an adaptation to the effects of chronic hypoxia and it appears to be independent of

Table 2. *Maximal response (E_{max}) and sensitivity (pD_2) for potassium chloride (KCl), sodium nitroprusside (SNP), norepinephrine (NE) and phenylephrine (Phe) in small pulmonary and femoral arteries in LLNB and HLNB*

	Pulmonary				Femoral			
	LLNB (n = 6)		HLNB (n = 6)		LLNB (n = 7)		HLNB (n = 7)	
	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2
KCl	1.33±0.20	1.40±0.03	2.62±0.20*	1.42±0.02	13.5±0.8	1.43±0.02	9.0±0.7*	1.49±0.02
SNP	100	6.08±0.14	100	6.52±0.11*	100	6.07±0.20	100	6.54±0.10*
NE	57±7	6.2±0.3	46±6	5.7±0.2	108±9	4.58±0.23	139±7*	5.61±0.19*
Phe	53±4	5.83±0.14	36±4*	5.2±0.4	52±3	5.49±0.11	118±5*	5.9±0.1*

Values are means ± SE. E_{max} values for KCl are expressed as tension in mN/mm; E_{max} for NE and Phe expressed as % maximal response to KCl; and E_{max} for SNP is expressed as % tone reduction in preconstricted arteries. Significant differences $P < 0.05$: *vs. LLNB.

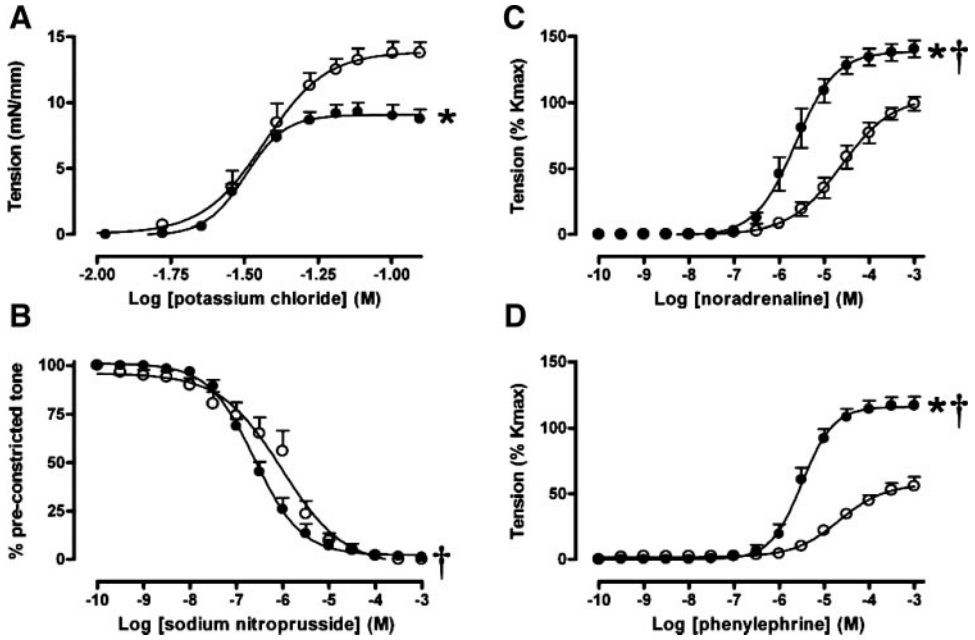

Fig. 5. Responses to potassium chloride (*A*), sodium nitroprusside (*B*), noradrenaline (*C*), and phenylephrine (*D*) in femoral small arteries from LLNB (white circles) and HLNB (black circles). Values are shown as means ± SE. Significant differences $*P < 0.05$ for maximal response; $\dagger P < 0.05$ for sensitivity tested by two-sample *t*-test HLNB vs. LLNB.

nutrition since the highland and lowland pregnant ewes were maintained with the same food intake per day.

