Role of the α-adrenergic system in femoral vascular reactivity in neonatal llamas and sheep: a comparative study between highland and lowland species

Fernando A. Moraga,1,2* Roberto V. Reyes,1* Emilio A. Herrera,1,3,6 Raquel A. Riquelme,4 Germán Ebensperger,1 Víctor M. Pulgar,1 Julian T. Parer,5 Dino A. Giussani,6 and Aníbal J. Llanos1,3,7

1Laboratorio de Fisiología y Fisiopatología del Desarrollo, Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile; 2Laboratorio de Fisiología, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile; 3Centro Internacional de Estudios Andinos (INCAS), Universidad de Chile, Santiago, Chile; 4Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile; 5Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, California; 6Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom; and 7Departamento de Biología, Facultad de Ciencias, Universidad de Tarapacá, Arica, Chile

Submitted 19 February 2010; accepted in final form 26 July 2011

Moraga FA, Reyes RV, Herrera EA, Riquelme RA, Ebensperger G, Pulgar VM, Parer JT, Giussani DA, Llanos AJ. Role of the α-adrenergic system in femoral vascular reactivity in neonatal llamas and sheep: a comparative study between highland and lowland species. Am J Physiol Regul Integr Comp Physiol 301: R1153–R1160, 2011. First published July 27, 2011; doi:10.1152/ajpregu.00124.2010.—Using an integrative approach at the whole animal, isolated vessels, and molecular levels, we tested the hypothesis that the llama, a species that undergoes pregnancy under the influence of the chronic hypoxia of high altitude, delivers offspring with an increased α-adrenergic peripheral vascular reactivity compared with neonates from lowland species. We studied the femoral vascular response to acute hypoxia in vivo, the reactivity of femoral vessels ex vivo, and the expression of femoral α1-adrenergic receptor subtypes using RT-PCR in vitro. The increase in femoral resistance during hypoxia was 3.6 times greater in newborn llamas than newborn sheep (P < 0.05). The sensitivity of the contractile response to noradrenaline (pD2 = 5.18 ± 0.06 vs. 4.84 ± 0.05, P < 0.05) and the maximal response (Rmax = 101.3 ± 1.4 vs. 52.4 ± 1.4% K+ max, P < 0.05) to phenylephrine were higher in femoral vessels from newborn llamas than newborn sheep. Competitive inhibition with prazosin of noradrenaline-induced contraction followed by Schild analysis showed higher affinity in the llama than the sheep (pA2 = 10.08 ± 0.093 vs. 8.98 ± 0.263, respectively, P < 0.05), consistent with greater α1A-adrenergic receptor transcript expression observed in small femoral arteries from neonatal llama. The llama newborn demonstrates significantly greater α-adrenergic peripheral vascular reactivity compared with neonates from lowland species that could be partially explained by preferential expression of α1B-adrenergic receptor subtype.

Newborn; hypoxia; small resistance artery; femoral resistance; α-adrenergic receptors

The llama, a South American Camelidae (Lama glama), has evolved in the low O2 milieu of the Andean altiplano at altitudes >4,000 m above sea level. This environment has likely exerted a selective pressure for the development of physiologic and cellular defense strategies to help this species thrive even at an atmospheric PO2 close to half that at sea level. During pregnancy, the llama fetus is able to grow and develop normally despite its mother being exposed to chronic hypobaric hypoxia. This is due to maternal, placental, and fetal adaptations, which have become genetically determined in the llama over generations (26, 29). For instance, maternal hemoglobin in the llama has a greater affinity for oxygen than maternal hemoglobin in sheep (30). This leftward shift in the maternal oxygen dissociation curve in the llama persists even in animals born and raised at sea level (30). Similarly, the fetal peripheral vasoconstriction during acute hypoxia, a physiologic defense-response that contributes to the redistribution of the fetal combined cardiac output away from peripheral and toward essential circulations, is much greater in the llama than in the sheep fetus, particularly in the fetal femoral circulation, a characteristic that persists in llama fetuses undergoing pregnancy at sea level (11, 25, 26).

