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REVIEW | *Hypoxia*

Perinatal cardiopulmonary adaptation to the thin air of the Alto Andino by a native *Altiplano* dweller, the llama

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Reyes RV, Herrera EA, Ebensperger G, Sanhueza EM, Giussani DA, Llanos AJ. Perinatal cardiopulmonary adaptation to the thin air of the Alto Andino by a native *Altiplano* dweller, the llama. *J Appl Physiol* 129: 152–161, 2020. First published June 25, 2020; doi:10.1152/japplphysiol.00800.2019.—Most mammals have a poor tolerance to hypoxia, and prolonged O_2 restriction can lead to organ injury, particularly during fetal and early postnatal life. Nevertheless, the llama (*Lama Glama*) has evolved efficient mechanisms to adapt to acute and chronic perinatal hypoxia. One striking adaptation is the marked peripheral vasoconstriction measured in the llama fetus in response to acute hypoxia, which allows efficient redistribution of cardiac output toward the fetal heart and adrenal glands. This strong peripheral vasoconstrictor tone is triggered by a carotid body reflex and critically depends on α-adrenergic signaling. A second adaptation is the ability of the llama fetus to protect its brain against hypoxic damage. During hypoxia, in the llama fetus there is no significant increase in brain blood flow. Instead, there is a fall in brain O_2 consumption and temperature, together with a decrease of Na^+-K^+-ATPase activity and Na^+ channels expression, protecting against seizures and neuronal death. Finally, the newborn llama does not develop pulmonary hypertension in response to chronic hypoxia. In addition to maintaining basal pulmonary arterial pressure at normal levels the pulmonary arterial pressor response to acute hypoxia is lower in highland than in lowland llamas. The protection against hypoxic pulmonary arterial hypertension and pulmonary contractile hyperreactivity is partly due to increased hemoxygenase-carbon monoxide signaling and decreased Ca^{2+} sensitization in the newborn llama pulmonary vasculature. These three striking physiological adaptations of the llama allow this species to live and thrive under the chronic influence of the hypobaric hypoxia of life at high altitude.

fetal; hypoxia; llama; neonatal; tolerance

INTRODUCTION

Exposure to high-altitude hypoxia is a great challenge for humans and for most animal species that have evolved near sea level, with an atmospheric pressure close to 760 mmHg, and a resulting partial pressure of oxygen (P_{O_2}) of ~150 mmHg. The decrease in barometric pressure as we move to high altitude results in a proportional decay of P_{O_2} in the atmosphere and in the partial pressure of oxygen in the arterial blood (Pa_{O_2}). As the curve of dissociation of hemoglobin (Hb) with O_2 (HbO_2) has a sigmoidal shape, O_2 saturation and tissue oxygenation begin to markedly fall above 2,500 m altitude (63). The effects of high altitude on oxygenation in the adult resemble the oxygenation in the fetus in lowland species even in healthy pregnancy. This is because the fetal Pa_{O_2} ranges between 20 and 30 mmHg, thereby being approximately one-third to one-quarter of the maternal Pa_{O_2}. This resembles the Pa_{O_2} of a mountaineer at 8,848 m on the top of Everest (62, 93), prompting Joseph Barcroft to coin his famous phrase "Everest in utero" to describe the oxygenation of the unborn child (31). During healthy pregnancy, the fetus obtains enough O_2 to satisfy its tissue metabolic demands because the fetal HbO_2 dissociation curve has a leftward shift relative to the mother, as fetal Hb has a higher affinity for O_2 and is usually present in greater concentrations compared with blood in the adult individual (56). Nevertheless, the physiological values of fetal Pa_{O_2} fall on the steep portion of the HbO_2 dissociation curve. Hence, a small decrease in fetal Pa_{O_2} below basal values will result in a much greater significant fall in fetal HbO_2, leading to a fall in the O_2 supply to the fetal tissues. This makes the fetus particularly vulnerable to hypoxia even when the pregnancy

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occurs near sea level, and this fetal margin of safety markedly decreases when the pregnancy occurs at high altitude (56, 59). Depending on intensity and duration, O_2 deprivation during fetal and neonatal life may induce hypoxic brain and/or heart damage, slowed fetal growth, low birth weight, neonatal pulmonary arterial hypertension, and developmental perturbations with increased risk of cardiovascular, metabolic, or neurological diseases in later life (20, 29, 57). Regions of the world with large plateaus over 3,000 m altitude include the Tibetan highlands of Central Asia, the Ethiopian plateau of East Africa, and the Andean *Altiplano* of South America. These areas provide a natural laboratory to study human and other animal adaptations to chronic hypobaric hypoxia conditions (63). The llama (*Lama glama*) is one of the four species of South American *Camelidae* whose evolution has taken place at high altitude for 2 million years, permitting the species to withstand this hypobaric and low-oxygen milieu (87). The fetal llama develops from the beginning to the end of gestation not only under the influence of the low oxygenation associated with embryonic and fetal life, but also under the additional superimposed low P_{O_2} of the environment in which its mother lives. This combined selective environmental pressure has enforced efficient physiological compensatory strategies to withstand fetal and postnatal hypoxia in the llama. Many of these strategies are adaptive and genetically determined since they persist when highland llamas are transported to live near sea level.