At high altitude, the HLNB showed a higher $\dot{V}O_2$, while having lower oxygen content under basal conditions. We propose that this higher $\dot{V}O_2$ in HLNB may result from the higher respiratory effort of hyperventilation at high altitude. In fact, when the acute hypoxic episode was induced, the $\dot{V}O_2$ reached similar levels in both groups of lambs. This shows that these lambs have a physiological adaptation, which allows the maintenance of $\dot{V}O_2$ even under acute hypoxia (24).

Pulmonary Vascular Bed

A variety of investigations have found a reduced (17, 18, 25, 39), no change, or enhanced (4, 6, 31, 39) in pulmonary hypoxic response after long-term exposure to low P_{O_2}. Here, we found that the pulmonary vasoconstriction under basal and hypoxic conditions is enhanced in newborns that have developed in utero and been born in the chronic hypoxia of the Andean altiplano.

The basal higher PAP in HLNB than in LLNB may be explained by our findings that pulmonary small arteries show a greater maximal response to KCl, with the same sensitivity, suggesting a greater vascular smooth-muscle mass in HLNB (23, 33). Chronic hypoxia induces overexpression of vasoconstrictors in pulmonary tissue, as well smooth muscle cell mechanisms that could be acting in the regulation of the pulmonary vascular tone producing the basal increase in PAP in our HLNB (5, 8, 21, 33). Besides, we observed a higher relaxant effect of exogenous nitric oxide in isolated pulmonary arteries, indicating a higher expression and/or function of the nitric oxide-cGMP-PKG transduction pathway located in the arterial smooth muscle.

Acute hypoxia increases PAP, PVR, and heart rate, in LLNB and HLNB, but with a lower increment in HLNB compared with their basal period. This is explained by the different basal P_{O_2} of each group. Furthermore, as we induce an isocapnic hypoxemia, HLNB showed a higher PAP in acute hypoxemia at a lower P_{CO_2} and higher pH compared with LLNB. Therefore, we would expect a higher pulmonary vascular tone and arterial pressure at same P_{CO_2} and pH at the highlands. To evaluate the pulmonary vascular response at a specific P_{O_2}, we designed a graded hypoxia/oxygenation experiment. This study revealed that in the pulmonary bed there is indeed a greater degree of contraction in HLNB. This higher PAP in HLNB at the same P_{O_2} could be the result of vascular remodeling with more muscularization of the pulmonary vessels (32).

The lower maximal response to phenylephrine observed in the isolated pulmonary arteries in HLNB agrees with the minor role of catecholamines in the pulmonary circulation (29), particularly in hypoxic newborns (30).

Systemic Cardiovascular Responses

In the femoral vascular bed, HLNB presents a higher FVR than LLNB during hipoxia. Moreover, the initial response to hypoxia was a brisk vasoconstriction in HLNB, not seen in LLNB. This marked vasoconstriction may represent persistence of a chemoreflex in origin followed by catecholamine action on the femoral vessels, as seen in fetal lambs (9, 16). The higher sensitivity and maximal response observed for noradrenaline and phenylephrine in HLNB suggests that adrenergic responses are playing a major role in the peripheral circulation in high-altitude-exposed animals, as is also observed in animals chronically exposed to hypoxia, such as the llama (10, 16), chronically hypoxic chicken embryos (27), and rats gestated under hypoxia (38). A higher response to sodium nitroprusside may indicate a major role for nitric oxide in the systemic circulation of high-altitude-exposed animals, to counteract the intense femoral vasoconstriction during hypoxia in HLNB.

In conclusion, the chronic hypoxia during pregnancy and early postnatal life at high altitude enhances the vascular contractile responses in the pulmonary and femoral beds, during basal conditions and during a superimposed hypoxic challenge. These changes determined in intrauterine life and

early after birth at altitude could be adaptive to the chronic hypoxic milieu of the Andean altiplano.

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