The fetal femoral constrictor response to acute hypoxia is triggered by a carotid chemoreflex and is mediated via activation of the sympathetic chain, in lowland species like the sheep and highland species like the llama (3, 11, 14, 15, 16). Once triggered, the fetal femoral vascular resistance is maintained during acute hypoxia by the release of vasoconstrictor agents into the fetal circulation, including catecholamines (20, 37, 39). Some studies suggest that compared with the sheep fetus, the contribution of the α-adrenergic system is upregulated in the llama fetus or in fetal sheep during development under chronic hypoxic conditions. First, treatment with phentolamine of fetal llamas or chronically hypoxic fetal sheep blocked the peripheral vascular resistance increase during acute hypoxia leading to fetal death (4, 16). In contrast, treatment of lowland fetal sheep with the α-adrenergic antagonist phentolamine only diminished the increase in femoral vascular resistance during acute hypoxia (14). Second, the concentration of plasma catecholamines during basal and hypoxic conditions is much greater in the llama than in the sheep fetus (9, 25, 37). Third, the contractile response to α-adrenergic agonists of femoral vessels isolated from chronically hypoxic fetal sheep (22) is much greater than that of normoxic counterparts.

The cardiovascular strategies to withstand hypoxia differ between fetal and postnatal animals. While the fetal cardiovascular strategy is designed to make the best use of the finite
isolated femoral vessels ex vivo, and inserted into the femoral artery, and vein and an ultrasonic blood flow transducer (Transonic, Ithaca, NY) was implanted around the contralateral femoral artery. The catheters and the flow probe lead were subcutaneously exteriorized through a keyhole incision at the newborn flank and kept in a pouch sewn onto the skin. The catheters were filled with a heparinized solution of 0.9% NaCl and plugged with a copper pin. At the end of the surgery and daily after the surgery, 10 mg/kg im Ampicilina (Laboratorio Best-Pharma, Santiago, Chile) and 4 mg/kg im Gentamicina Sulfato (Laboratorio Biosano, Santiago, Chile), were administered every 12 h for 4–5 days. Following surgery, the animals were returned to the yard, and the experiments were not commenced until at least 3 days postsurgery. Patency of the catheters was maintained by daily flushing with heparinized 0.9% NaCl (200 IU/ml).

In vivo femoral vascular response to an episode of acute hypoxia. Experiments were based on a 3-h protocol divided into three periods of 60 min each: 1 h of normoxia (N), 1 h of hypoxia (H), and 1 h of recovery (R). Following 1 h of basal recording in which the animal breathed air, a transparent respiratory hood was placed over the newborn’s head and loosely tied (18, 19). A controlled mixture of air, N2 and CO2 (~9% O2 and 2–3% CO2 in N2) was passed at ~30 l/min, designed to reduce the animal’s PaO2 from ~90 to 30 mmHg and oxygen supply, the neonatal cardiovascular strategy can afford to maintain blood flow to most organs with adequate total oxygen consumption (18). Consequently, in contrast to the systemic vasoconstrictor response to acute hypoxia in the fetus (35), systemic vascular resistance is either maintained or even decreased in response to acute hypoxia in postnatal animals (18). However, the postnatal peripheral hemodynamic response to acute hypoxia in highland species is completely unknown. Neonautes of highland species compared with lowland species likely use a different cardiovascular strategy to withstand episodes of acute reductions in oxygenation. We hypothesize that in the llama the enhanced α-adrenergic peripheral vascular reactivity in fetal life will persist through to neonatal life. We further hypothesize that this enhancement of the α-adrenergic tone may have become genetically determined such that it will persist even in episodes of acute hypoxia in animals born and raised at sea level for several generations. The hypothesis was tested using an integrative approach at the whole organism, isolated arteries, and molecular levels, by comparing in newborn llamas and sheep: 1) the femoral vascular response to an episode of acute hypoxia in vivo, 2) the vascular reactivity in isolated femoral vessels ex vivo, and 3) the expression of α1-adrenergic receptor subtypes in femoral vessels using RT-PCR in vitro.

### MATERIALS AND METHODS

All animal care, maintenance, procedures, and experimentation were performed in accordance with the United Kingdom’s Animals (Scientific Procedures) Act 1986, and the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings (American Physiological Society, 2002) and were reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Chile.