HEMATOLOGICAL ADAPTATIONS IN THE LLAMA

To withstand O_2 shortage, the llama has developed hematologic adaptations allowing efficient O_2 transport and delivery to tissues. The red blood cells of adult llama are elliptic and smaller than those observed in lowland species, offering a higher surface for O_2 exchange (50). The O_2 tissue extraction is greater in the adult llama than in species without ancestry at high altitude like the sheep (6). This is in part consistent with low activity of the carbonic anhydrase isoform, which is preferentially expressed in the red blood cells of the llama (94). This efficient total O_2 extraction is also observed in the llama fetus at ~70% of gestation (7, 56, 57). This improved O_2 extraction could result from shorter distance between capillary and cells, increased density, and higher myoglobin content in muscle. Nevertheless, these mechanisms need to be demonstrated in fetal llama. It also remains to be elucidated to which extent these adaptations change during fetal and neonatal development in this species. When Hb concentration, its O_2 affinity (P_{50}), and blood O_2 content in both the maternal and fetal llama are compared with those in maternal and fetal sheep, the mothers of both species have a similar Hb concentration (maternal Hb ~10.3 g/dL in llama versus ~10.1 g/dL in sheep), but the maternal llama has a lower P_{50} (maternal P_{50} ~24.6 mmHg in llama versus 40.0 mmHg in sheep), while the fetal llama has a higher Hb concentration (fetal Hb ~13.1 g/dL in llama versus ~11.4 g/dL in sheep) but similar P_{50} (fetal P_{50} ~19.7 mmHg in llama versus ~19.8 mmHg in sheep). These combined hematological adaptations result in a higher O_2 blood content in either maternal or fetal llama compared with the maternal or fetal sheep at any given Pa_{O_2}. For instance, at the normal fetal Pa_{O_2} of 24 mmHg, the fetal blood O_2 contents calculated from the corresponding blood O_2 dissociation curves deduced from Hill's equation are 12.0 mL/dL and 10.5 mL/dL for llama and sheep, respectively (64).

CARDIORESPIRATORY FUNCTION OF THE LLAMA UNDER BASAL CONDITIONS AND HEMODYNAMIC RESPONSE TO ACUTE HYPOXIA

The cardiorespiratory function of the llama fetus gestated at ~4,500 m but measured near term at low altitude (~586 m) shows that under basal conditions, heart rate, HbO_2, pH, and arterial partial carbon dioxide tension (Pa_{CO_2}) are lower compared with the sheep fetus at an equivalent gestational age (7). The basal combined ventricular output and umbilical blood flow, as well as net organ blood flow and calculated O_2 delivery for most of fetal organs and territories, with the exception of adrenal glands and liver, are also lower in the llama than in the sheep. Despite this lower O_2 delivery, the fetal O_2 extraction in the llama is higher than in the sheep, as indicated by the lower Hb saturation with O_2 in the umbilical artery (7). Conversely, total peripheral vascular resistance, as well as resistance in most regional vascular beds, is higher in the llama than in the sheep fetus under basal conditions (7). The higher vascular resistances in the llama probably result from differences in cardiovascular, local, humoral, and neural factors that favor an enhanced vasoconstrictor tone, since the arterial O_2 content is similar in both llama and sheep fetuses at lowlands (7). When subjected to acute hypoxia for 1 h, the llama fetus does not change its mean systemic arterial pressure and combined cardiac output or umbilical blood flow. There is an initial transient bradycardia, but after a few minutes, the heart rate normalizes. Blood flow to fetal heart and adrenal glands increases, while it decreases in the kidney, spleen, and carcass, and it is maintained in the fetal brain (27, 53, 56). In contrast, fetuses of lowland species like the sheep react to acute hypoxia with a significant bradycardia, an increase in systemic arterial pressure, and increases in blood flow to the brain, heart, and in particular to the adrenal glands. Indeed, the fall in blood flow to the kidney and the carcass is less pronounced in sheep than in the llama fetus (11, 56). Therefore, while in both species, there is redistribution of blood flow away from peripheral circulations toward the adrenal glands and the heart during acute hypoxia, in the llama fetus there is a mild or no increase in cerebral blood flow and the peripheral vasoconstriction is much more intense (27, 56). Of note, these hemodynamic responses to acute hypoxia seem to persist in the llama even into the late-gestation and the early postnatal period (54, 65).

NEURAL, ENDOCRINE AND LOCAL MECHANISMS MEDIATING THE HEMODYNAMIC DEFENSE TO ACUTE HYPOXIA IN THE LLAMA COMPARED WITH THE SHEEP FETUS

The carotid chemoreflex is an important regulator of peripheral vascular resistance in lowland fetuses like the sheep, since carotid chemodenervation markedly diminishes the hypoxic femoral vasoconstriction (24, 25). In sheep, once initiated, the fetal peripheral vasoconstriction during acute hypoxia is maintained by release of hormones into the fetal circulation, such as arginine-vasopressin (AVP) and catecholamines (19, 25), through activation of V1-receptors and α1-adrenergic receptors, respectively. Blockade of either receptor type diminishes

the peripheral vasoconstriction and attenuates the rise in fetal arterial blood pressure (24, 25, 56, 75, 76). We now understand that chemoreflex and endocrine vasoconstrictor influences on the peripheral circulation in the sheep fetus are offset by hypoxia-induced increases in local factors, such as nitric oxide (NO) at the level of the endothelium. This is because NO blockade with a NO clamp enhances the peripheral vasoconstrictor response to acute hypoxia in the sheep fetus (66). Further, the bioavailability of NO during acute hypoxia is itself limited by free radical generation, such as an increase in the superoxide anion ($O_2^{\cdot -}$), during acute hypoxia in the sheep fetus (89). Hence, in the late gestation sheep fetus a vascular oxidant tone, determined by the interaction between $O_2^{\cdot -}$ and NO, contributes to the chemoreflex and endocrine constrictor influences in the peripheral circulation, part of the fetal brain-sparing response in the sheep fetus (30).

