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

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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) and sensitivity (pD2 = 5.47 ± 0.03 vs. 4.57 ± 0.05, 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 α1H-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 α1H-adrenergic receptor subtype.

newborn; hypoxia; small resistance artery; femoral resistance; α-adrenergic receptors
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. Neonates 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 new-born 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 Diazepam; Lab Biosano, Santiago, Chile) with additional local infiltration of 2% lidocaine hydrochloride (Dimecaína; Laboratorio Chile, 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).

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 animal’s head, and the animal breathed a gas mixture of 60% N₂ and 30% O₂ and 2–3% CO₂ in N₂) was passed at 30 l/min, with a respiratory frequency of 30 breaths/min. MAP, mean arterial pressure; FBF, femoral blood flow; SaO₂, saturation of hemoglobin; Hb, hemoglobin. Significant differences (P < 0.05): *vs. NB sheep; †vs. normoxia.

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>α₁A-Adrenergic receptor</td>
<td>(A) GGGTGATCGACGGCTGGCTGCA (S) AAGTGACGCTCCGCATCCA</td>
<td>53°C</td>
<td>35</td>
<td>277 bp</td>
</tr>
<tr>
<td>α₁B-Adrenergic receptor</td>
<td>(A) CAAATAGATGAAATGCGAGGCTAGTACC (S) CATTGACCGCTACATTGGGGTG</td>
<td>53°C</td>
<td>35</td>
<td>502 bp</td>
</tr>
<tr>
<td>18S-rRNA</td>
<td>(A) CCATCCAATCGGTAGTAGCG (S) GGTGATGCAGCTGTTTAGGTA</td>
<td>55°C</td>
<td>20</td>
<td>152 bp</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>NB 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>NB 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>NB 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>NB 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>NB sheep</td>
<td>80 ± 2</td>
<td>31 ± 1 †</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
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</tr>
<tr>
<td>NB llama</td>
<td>39 ± 2</td>
<td>38 ± 2</td>
<td>36 ± 2*</td>
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<tr>
<td>NB sheep</td>
<td>37 ± 1</td>
<td>36 ± 1</td>
<td>32 ± 1†</td>
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<tr>
<td>SaO₂, %</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NB llama</td>
<td>97 ± 2</td>
<td>67 ± 5†*</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>NB 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>NB 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 thiopentone, 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 mol/L.

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^{-3} M of the nitric oxide (NO) synthase inhibitor N\textsuperscript{-}nitro-l-arginine methyl ester (l-NNAME) were performed. Concentration response curves to noradrenaline were also repeated in vessels preincubated with the \(\alpha\)-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 (R_{max}) and sensitivity (EC_{50} or pD\textsubscript{2}) to the different vasoactive agents tested were obtained by fitting the concentration response curves to a Boltzmann function (Prism 4.0; Graphpad). R_{max} was expressed as tension (N/m) for K\textsuperscript{+} and for the adrenergic agonists as percentage of R_{max} to K\textsuperscript{+} (% K_{max}). Sensitivity was expressed as EC_{50} (the concentration at which 50\% of R_{max} was obtained) for K\textsuperscript{+} or for the adrenergic agonists as pD\textsubscript{2} (-\log EC_{50}). The data using inhibition with prazosin were analyzed using the global Schild method to generate an estimate of pA\textsubscript{2}. Schild analysis was performed by plotting Log (R-1) 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 antagonist. Antagonist pA\textsubscript{2} values were then taken to be the x-intercept to the Schild slope (32).

KRB contained (in mM): 118.5 NaCl, 25 NaHCO\textsubscript{3}, 4.7 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 1.2 MgSO\textsubscript{4}, 2.5 CaCl\textsubscript{2}, 5.5 glucose with a pH of 7.4. In K-KRB (125 mM K\textsuperscript{+}) 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 \(\alpha\)-adrenergic receptors. 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 \(\alpha\textsubscript{1A}\) and \(\alpha\textsubscript{1B}\)-adrenergic receptors and 18S-rRNA (27) and the amplification conditions indicated in Table 1.