**Animals.** The study used 13 neonatal llamas (10.9 ± 0.5 days old and weighing 12.6 ± 0.5 kg at the time of the study) and 13 neonatal Merino lambs (10.7 ± 0.4 days old and weighing 6.9 ± 0.4 kg at the time of the study) gestated and born at the University of Chile farm at 580 m above sea level. Upon arrival to the city of Santiago (585 m above sea level), the newborn llamas and sheep and their mothers were housed in an open yard with access to food and water ad libitum.

**Chronic instrumentation of newborns.** Six newborn llamas and five newborn sheep underwent surgery between 3 and 4 days of age. The animals were premedicated with atropine (0.04 mg/kg im, Atropina Sulfato; Laboratorio Chile, Santiago, Chile). Under general anesthesia (10 mg/kg im Ketostop; Drag Pharma-Invetec, Santiago, Chile and 0.5 mg/kg im Dizepam; Lab Biosano, Santiago, Chile) with additional local infiltration of 2% lidocaine hydrochloride (Dimecaína; Laboratorio Beta, Santiago, Chile) polyvinyl catheters (0.8 mm ID) were inserted into the femoral artery, and vein and an ultrasonic blood flow transducer (Transonic, Ithaca, NY) was implanted around the contralateral femoral artery. The catheters and the flow probe lead were subcutaneously exteriorized through a keyhole incision at the newborn flank and kept in a pouch sewn onto the skin. The catheters were filled with a heparinized solution of 0.9% NaCl and plugged with a copper pin. At the end of the surgery and daily after the surgery, 10 mg/kg im Ampicilina (Laboratorio Best-Pharma, Santiago, Chile) and 4 mg/kg im Gentamicina Sulfato (Laboratorio Biosano, Santiago, Chile), were administered every 12 h for 4–5 days. Following surgery, the animals were returned to the yard, and the experiments were not commenced until at least 3 days postsurgery. Patency of the catheters was maintained by daily flushing with heparinized 0.9% NaCl (200 IU/ml).

### Table 1. Sequence and primers and PCR conditions

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>Sequence (5′ → 3′)</th>
<th>Annealing Temperature</th>
<th>Amplification Cycles</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1α-Adrenergic receptor</td>
<td>(S) AAGTGAACCCGCTGGCATCCA</td>
<td>53°C</td>
<td>35</td>
<td>277 bp</td>
</tr>
<tr>
<td></td>
<td>(A) GGTTGTATCGGAGCTTTAGGTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1β-Adrenergic receptor</td>
<td>(S) CATTGACGCTACATTGGGGTG</td>
<td>55°C</td>
<td>35</td>
<td>502 bp</td>
</tr>
<tr>
<td></td>
<td>(A) CCACACTTAGTGAACTGGCGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18S-rRNA</td>
<td>(S) GTAACCCGTTAAGCCCGATT</td>
<td>55°C</td>
<td>20</td>
<td>152 bp</td>
</tr>
<tr>
<td></td>
<td>(A) CCGATCGAATCGGTAGTACGG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For all of the reactions, preliminary experiments were performed to determine the number of PCR cycles at which saturation occurred. Experiments were carried out with the number of cycles that precedes saturation as indicated. S, sense; A, antisense.

### Table 2. Cardiovascular variables, pH, and blood gases in newborn (NB) llamas and sheep during normoxia, acute hypoxia, and recovery