In the llama fetus, bilateral section of the carotid sinus nerve prevents the transient bradycardia at the beginning of hypoxia, but it does not modify the fall in femoral blood flow or the increase in femoral vascular resistance. These findings suggest that unlike the sheep, in the llama, the carotid chemoreflex is not responsible for triggering the important redistribution of blood flow away from the peripheral circulations (27). In the llama fetus, α-adrenergic blockade with phentolamine treatment during normoxia produces hypotension, tachycardia, and vasodilatation in both the carotid and the femoral circulations (28, 34). During hypoxemia, llama fetuses treated with phentolamine do not elicit the pronounced femoral vasoconstriction, develop a strong hypotension followed by a cardiovascular collapse, and all die within 20 min of the onset of hypoxemia. Conversely, a V1-receptor antagonist produced a femoral vasodilatation during normoxia but does not affect the fetal cardiovascular responses to acute hypoxemia in the llama fetus (28). When prazosin, a specific α1-adrenergic antagonist, is infused, the peripheral vasoconstrictor response is also abolished, and the llama fetuses shortly die under an acute hypoxic challenge (57). It is interesting to note that in lowland sheep, the α-adrenergic blockade also abolishes the hypoxic femoral vasoconstriction but does not compromise fetal survival unless it is combined with carotid sinus nerve section (24), while in chronically hypoxic sheep fetuses, the α-adrenergic blockade alone provokes severe hypotension and fetal death during acute hypoxia (9, 57). Collectively, these data support that α-adrenergic and V1-vasopressinergic mechanisms contribute to a basal vasoconstrictor tone in the femoral circulation in the llama fetus. The enhanced femoral vasoconstriction during acute hypoxemia in the llama fetus is not mediated by stimulation of V1-vasopressin receptors but is markedly dependent on α-adrenergic receptor stimulation. Such α-adrenergic efferent mechanisms are therefore indispensable to fetal survival during hypoxemia in the llama since their abolition leads to cardiovascular collapse and death (28, 34). Since chronically hypoxic sheep fetuses also show an enhanced dependence on alpha-adrenergic responses to acute hypoxic stress, it seems that upregulation of alpha adrenergic signaling is an adaptive response to chronic hypoxia in lowland species, like the sheep, that may become genetically determined in fetuses of highland species, like the llama, and be present in South American camelids even when born and living at sea level.

Interestingly, the high peripheral vascular vasoconstrictor tone under basal conditions and in response to acute hypoxia in the fetal llama persists in the newborn period. The femoral vascular resistance is higher under normoxia and under acute hypoxia in the lowland newborn llama than in the newborn sheep. As in fetal sheep, the high vasoconstrictor femoral tone of the newborn llama is probably dependent of enhanced α1-adrenergic signaling, as suggested by the greater maximal contractile response and potency of isolated femoral vessels to phenylephrine in the newborn llama than the newborn sheep (65). Moreover, prazosin, α1-adrenergic receptor antagonist, elicits a rightward shift in α-adrenergic contraction curves of femoral vessels from both newborn sheep and llama, but decreases the maximal contraction only in llama, suggesting a different subtype of α1-adrenergic receptor subtype expression in both species. This is consistent with the preferential expression of the high-affinity and hypoxia-inducible α1B-receptor in the newborn llama, whereas the lower-affinity α1A-receptor expression is found in the newborn sheep (65).

As in fetal sheep, local endothelial NO production appears to have an important role in maintaining basal blood flow in several circulations and modifying perfusion during acute hypoxemia in the llama fetus. The blockade of NO synthesis with L-NAME under basal conditions in the llama fetus increases the mean systemic arterial pressure and the cerebral, carotid, and femoral beds vascular resistances. During acute hypoxia, suppression of NO synthesis markedly increases vascular resistance in the cerebral vascular bed and enhances the increase in resistance in the peripheral circulation of the llama fetus (83). These results suggest an important role of NO signaling in the maintenance of a dilator tone in the cerebral and femoral vascular beds, counteracting vasoconstrictor influences during acute hypoxia, in the llama fetus. Other studies have also reported an important role of the carotid chemoreflex and of NO in modifying adrenal blood flow and adrenocortical sensitivity in the llama fetus to a greater extent than in the sheep fetus (26, 79, 80). ET-1 is probably one of most powerful vasoconstrictors released by the endothelium, and it acts through its receptors ET_A and ET_B expressed in the vascular smooth muscle. ET-1 is also involved in the hypoxic increase of peripheral resistance in the llama fetus, since the infusion of BQ123, an ET_A receptor blocker, practically abolished the increase of femoral vascular resistance under acute hypoxia without modifying the basal femoral tone (57). Figure 1 summarizes the principal cardiovascular and hemodynamic responses to acute hypoxia in the llama fetus.

THE BRAIN STRATEGY TO COPE WITH HYPOXIA IN THE LLAMA FETUS

Cerebral vasodilatation in fetal animals of lowland species is a primary defense against hypoxia to maintain O_2 delivery and consumption by neurons that have a high metabolic rate (11, 46). In the sheep fetus, the brain blood flow matches the O_2 extraction in such a way that cerebral O_2 consumption remains stable until degrees of hypoxemia equivalent to 2.2 mL/dL of O_2 content in the ascending aorta (17). At lower levels of O_2 content, a fall of fetal brain O_2 consumption is measured, and if values persist at 50% lower than the basal O_2 consumption, nonreversible fetal brain damage occurs. This is evidenced by seizure activity in the electrocorticogram and/or histological signs of neuronal death, suggesting that compensatory mechanisms are no longer able to preserve the neuronal O_2 demand