The \(\alpha\textsubscript{1A}\) and \(\alpha\textsubscript{1B}\)-adrenergic receptors’ primers were designed to amplify nucleotide sequences (277 bp and 502 bp for \(\alpha\textsubscript{1A}\) and \(\alpha\textsubscript{1B}\)-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 \(\alpha\textsubscript{1A}\) and \(\alpha\textsubscript{1B}\)-adrenergic receptors (accession nos. NM017191 and NM016991, respectively). The amplicons obtained from sheep cDNA by using the \(\alpha\textsubscript{1A}\)-adrenergic primer showed 98\% identity with the recently published sheep sequence (24) (accession no. EU723257), while the one obtained in the llama had 93.5\% identity with sheep (24), and 92\% with bovine and human \(\alpha\textsubscript{1A}\)-adrenergic, respectively (accession nos. NM_174498 and NM_000680). The amplicons obtained in sheep and llama using the rat \(\alpha\textsubscript{1A}\)-adrenergic receptor primers showed 97\% identity with the corresponding bovine and human \(\alpha\textsubscript{1B}\)-adrenergic sequences (accession nos. NM_001191139 and BC136569, respectively). Once the common sequences in sheep and llama \(\alpha\textsubscript{1A}\)- and \(\alpha\textsubscript{1B}\)-adrenergic receptor amplicons were verified, specific sheep and llama primers for each adrenoceptor 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 \(\mu\)g 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 MgCl\textsubscript{2}, 0.2 mM dNTP mix, and 0.3 \(\mu\)M of each one of the primers under the conditions indicated in Table 1. The PCR products and molecular weight standard 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 pA\textsubscript{2} (intercept) values, the slope, and the correlation coefficient (r and \(r^2\)) 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, \( P_{\text{aO}_2} \) was higher and hemoglobin (Hb) concentration lower in newborn llamas compared with newborn sheep. Percent \( \text{SaO}_2 \), pH, and \( P_{\text{aCO}_2} \) were similar in both species (Table 2). During the episode of acute hypoxia, \( P_{\text{aO}_2} \) was significantly reduced to similar levels in both groups. However, compared with the newborn sheep, this fall in \( P_{\text{aO}_2} \) in the newborn llama was achieved with a smaller decrement in \( \text{SaO}_2 \) 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, \( P_{\text{aO}_2} \), \( \text{SaO}_2 \), and Hb concentration returned to basal levels in both groups. However, \( P_{\text{aCO}_2} \) 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 \( \mu \text{m} \) for newborn llamas and 370 ± 43 \( \mu \text{m} \) for newborn sheep. There were no significant differences in the \( R_{\text{max}} \) or in the sensitivity to \( K^+ \) between newborn llamas and newborn sheep (\( R_{\text{max}} \): 12.1 ± 0.8 N/m vs. 12.6 ± 0.9 N/m; pEC50: 1.62 ± 0.46 vs. 1.56 ± 0.4, respectively).

Although the \( R_{\text{max}} \) for noradrenaline between newborn llamas and newborn sheep was similar (99.3 ± 0.8% vs. 102.0 ± 1.5% \( K_{\text{max}} \)), the former showing greater sensitivity to noradrenaline (pD2: 5.18 ± 0.06 in newborn llama vs. 4.84 ± 0.05 in newborn sheep; \( P < 0.05 \), Fig. 2A). In addition, both the \( R_{\text{max}} \) and the sensitivity to phenylephrine were greater in newborn llamas (\( R_{\text{max}} \): 101.3 ± 1.4% \( K_{\text{max}} \) and pD2: 5.47 ± 0.03) compared with newborn sheep (\( R_{\text{max}} \): 52.4 ± 1.4% \( K_{\text{max}} \) 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 \( R_{\text{max}} \) 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 \( R_{\text{max}} \) 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...
**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 intense 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 vasoconstrictor sensitivity to \( \alpha \)-adrenergic stimulation, decreased vascular reactivity to \( \alpha \)-adrenergic-induced NO-dependent vasodilatation, and differences in the population of \( \alpha \)-adrenergic receptors subtypes expressed in the femoral vascular bed. Combined, the in vivo, ex vivo, and in vitro data strongly support the hypothesis tested that the llama newborn demonstrates greater \( \alpha \)-adrenergic peripheral vasoconstriction compared 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 oxygenation may differ markedly before and after birth. After birth, a reduction in oxygen supply triggers balanced cardiovascular and ventilatory responses. In response to acute hypoxia, 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 \( \text{PO}_2 \) 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...
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 vasodilation 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). Schild 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 α₁-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 the balance between physiological 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.

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DISCLOSURES

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

REFERENCES

α-ADRENERGIC SYSTEM AND FEMORAL VASCULAR REACTIVITY