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>94 ± 4*</td>
<td>102 ± 5*</td>
<td>98 ± 6*</td>
</tr>
<tr>
<td>sheep</td>
<td>83 ± 2</td>
<td>81 ± 1</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>110 ± 7*</td>
<td>146 ± 7†*</td>
<td>115 ± 7*</td>
</tr>
<tr>
<td>sheep</td>
<td>205 ± 8</td>
<td>263 ± 17†*</td>
<td>206 ± 8</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·kg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>4.6 ± 0.6*</td>
<td>3.7 ± 0.8†*</td>
<td>3.7 ± 0.9†*</td>
</tr>
<tr>
<td>sheep</td>
<td>6.1 ± 0.4</td>
<td>5.2 ± 0.4†</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>7.43 ± 0.01</td>
<td>7.45 ± 0.02</td>
<td>7.48 ± 0.02*</td>
</tr>
<tr>
<td>sheep</td>
<td>7.41 ± 0.01</td>
<td>7.39 ± 0.02</td>
<td>7.42 ± 0.01</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>90 ± 1*</td>
<td>33 ± 2†</td>
<td>94 ± 7*</td>
</tr>
<tr>
<td>sheep</td>
<td>80 ± 2</td>
<td>31 ± 1†</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>39 ± 2</td>
<td>38 ± 2</td>
<td>36 ± 2*</td>
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<tr>
<td>sheep</td>
<td>37 ± 1</td>
<td>36 ± 1</td>
<td>32 ± 1†</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NB llama</td>
<td>97 ± 2</td>
<td>67 ± 5†*</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>sheep</td>
<td>95 ± 1</td>
<td>53 ± 3†</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>9.5 ± 0.3*</td>
<td>11.2 ± 0.8†</td>
<td>9.6 ± 0.4</td>
</tr>
<tr>
<td>sheep</td>
<td>10.9 ± 0.5</td>
<td>11.3 ± 0.4</td>
<td>10.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. NB llama (n = 6), NB sheep (n = 5). MAP, mean arterial pressure; FBF, femoral blood flow; SaO₂, saturation of hemoglobin; Hb, hemoglobin. Significant differences (P < 0.05): *vs. NB sheep, †vs. normoxia.
hemoglobin saturation (SaO₂) to ~60%, without affecting PaCO₂. Following 1 h of hypoxia, the newborn animal was allowed to recover by breathing air for an additional 60 min. Femoral arterial and central venous pressures and femoral blood flow were recorded continuously throughout the experiments using a data acquisition system connected to a computer (Powerlab/8SP; AD Instruments, New South Wales, Australia). Heart rate and mean arterial pressure (MAP) and central venous pressure (the transducer membrane placed at the level of neonatal heart) were calculated and measured from these records. Samples of heparinized arterial blood samples (0.3 ml) were taken from the animals at 15 and 45 min of normoxia, at 15 min intervals during the hypoxic hour, and after 15 and 45 min of the recovery. Arterial pH, PO₂, PCO₂ (model ABL 555 blood gas monitor; Radiometer, Copenhagen, Denmark; measurements corrected at 38°C), SaO₂, and hemoglobin concentration (OSM3 Hemoximeter; Radiometer, Copenhagen, Denmark) were measured. Femoral vascular resistance was calculated using Ohm’s approximation by dividing the perfusion pressure (MAP – venous pressure) by femoral blood flow.

*Ex vivo* small vessel wire myography. Six newborn llamas and six newborn sheep (11 to 14 days old) were killed using intravenous sodium thiopenthone, and small-resistance arteries (third branch, 370-μm internal diameter) were dissected from the femoral vascular bed and placed in ice-cold saline. Arterial segments of ~2-mm lengths were mounted in a four-channel small vessel myograph (610M Multimyograph; Danish Myotechnology, Aarhus, Denmark) for measurement of isometric force. The vessel segments were incubated in Krebs Ringer bicarbonate (KRB) at 37°C and gassed with a mixture of 5% in CO₂ balanced with O₂. Following 1 h of incubation, optimal diameter was determined for each artery, at which the artery displayed maximal contractile responses to Krebs buffer with equimolar replacement of Na⁺ with 125 mM K⁺ (K-KRB) (19).

Following an equilibration period of at least 30 min, concentration response curves to potassium, α-adrenergic agonist noradrenaline, α₁-adrenergic agonist phenylephrine, and α₂-adrenergic agonist clonidine were obtained at concentrations ranging from 10⁻¹⁰ to 10⁻³ M.

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Fig. 1. Femoral vascular resistance (FVR) in newborn (NB) llama (n = 6) and NB sheep (n = 5) during normoxia, acute hypoxia, and recovery. Values are means ± SE. Significant differences (P < 0.05) †vs. normoxia, *vs. NB sheep.

Fig. 2. Adrenergic-induced contraction in femoral resistance arteries. **A:** concentration response curve to noradrenaline (NA) in NB llama (n = 6) and NB sheep (n = 6), maximum NA-induced contraction with respect to K⁺ max and sensitivity to this agonist. **B:** concentration response curve to phenylephrine (Phe) in NB llama (n = 6) and NB sheep (n = 6), maximum response to Phe is expressed as % K⁺ max and sensitivity. Values are means ± SE. *Significant differences (P < 0.05) vs. NB sheep.
M. In addition, concentration response curves to phenylephrine in vessels preincubated for 30 min with 10⁻⁵ M of the nitric oxide (NO) synthase inhibitor N⁵-nitro-L-arginine methyl ester (L-NNAME) were performed. Concentration response curves to noradrenaline were also repeated in vessels preincubated with the α₁-adrenergic receptor blocker prazosin (10⁻⁹ M to 10⁻⁷ M). Between each experiment, the arteries were allowed to recover for at least 30 min.