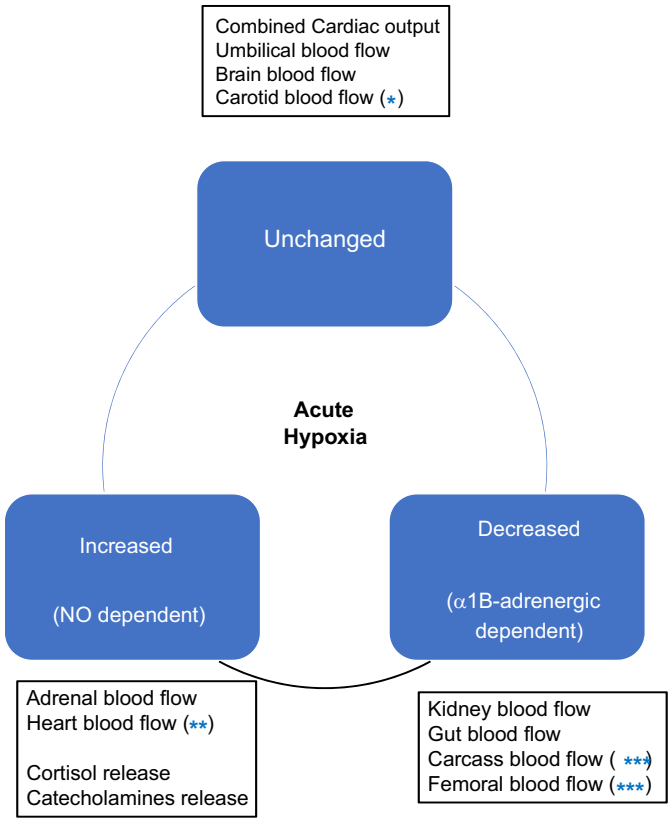

Fig. 1. Blood flow redistribution of the fetal llama in response to acute hypoxia. The main regional blood flow redistribution responses of the fetal llama to an acute hypoxic challenge of 1 h of duration are depicted. Carotid blood flow augmented if the hypoxic challenge is sustained for more than 8 h (*). Very high increase compared with fetuses from lowland species (**). Very marked decrease compared with fetuses from lowland species (***). The marked peripheral vasoconstrictor tone is also present in the neonatal llama.

(4, 17, 32, 56, 72). The neurons have a high metabolic rate because the repetitive firing of action and synaptic potentials results in a significant increased activity of the Na^+-K^+-ATPase pump, necessary to restore the ion gradients and neurotransmitter reuptake. Further, in neurons, ATP synthesis is essentially carried out through oxidative phosphorylation. The hypoxic brain damage is in great part the result of an energetic collapse, with a decrease of ATP that sequentially leads to the gradual dissipation of ion gradients, the release of excitotoxic neurotransmitters, the increase of intracellular Ca^{2+}, and the activation of multiple cascades that ultimately generate apoptotic and necrotic cell death, leading to hypoxic ischemic encephalopathy of the newborn (13, 49, 51). Unlike fetal sheep and other fetuses from lowland species, the fetal llama does not increase brain blood flow under acute hypoxia. Moreover, the llama fetus progressively decreases cerebral O_2 consumption in parallel with decreasing oxygenation. When submitted to gradual hypoxemia in 20-min steps, ranging from basal values in the fetal ascending aorta of Pa_{O_2} ~24 mmHg to a final Pa_{O_2} of ~14 mmHg, the llama fetus keeps constant the O_2 extraction but decreases the O_2 delivery and consumption. The cerebral hemispheric O_2 consumption progressively decreases as carotid blood O_2 content falls from ~10 mL/dL to ~2 mL/dL (55). This progressive decrease in O_2 consumption in the fetal llama brain during acute hypoxia is the hallmark of its differential adaptive strategy to withstand hypoxia. That is to say that the llama fetus matches cerebral O_2 consumption to the impaired cerebral O_2 supply by decreasing the fetal brain metabolic rate. Indeed, in the electrocorticogram of the llama fetus, a high voltage-low frequency pattern associated with a lower cerebral O_2 consumption prevails over a low voltage-high frequency pattern associated with higher brain O_2 consumption, when a lower carotid blood flow is observed (8). This again is a cerebral signature of an adaptive strategy for O_2 economy. When the llama fetus is submitted to hypoxia, the electrocorticogram flattens and becomes isoelectric, but in contrast to fetal sheep, no seizures are observed (57). Moreover, when the llama fetus is submitted to 24 h of continuous hypoxia resulting at a Pa_{O_2} ~12 mmHg equivalent to a ~50% decrease of arterial blood O_2 content, there is a fall of 0.56°C in the fetal brain cortex temperature. This hypoxic fetal brain cortex cooling is selective since the fetal core temperature does not change. Furthermore, the brain temperature is higher than the core in normoxic fetuses but becomes colder than core temperature in hypoxemic fetuses, suggesting a selective decrease of cerebral heat production and metabolism. Indeed, this selective brain cooling takes place together with a ~50% decrease in the cortical Na^+-K^+-ATPase activity, thereby consistent with a ~30% decrease in pump density, and a downregulation of the NaV1.1 channel. No functional evidence of brain damage such as seizures, no biochemical signs of increased apoptotic, or necrotic cell death evaluated through poly-ADP-ribose-polymerase (PARP) proteolysis are found in hypoxic llama fetuses (15). It is interesting to note that while carotid blood flow in the llama fetus does not change during the first 6 h of sustained hypoxia, carotid blood flow increases significantly from the 7th hour until the end of the chronic hypoxic insult. This suggests that other parts of the upper fetal body may need to preserve their O_2 delivery through increasing flow. When the Na^+-K^+-ATPase activity of the fetal llama brain is compared regionally, it is noteworthy that in addition to the brain cortex, the sodium pump also decreases its activity in the mesencephalon, but it does not change in the brain stem under hypoxia. The latter suggests that brain regions that control autonomic functions need to preserve their metabolic activity under conditions of chronic hypoxia (Reyes RV, unpublished results). Although NO is a normal vasodilator and regulator of synaptic transmission, its overproduction during long-term hypoxia may be neurotoxic (41). Indeed, chronic hypoxia induces an increase of nitric oxide synthase activity in the brain of sheep fetuses, while NOS activity in the brain cortex and cerebellum of the llama is not modified during a 24-h hypoxic challenge, suggestive of an adaptive cerebral cytoprotective mechanism in the highland species (1, 23). Taken together, these data indicate that the fetal llama brain triggers a hypometabolic response as an adaptive mechanism to preserve brain indemnity against hypoxic damage mediated in part via downregulation of Na^+-K^+-ATPase, the main consumer of ATP (and consequently of O_2), and of Na^+ channels, in order to reduce brain electrical activity and energy expenditure. We cannot exclude another additional neuroprotective consequence of the decrease of Na^+-K^+-ATPase activity: a slight increase in brain water content that could in turn help to dilute excitatory neurotransmitters. Such a strategy involving channel arrest mechanisms to enhance hypoxia tolerance has