Maximal responses (Rₘₐₓ) and sensitivity (EC₅₀ or pD₂) to the different vasoactive agents tested were obtained by fitting the concentration response curves to a Boltzmann function (Prism 4.0; Graphpad). Rₘₐₓ was expressed as tension (N/m) for K⁺ and for the adrenergic agonists as percentage of Rₘₐₓ at 10⁻⁷ M. Sensitivity was expressed as EC₅₀ (the concentration at which 50% of Rₘₐₓ was obtained) for K⁺ or for the adrenergic agonists as pD₂ (−logEC₅₀). The data using inhibition with prazosin were analyzed using the global Schild method to generate an estimate of pA₂. Schild analysis was performed by plotting Log (R/I) values for individual vessels against the antagonist concentration (log [A]), where R is defined as ratio of the EC₅₀ values in the presence and absence of the antagonist (2). Analysis was performed using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Slope analysis was performed in each curve, supporting the presence of competitive antagonism. Antagonist pA₂ values were then taken to be the x-intercept to the Schild slope (32).

KRB contained (in mM): 118.5 NaCl, 25 NaHCO₃, 4.7 KCL, 1.2 KH₂PO₄, 1.2 MgSO₄, 2.5 CaCl₂, 5.5 glucose with a pH of 7.4. In K-KRB (125 mM K⁺) all NaCl was replaced by an equimolar amount of KCl. Noradrenaline, phenylephrine, clonidine, L-NNAME, and prazosin were obtained from Sigma (St. Louis, MO).

In vitro RT-PCR expression of α₁-adrenergceptors. Total RNA from femoral resistance arteries from five newborn llamas and five newborn sheep was prepared using the SV Total RNA Isolation kit (Promega, Madison, WI). cDNA was synthesized by reverse transcription using random hexamers and the Superscript First Strand Synthesis System for RT-PCR kit (Invitrogen Life Technologies). Procedures were carried out according to the manufacturer’s instructions. PCR was performed using the primers corresponding to α₁A- and α₁B-adrenergic receptors and 18S-rRNA (27) and the amplification conditions indicated in Table 1.

The α₁A- and α₁B-adrenergic receptors’ primers were designed to amplify nucleotide sequences (277 bp and 502 bp for α₁A- and α₁B-adrenergic receptor, respectively). In brief, these common sequences were identified in PCR products initially obtained by amplification of sheep and llama cDNAs with primers for rat α₁A- and α₁B-adrenergic receptors (accession nos. NM_017191 and NM_016991, respectively). The amplicons obtained from sheep cDNA by using the rat α₁A-adrenergic primer showed 98% identity with the recently published sheep sequence (24) (accession no. EU 723257), while the one obtained in the llama had 93.5% identity with sheep (24), and 92% with bovine and human α₁A-adrenergic, respectively (accession nos. NM_174498 and NM_000680). The amplicons obtained in sheep and llama using the rat α₁B-adrenergic receptor primers showed 97% identity with the corresponding bovine and human α₁B-adrenergic sequences (accession nos. NM_001191139 and BC136569, respectively). Once the common sequences in sheep and llama α₁A- and α₁B-adrenergic receptor amplicons were verified, specific sheep and llama primers for each adrenergic receptor subtype were designed, and semiquantitative RT-PCR was carried out under the conditions indicated in Table 1.

Assay conditions were established for each pair of primers and for both sheep and llama cDNA. For every sample, two independent preparations were performed to determine the range of cycles between the thresholds at which there is no detectable amplification and until saturation, as a result of which there is no further increase in the amplicon intensity with increasing amplification cycles. PCR amplification was carried out from cDNA synthesized from 0.1 µg of total RNA, with 1 unit of Taq polymerase (Promega), 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.2 mM dNTP mix, and 0.3 µM of each one of the primers under the conditions indicated in Table 1. The PCR products and molecular weight standards were separated by electrophoresis on ethidium bromide agarose gels and visualized under UV light. The optical density of the bands of the PCR products and the 300 bp standard were quantified by densitometry using Scion Image Software (Beta 4.02 Win; Scion Image). Sample intensity was calculated using the 300 bp band as standard. Samples were measured in two or three cDNA dilutions and at least in two separate RT-PCR assays.

Statistical analysis. Data are expressed as means ± SE. Two-way ANOVA for repeated measures or the Student’s t-test for unpaired data were used to compare data, as appropriate. Linear regression analysis of the Schild plot was used to estimate pA₂ (intercept) values, the slope, and the correlation coefficient (r and r²) of the regression line. Comparison of slopes and intercepts of regression lines were performed by unpaired t-test (41). For all comparisons, differences were considered significant when P < 0.05.
RESULTS

In vivo responses to an episode of acute hypoxia. Under normoxic conditions, PaO₂ was higher and hemoglobin (Hb) concentration lower in newborn llamas compared with newborn sheep. Percent SaO₂, pH, and PaCO₂ were similar in both species (Table 2). During the episode of acute hypoxia, PaO₂ was significantly reduced to similar levels in both groups. However, compared with the newborn sheep, this fall in PaO₂ in the newborn llama was achieved with a smaller decrement in SaO₂ during acute hypoxia. While Hb concentration was significantly increased in the newborn llama during acute hypoxia, it remained statistically similar to baseline in newborn sheep (Table 2). During recovery, pH, PaO₂, SaO₂, and Hb concentration returned to basal levels in both groups. However, PaCO₂ was lower in newborn sheep than newborn llamas by the end of the protocol, suggesting that sheep are hyperventilating during the recovery from hypoxia (Table 2).

During normoxia, systemic MAP was higher and femoral blood flow lower in newborn llamas than in newborn sheep (Table 2). Consequently, calculated femoral vascular resistance was greater in newborn llamas than in newborn sheep (Fig. 1). During acute hypoxia, heart rate increased and MAP remained unchanged from baseline with a significant fall in femoral blood flow in both species (Table 2). During recovery, the femoral vascular resistance remained significantly elevated in neonatal llamas relative to newborn sheep, but not compared with the llama normoxic period (Fig. 1).

Ex vivo wire myography. The optimal diameter of femoral arteries was 375 ± 50 μm for newborn llamas and 370 ± 43 μm for newborn sheep. There were no significant differences in the Rmax or in the sensitivity to K⁺ between newborn llamas and newborn sheep (Rmax: 12.1 ± 0.8 N/m vs. 12.6 ± 0.9 N/m; pECs0: 1.62 ± 0.46 vs. 1.56 ± 0.4, respectively).

Although the Rmax for noradrenaline between newborn llamas and newborn sheep was similar (99.3 ± 0.8% vs. 102.0 ± 1.5% K⁺max), the former showing greater sensitivity to noradrenaline (pD₂: 5.18 ± 0.46 vs. 4.84 ± 0.05 in newborn sheep; P < 0.05, Fig. 2A). In addition, both the Rmax and the sensitivity to phenylephrine were greater in newborn llamas (Rmax: 101.3 ± 1.4% Kmax and pD₂: 5.47 ± 0.03) compared with newborn sheep (Rmax: 52.4 ± 1.4% K⁺max and pD₂: 4.57 ± 0.05; Fig. 2B).

While stimulation with phenylephrine during NO synthesis blockade did not affect the vascular response in newborn llamas, it recovered the Rmax to values measured during stimulation with K⁺ or noradrenaline in newborn sheep (Fig. 3, A and B). Treatment with clonidine did not elicit any response in femoral resistance arteries isolated from newborn llamas and newborn sheep (data not shown).

Treatment with prazosin in newborn llamas decreased the Rmax to 80% at 10 nM and led to a concentration-dependent rightward shift in the sensitivity to noradrenaline (Fig. 4A). In contrast, this treatment in newborn sheep did not affect the
pared with lambs and that this may be an adaptive vascular
support the hypothesis tested that the llama newborn demon-
Combined, the in vivo, ex vivo, and in vitro data strongly
receptors subtypes expressed in the femoral vascular bed.