been described in turtles (*Thrachemys scripta*) and to a lesser extent in the arctic ground squirrel (*Urocitellus parryii*) and the naked mole rat (*Heterocephalus glaber*) (49, 70). A protective lower brain metabolic rate has been also described in adult humans under prolonged apnea as well as in Quechua Indians of South America, with more than 10,000 years of living ancestry at high altitude, but it remains to be elucidated if a mechanism of channel arrest also participates in this adaptive strategy (3, 40). Figure 2 summarizes the main findings and evidence regarding fetal llama brain blood flow and cerebral metabolism under hypoxia.

THE NEWBORN LLAMA PULMONARY CIRCULATION: AVOIDANCE OF HYPOXIC PULMONARY HYPERTENSION

A unique feature of the pulmonary arteries is their intrinsic sensitivity to hypoxia: they have a vasoconstrictor response to acute hypoxia, the so called "hypoxic pulmonary vasoconstriction" (HPV), which is present in fetal and postnatal arteries during the entirety of life. The HPV is a rapid and reversible contraction of the pulmonary arteries, the intensity of which is proportional to the degree of hypoxia, and it occurs independently of neural or humoral factors. The HPV contributes to the maintenance of high pulmonary vascular resistance (PVR) state at the physiologically low Pa_{O_2} during fetal life, to redirect fetal blood to the placenta, while it allows matching of ventilation and perfusion during the postnatal life (81, 86). Pulmonary arteries also mount a maladaptive response to chronic hypoxia, inducing pathological pulmonary arterial remodeling, mainly characterized by the hyperplasic/hypertrophic thickening of medial layer with later involvement of adventitia and intima, with increased extracellular matrix deposition. An example of the latter results from exposure of the individual to chronic hypoxia during pregnancy, early postnatal life or in life at adulthood, resulting in pulmonary hypertension. This condition is characterized by increased basal mean pulmonary arterial pressure (mPAP) and PVR, increased pulmonary arterial response to vasoconstrictor stimuli (like superimposed hypoxia itself), decreased responsiveness to vasodilators, pathological pulmonary arterial remodeling, often complicated with right ventricular hypertrophy, and cardiac failure. The hypoxic pulmonary hypertension has been extensively documented in rodents, sheep, cattle, and humans (reviewed in 77).

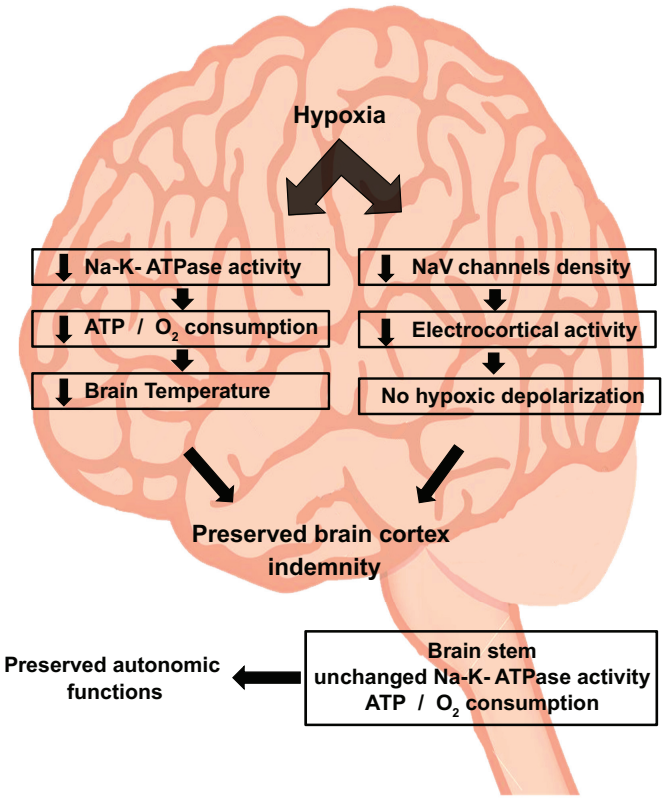

Fig. 2. Fetal brain hypometabolism of the llama as an adaptive response to hypoxia. In response to a hypoxic challenge of 24 h, the fetal llama's brain cortex decreases Na-K-ATPase activity and voltage-dependent sodium channel (NaV) density, decreasing ATP/O_2 consumption and electrical activity. In the brain stem, the decrease of Na-K-ATPase does not ensue, suggesting that in this particular region the hypometabolic response does not occur.