**DISCUSSION**

The data show that the postnatal hemodynamic response to
acute hypoxia in the llama resembles a fetal-like cardiovascular
defense. Therefore, in response to acute hypoxia, there is an
tense femoral vasoconstriction in the newborn llama, while
femoral vascular resistance remains unchanged in newborn
sheep. The physiologic mechanisms underlying this response
in the newborn llama include enhanced peripheral vasocon-
strictor sensitivity to α1-adrenergic stimulation, decreased vas-
cular reactivity to α-adrenergic-induced NO-dependent vaso-
dilatation, and differences in the population of α1-adrenergic
receptors subtypes expressed in the femoral vascular bed.
Combined, the in vivo, ex vivo, and in vitro strongly support the hypothesis tested that the llama newborn demon-
strates greater α-adrenergic peripheral vasocostriction com-
pared with lambs and that this may be an adaptive vascular
response that persists even in animals gestated and born at sea
level.

Overall, the defense strategies to episodes of reduced oxy-
genation may differ markedly before and after birth. After
birth, a reduction in oxygen supply triggers balanced cardio-
vascular and ventilatory responses. In response to acute hyp-
oxia, there is an increase in minute ventilation and an increase
in cardiac output in postnatal animals and humans (18). The
vast supply of atmospheric oxygen allows the cardiovascular
system in postnatal individuals to increase perfusion even to
peripheral circulations, maintaining their oxygenation during
periods of systemic hypoxia (18, 38). Within the womb, the
supply of oxygenated blood to the fetus is dependent on the
placenta rather than on pulmonary ventilation. Consequently,
during acute hypoxia, fetal breathing movements cease (5, 6)
and the fetal defense-strategy is primarily dependent on the
cardiovascular system. The fetal cardiovascular strategy during
episodes of reduced oxygenation concentrates on decreasing
oxygen consumption by the tissues and prioritizing the fetal
cardiac output away from peripheral and toward essential
circulations (25). The increase in femoral vascular resistance is
an important index of this fetal cardiovascular strategy as it not
only decreases oxygen consumption by the hindlimbs but it
also contributes to the redistribution of the cardiac output away
from peripheral circulations (7, 25).

The neonatal llamas living in the Andean altiplano, at an
atmospheric PO2 close to half that at sea level, may rely more
on cardiovascular than ventilatory defenses to withstand an
acute episode of reduced oxygenation. The present study is the
first to measure the femoral hemodynamic response to acute
hypoxia in the neonatal period in species genetically adapted to
the chronic hypobaric hypoxia at high altitude (26). Persistence
of the in vivo femoral constrictor response to acute hypoxia
and a greater fall in oxygen delivery to the hindlimbs in

![Fig. 5. RT-PCR products obtained for α1A- and
α1B-adrenergic receptor and 18S r-RNA in NB
llama and NB sheep femoral resistance arteries.
A: α1A-adrenergic receptor expression in femoral
resistance arteries from NB llama (n = 5) and NB
sheep (n = 5). B: α1B-adrenergic receptor expres-
sion in femoral resistance arteries from NB llama
(n = 5) and NB sheep (n = 5). St, standard. Values
are means ± SE. *Significant differences (P < 0.05)
vs. NB sheep.](Image)
newborn llamas supports the concept that the highland neonate is more dependent on its cardiovascular defenses, adopting a fetal-like cardiovascular strategy to withstand an episode of hypoxia (25, 26). While this fetal-like cardiovascular defense response to hypoxia may be beneficial in the short term, it does not seem like a good strategy for postnatal life in the longer term if the peripheral vasoconstriction persists during chronic hypoxia. The cardiovascular defense responses to acute and chronic hypoxia in the newborn llama are likely very different, and it is possible that the fetal-like cardiovascular defense response reverses during sustained or chronic hypoxia. Alternatively, the increase in femoral vascular resistance during acute hypoxia in newborn llamas may not be adaptive, but instead it may represent a consequence of phylogenetic history or adaptive change in a different but interacting physiological trait (13, 23). Clearly, more studies are necessary to elucidate these alternatives.