Both the adult and the newborn llama are resistant to hypoxic pulmonary hypertension. Early studies carried out either in young adult llamas, or in a mixed population of adult llama and alpaca, show that mPAP at altitude ranging between 3,420 m and 4,200 m does not increase or modestly increases compared with their lowland controls, while pathological pulmonary arterial remodeling and right ventricular hypertrophy are also mild or undetectable (5, 33). Newborn lambs exposed to gestational and early postnatal life to hypobaric hypoxia at Putre, at 3,600 m altitude, are hypoxemic, and they develop pulmonary arterial hypertension (35). Newborn lambs that spent their last 70% of gestation at high altitude until delivery and studied at low altitude at an early neonatal stage also develop pulmonary arterial hypertension despite their Pa_{O_2} being normal (38). Both models of ovine hypoxic pulmonary hypertension have increased basal mPAP, PVR, and greater HPV response compared with their lowland controls, as well as right ventricular hypertrophy and pathological pulmonary arterial remodeling (34–37, 57, 70). Unlike newborn lambs, newborn llamas gestated, born, and raised at high altitude do not develop hypoxic pulmonary hypertension. Despite their lower Pa_{O_2}, newborn llamas gestated, born, and raised at 3,600 m altitude show a similar mPAP and PVR under basal conditions than newborn llamas dwelling in the lowlands for more than fifteen years. Cardiac output, systemic arterial pressure, and resistance are also similar when lowland and highland llamas are compared (37, 78). It is particularly interesting to note that protection against hypoxic pulmonary hypertension in the newborn llama is probably the result of increased vasodilator/decreased vasoconstrictor signaling mechanisms since highland newborn llamas have slightly thicker pulmonary artery walls than lowland llamas. Nevertheless, the medial layer area of small pulmonary arteries of newborn llama are thinner than those of newborn sheep at equivalent altitude (36, 37, 78).

In general terms, vasoconstriction occurs as a result of phosphorylation of the myosin light chain (MLC) on Ser19, promoted by myosin light chain kinase through a Ca^{2+} and calmodulin dependent reaction. Conversely, dephosphorylation of PSer19-MLC catalyzed by myosin light chain phosphatase decreases the tension developed by the contractile machinery to a given Ca^{2+} concentration (Ca^{2+} desensitization) and

promotes vasodilatation (45). NO-cGMP vasodilator signaling decreases both Ca^{2+} concentration and Ca^{2+} sensitivity at the smooth muscle cell, while RhoA-Rho kinase (ROCK) signaling increases Ca^{2+} sensitivity and promotes vasoconstriction (45, 91). The relative activity of both pathways is regulated by the complex integration of multiple factors like arginine availability for NO synthesis and cGMP production, endogenous inhibitors like asymmetric dimethyl arginine (ADMA), and phosphorylation of key proteins like endothelial nitric oxide synthase (eNOS), phosphodiesterase 5 (PDE5), BKCa potassium channels, RhoA, myosin phosphatase target 1 (MYPT1), and C-kinase potentiated protein phosphatase-1 Inhibitor (CPI-17) (18, 22, 82, 90–92). These phosphorylations are mainly mediated by protein kinase G (PKG-1) and ROCK, but also by protein kinases A and C (PKA and PKC), and account for the regulation of these cross-talking pathways to finally regulate the amount of Pser19-MLC and to evoke smooth muscle contraction or relaxation (16, 45, 52, 69, 84). The function and expression of many of the molecular components of these pathways differ in the low- and high-altitude sheep and llama.