The ex vivo data show that the physiologic mechanisms underlying the maintenance of the femoral vasoconstrictor response to acute hypoxia in the neonatal llama involve up-regulation of the α-adrenergic system. The data show greater vasoconstrictor sensitivity and capacity to α1-adrenergic agonists in newborn llama than in newborn sheep. While the maximal phenylephrine- and the noradrenaline-induced contractions in the femoral vasculature were similar in newborn llamas, the maximal response to phenylephrine in newborn sheep was only 50% of the noradrenaline-induced contraction. This difference not only highlights that the enhanced femoral vascular responsiveness in newborn llamas is mainly mediated via α1-adrenergic receptors, but it suggests that in sheep there may be functional α2-adrenergic receptors in the femoral vessels. However, we did not find any contractile responses to the selective α2-adrenergic agonist clonidine in femoral vessels isolated from newborn sheep.

Since vascular contraction induced by α1-adrenergic receptor activation may be offset by a simultaneous NO release from the endothelium (10, 34, 40), we explored the possibility that a decrease in this NO-dependent vasodilatation opposing α1-adrenergic-induced constriction may explain, in part, the enhanced femoral vascular response in the newborn llama relative to the newborn sheep. In support of this contention, vascular contraction to phenylephrine was markedly enhanced after NO blockade in femoral vessels isolated from newborn sheep. In contrast, no effect of NO blockade was observed in femoral vessels isolated from newborn llamas. Further vascular experiments of inhibition of noradrenaline-induced contraction at different concentrations of prazosin shifted the noradrenaline-concentration response curves to the right in both species, confirming competitive inhibition as it has been shown in other species (17, 28, 31, 33, 36). Schiold analysis of these competitive inhibition curves revealed α1-adrenergic receptors of high affinity for prazosin in the llama and low affinity in sheep. These differences in affinity for prazosin may also reflect differences in the α1-adrenergic receptor subtype between llama and sheep that contribute to the marked increase in femoral vascular resistance during acute hypoxia in the newborn llama. Having shown ex vivo differences in the α1-adrenergic-induced contraction and different prazosin affinities for these receptors, we then used RT-PCR to compare which of the α1-adrenergic receptors subtypes were present in the femoral arteries in both species. The data show a greater expression of α1B-adrenergic receptor in newborn llama compared with newborn sheep, a finding consistent with a greater affinity for prazosin in newborn llamas (1, 8, 12, 21, 31, 36).

In conclusion, in vivo data in the present study show that basal femoral vascular resistance is greater in the newborn llama than in the newborn sheep and that femoral vascular resistance increases in response to acute hypoxia in the newborn llama but it remains unchanged from baseline in newborn sheep. The ex vivo data show that the physiology underlying this difference between the species is due to: 1) enhanced vasoconstrictor reactivity and capacity to α-adrenergic agonists; 2) diminished NO-mediated vasodilatation in response to α1-adrenergic-induced constriction; and 3) a greater expression of high affinity α1B-adrenergic receptors in the newborn llama femoral vasculature.

**Perspectives and Significance**

Neonates of a highland species, the llama, contrasted to a lowland species, the sheep, utilize a different cardiovascular strategy to withstand episodes of acute hypoxia. The enhanced α-adrenergic femoral vascular reactivity present in vivo in the neonatal llama could warrant better blood flow to the heart, brain, and adrenals, particularly in the Alto Andino, its natural habitat, where there is a limited supply of atmospheric oxygen. This is one of many examples that represent how nature withstands extreme environments. Future research on this area should give clues to understand the mechanisms by which this remarkable response is achieved and, at the same time, understand further the biological basis of pathological conditions in humans and lowland animals either living in high altitude or undergoing sea level pregnancy complicated by sustained reductions in oxygen delivery to the fetus.

**ACKNOWLEDGMENTS**

We thank Dr. César E. Ulloa for help reading and editing this paper and to Carlos Brito for technical assistance.

**GRANTS**

This work was supported by National Fund for Scientific and Technological Development (FONDECYT), Grants 1090355, 1080663, and 1050479 and Welcome Trust Grant CRG 072256 UK. E. Herrera is a Fellow of Beqa, Presidente de la República, Gobierno de Chile. Dino A. Giussani is a Wolfson Research Merit Award Holder of The Royal Society.

**DISCLOSURES**

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

**REFERENCES**


α-ADRENERGIC SYSTEM AND FEMORAL VASCULAR REACTIVITY