The role of NO signaling in both basal vasoconstrictor tone and in its response to acute hypoxia, in both normoxic and chronically hypoxic newborn llamas and sheep, has been investigated. Blockade of NO synthesis with L-NAME increases basal mPAP in both low- and high-altitude llamas, and in both species the increase is higher under high altitude. Nevertheless, this increase of mPAP after blockade of NO synthesis is lower in newborn llama than in sheep either at low or high altitude (37). Combined, these data indicate that NO synthesis or signaling downstream of NO is upregulated under chronic hypoxia in both species, but that this upregulation of the nitrergic tone, despite being greater in the sheep, does not prevent the increase in basal mPAP and PVR in these species, as it is not enough to explain the lack of increase in basal mPAP and PVR in the highland llama. In agreement with this interpretation, pulmonary nitric oxide synthase activity and expression are upregulated in highland sheep but not in highland llama, and the activity is lower in the llama than in the sheep at equivalent altitudes (37, 58). Nevertheless, the enzyme assays carried out in vitro do not reflect the presence of endogenous inhibitors or the physiological concentration of substrate. Both the plasma ADMA and the pulmonary arginase II expression are increased in the chronically hypoxic lamb compared with the normoxic controls, whereas they remain constant in the hypoxic newborn llama. Moreover, ADMA concentration is lower in llamas than in lambs at equivalent altitudes (61). Thus, the ability to keep low and constant concentration of endogenous inhibitors and degradation of eNOS substrate under chronic hypoxia may be also a factor to avoid pulmonary hypertension in the llama. Differential tolerance to hypoxic pulmonary hypertension between newborn sheep and llama may also result from optimization of signaling downstream of NO production. Indeed, the pulmonary expression of sGC is increased, while the expression and function of PDE5 are increased in the highland compared with the lowland newborn sheep, whereas sGC, PDE5 and the more active PSer92-PDE5 remain unchanged in highland compared with lowland newborn llamas (36–38, 58, 78). In other words, the pulmonary cGMP content at high altitude is probably decreased in the newborn sheep but not in the newborn llama. Such a reduced responsiveness to NO and potentially impaired sGC-cGMP action is also observed in chronically hypoxic piglets with neonatal pulmonary vascular disease (12). Moreover, the pulmonary cGMP content of the high-altitude newborn sheep has not been measured, but in the llama, it is unchanged at low and high altitude. Exploration of the NO signaling downstream of cGMP in the newborn llama lung shows that the expression of total MYPT1 is increased, while the expression of ROCK2 and PSer19-MLC are decreased, in highland llamas compared with lowland controls, suggesting that Ca^{2+} desensitization strengthens vasodilator tone and provides additional protection against pulmonary hypertension in this species, despite the hypoxic pulmonary arterial remodeling (78). In keeping with this statement, blunted ROCK is observed in the adult yak as a protective strategy against hypoxic pulmonary vasoconstriction, whereas a decrease of MYPT1 is reported in hypoxic fetal ovine pulmonary artery myocytes and increased RhoA-ROCK signaling in lung of highland newborn sheep as a possible explanation for pulmonary hypertension (39, 42, 60, 85). Another striking difference between newborn llama and sheep is their different pulmonary vasoconstriction in response to acute hypoxia both at low and high altitude. The pulmonary vasoconstriction in response to an acute hypoxic challenge of 60 min in the llama is biphasic, with a greater increase in mPAP and PVR during the first 15 min, followed by a less marked but still significant increase of both variables during the remaining 45 min of O_2 shortage. In contrast, in the newborn sheep, the hypoxic increase is not biphasic. Moreover, whereas the HPV is greater in highland than in lowland sheep, the opposite is observed in highland llama: their HPV response is lower in high- than in low-altitude animals in both phases of the response (10, 35, 36, 38, 73, 78). When the NO synthesis is blocked during acute hypoxia, there is additional increase of mPAP and PVR in low- but not in high-altitude newborn sheep, and HPV becomes similar in both groups, suggesting that NO action is maximally enhanced at high altitude and cannot limit the augmentation of PVR (39). ln the llama, the biphasic shape of HPV response is not modified by NO synthesis inhibition. Both lowland and highland llama increase mPAP and PVR during hypoxia, but the values of mPAP during acute hypoxia become greater in high- than low-altitude llama (78). In other words, the highland newborn llama optimizes its vasodilator tone through increasing the efficacy of NO signaling and this increased nitrergic tone mainly limits an excessive increase of mPAP to a superimposed acute hypoxic challenge, and partially contributes to a counteracting basal pulmonary vasodilator tone under chronic hypoxia. The nitrergic tone contributes also to regulation of mean systemic arterial pressure (mSAP) and resistance (SVR) in the newborn llama, but its hypoxic upregulation is restricted to pulmonary vascular bed. The upregulation of nitrergic tone under chronic hypoxia is restricted to the pulmonary vascular bed because L-NAME infusion increases similarly the mSAP and SVR values in high- and low-altitude llama (78).

Hemoxygenase-carbon monoxide (HO-CO) signaling has been also studied in both sheep and llama. Hemoxygenases 1 and 2 (HO1 and HO2) are expressed in vascular tissue and they catalyze the heme group degradation into equimolar amounts of CO, Fe^{2+}, and biliverdin. Fe^{2+} quickly binds to ferritin, while biliverdin is converted to bilirubin. Biliverdin, bilirubin, and ferritin have antioxidant properties (21, 47, 48, 68, 88,). On the other hand, CO has vasodilator, anti-inflammatory and

antiproliferative properties (21, 44, 45, 67, 71). It is proposed that CO exerts its vasodilator action through activation of sGC and BKCa potassium channels to increase smooth muscle cGMP and membrane potential, respectively, and its anti-remodeling action by decreasing the smooth muscle proliferation (58).

The highland newborn sheep show a decreased pulmonary production of CO, as well as a decreased pulmonary HO-1 expression compared with lowland controls (37). Moreover, newborn sheep that show pulmonary hypertension that persists at sea level as a result of gestation at high altitude have also both decreased pulmonary HO1 expression and CO production (38). Indeed, an inverse correlation between pulmonary CO and basal mPAP is observed when lowland lambs, highland lambs, or lambs with partial gestation at high altitude are compared (58). Conversely, highland newborn llama has both greater pulmonary CO production and HO1 expression, and moreover, the pulmonary CO production of the highland newborn llama is greater than in highland newborn sheep (37, 38, 58). Long-term administration of inhaled CO prevents or reverts the development of pulmonary hypertension in chronically hypoxic rodents, while acutely administered CO attenuates the HPV in adult sheep (14, 67, 95). Collectively, these data suggest that HO-CO signaling is another key adaption of the pulmonary circulation of the llama to prevent development of pulmonary hypertension. Both enhanced NO and HO-CO signaling are probably necessary to keep low basal mPAP and PVR and to limit excessive vasoconstriction under an acute superimposed hypoxic challenge in chronically hypoxic llama. Figure 3 summarizes the main findings regarding the adaptations of the pulmonary circulation of the llama to circumvent hypoxic pulmonary hypertension.

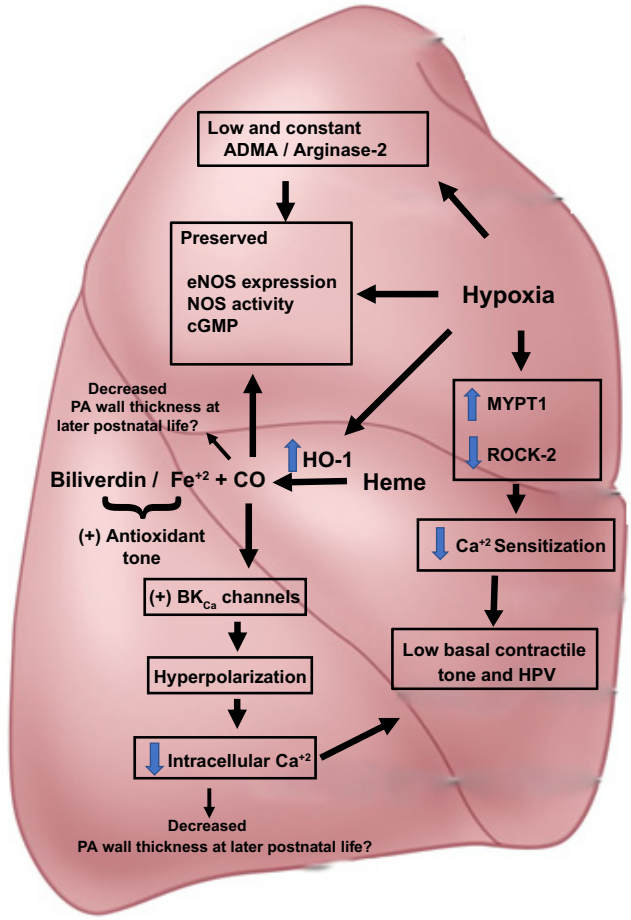

Fig. 3. Protective signaling mechanisms of the newborn llama against hypoxic pulmonary hypertension. The chronically hypoxic newborn llama maintains low and constant asymmetric dimethyl arginine (ADMA) levels and arginase-2 expression, preserves NO and cGMP synthesis, while signaling efficiency downstream cGMP is improved through upregulation of myosin phosphatase target 1 (MYPT1) and downregulation of Rho-associated kinase-2 (ROCK2) resulting in decreased Ca^{2+} sensitivity of the pulmonary artery smooth muscle contractile machinery. In parallel, upregulation of hemoxygenase 1 (HO1) increases CO production, contributing to preserve cGMP levels, stimulates BKCa channels and decreases intracellular Ca^{2+} The net results are both low hypoxic pulmonary vasoconstriction (HPV) and basal pulmonary arterial vasoconstrictor tone. Both increased CO and decreased intracellular Ca^{2+} that persist during llama's development may contribute to the thinning of pulmonary artery (PA) wall later in the adult llama.

CONCLUSIONS, OPEN QUESTIONS, AND PERSPECTIVES

The study of physiological adaptations selected to tolerate short- or long-term O_2 shortage by species that have evolved under environmental hypoxia is without any doubt one of the most promising avenues to understand and prevent animal and human diseases that are secondary to decreased O_2 supply or to diseases that reduce O_2 supply. The llama, as well as other south American *Camelidae*, have evolved for at least 2 million of years at the Andean *Altiplano*, thereby providing many remarkable examples of successful adaptations. Some of these adaptations are probably shared by other species with a different ancestry at high altitude as those found at the Tibetan plateau, such as the yak, as well as with some poikilotherms like the turtle, but with a different degree of intensity and small variations regarding the cellular mechanism to reach such adaptations. There are some general and specific questions related to the perinatal adaptations used by the llama to withstand hypoxia. First, it is necessary to know to which extent some of the adaptations discussed here, like the high peripheral vascular resistance or the brain hypometabolism, persist or are modified later in postnatal or adult life. Second, pulmonary arterial wall thickening is observed in highland newborn but not in adult llamas (33, 78). To know the stage of postnatal development of the llama in which the regression of pulmonary arteries wall occurs, and if other mechanisms in addition than those discussed here are involved, needs to be elucidated. Third, alternative protective mechanisms against pulmonary hypertension in the newborn llama need to be explored. Two candidates are prostacyclin and store/receptor operated channel signaling: prostacyclin signaling has vasodilator/anti-remodeling action whereas store and receptor operated channels have a vasoconstrictor/pro-remodeling action (2, 77). Preliminary results show that blockade of prostacyclin synthesis with indomethacin produces an increase of the second phase of hypoxic pulmonary vasoconstriction, whereas blockade of store operated channels with a 2-APB, does not modify the response in either low- or high-altitude newborn llama, suggesting that both upregulation upregulation of prostacyclin signaling, or silencing of store operated channels signaling, may be alternative strategies to circumvent hypoxic pulmonary hypertension (Reyes RV, unpublished results). These alternatives are currently under exploration.

The comparative physiology of the llama and other hypoxia-tolerant organisms could unfold many new mechanisms that may be of great help treating pathologies in animals and humans evolving with chronic hypoxia. An example of this potential translation to humans is the increasing use of brain cooling to limit brain damage in human newborns with ischemic hypoxic encephalopathy and the increasing interest to clinically evaluate many of the signaling proteins downstream to NO as complementary pharmacological targets to treat persistent pulmonary hypertension of the newborn, since the main treatment consisting of inhaled NO fails in at least 30% of cases (43, 74).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.V.R. and A.J.L. conceived and designed research; R.V.R. and A.J.L. prepared figures; R.V.R., E.A.H., G.E., E.M.S., D.A.G., and A.J.L. drafted manuscript; R.V.R., E.A.H., G.E., E.M.S., D.A.G., and A.J.L. edited and revised manuscript; R.V.R., E.A.H., G.E., E.M.S., D.A.G., and A.J.L. approved final version of manuscript.

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