Respiratory Physiology & Neurobiology 184 (2012) 186–191

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

The heme oxygenase–carbon monoxide system in the regulation of cardiorespiratory function at high altitude☆

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ARTICLE INFO

Article history:
Accepted 2 May 2012

Keywords:
Pulmonary vasodilatation
Vascular remodeling
hypoxia
CORM
Carbon monoxide
Heme oxygenase

ABSTRACT

Pulmonary arterial hypertension is one of the most serious pathologies that can affect the 140 million people living at altitudes over 2500 m. The primary emphasis of this review is pulmonary artery hypertension in mammals (sheep and llamas) at high altitude, with specific focus on the heme oxygenase and carbon monoxide (HO–CO) system. We highlight the fact that the neonatal llama has neither pulmonary artery hypertension nor pulmonary vascular remodeling in the *Andean altiplano*. These neonates have an enhanced HO–CO system function, increasing the HO-1 protein expression and CO production by the pulmonary vessels, when compared to llamas raised at low altitude, or neonatal sheep raised at high altitude. The neonatal sheep has high altitude pulmonary artery hypertension in spite of enhancement of the NO system, with high eNOS protein expression and NO production by the lung. The gasotransmitters NO and CO are important in the regulation of the pulmonary vascular function at high altitudes in both high altitude acclimatized species, such as the sheep, and high altitude adapted species, such as the llama.

"...carbon monoxide renders the blood crimson throughout the whole of the circulatory system."

Claude Bernard, 1846

Fair is foul and foul is fair

Act I. Scene II

Macbeth, W. Shakespeare

1. Introduction

Mothers, fetuses and neonates can experience acute and/or chronic hypoxia anywhere on the planet. However the possibility of episodes of acute or chronic hypoxia superimposed on the basal chronic hypoxia of life at high altitude is a substantial added risk. More than 140 million people live at altitudes greater than 2500 m (Moore et al., 2004) comprising the largest single human group at risk for perinatal complications due to low PO_2. This is a poorly recognized world-wide public health problem, and those affected are mainly located in developing countries.

One of the more serious pathologies associated with high altitude in various species is pulmonary hypertension, initially produced by pulmonary vasoconstriction and later exacerbated by vascular remodeling (Canepa et al., 1956; Gamboa and Marticorena, 1971; Penaloza and Arias-Stella, 2007). Under certain acute conditions increases in pulmonary arterial vascular resistance may be

Abbreviations: BKCa, big conductance calcium dependent potassium channel; cGMP, cyclic guanosine monophosphate; CO, carbon monoxide; COHb, carboxyhemoglobin; CORMs, carbon monoxide releasing molecules; eNOS, endothelial nitric oxide synthase; HHH, neonates whose conception, gestation, delivery and neonatal period took place at high altitude; HO, heme oxygenase; LLL, neonates whose conception, gestation, delivery and neonatal period took place at low altitude; LHL, neonatal lambs in which the conception and 30% gestation took place at low altitude, then 70% gestation and delivery occurred at high altitude and finally the neonatal period ensued at low altitude; L-NAME, L-arginine methyl ester; mPAP, mean pulmonary arterial pressure; NA, not available; NO, nitric oxide; NOS, nitric oxide synthase; PDE-5, phosphodiesterase 5; PO_2, arterial oxygen partial pressure; PVR, pulmonary vascular resistance; sGC, soluble isoform of guanylyl cyclase.

☆ This paper is part of a special issue entitled "Gasotransmitters and Respiration: Consequences in Health and Disease", guest-edited by Prem Kumar and Chris Peers.

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http://dx.doi.org/10.1016/j.resp.2012.05.003

beneficial, balancing pulmonary perfusion to the reduced alveolar oxygenation in specific areas of the lung. However, inappropriate adaptations to chronic exposure of the lung to the low PO_2 of high altitude may trigger significant sustained pulmonary hypertension that can produce acute pulmonary edema in infants and adult humans, subacute infantile mountain sickness (Sui et al., 1988; Anand and Wu, 2004), persistent pulmonary hypertension in newborn infants, and brisket disease in cattle (Abman, 1999; Abman and Steihorn, 2011; Rhodes, 2005).

Newborn sheep whose conception, gestation and delivery took place at high altitude showed marked pulmonary hypertension when compared with their lowland controls (Herrera et al., 2007). In contrast, in mammalian species adapted to high altitude as the result of dwelling at there for millions of years, such as the llama (*Lama glama*), the pulmonary vasculature has developed a blunted response to chronic hypoxia as a result of increased pulmonary vasodilator function and decreased pulmonary vascular remodeling. The gasotransmitters NO and CO are important in the regulation of the pulmonary vascular function at high altitude in both high altitude acclimatized species, such as the sheep, and high altitude adapted species, such as the llama.

Carbon monoxide administration to animals (either inhalatory or using carbon monoxide releasing molecules (CORMs)) had beneficial effects in several pathologic conditions, such as cardiovascular disease, sepsis, shock, cancer, organ transplantation, and acute injury of the lung, kidney and liver (Bauer and Pannen, 2009; Motterlini and Otterbein, 2010). However there is little information concerning the action of CORMs in the treatment of pulmonary hypertension (Motterlini and Otterbein, 2010).

Other aspects of the role of CO as a gasotransmitter will be addressed by others in this issue; we will discuss our results of HO–CO function in the pulmonary circulation in high altitude and low altitude llama and sheep neonates. We will also consider the role of the NOS–NO function in the same species.

2. HO–CO function at high altitude in llama neonates

Carbon monoxide reduces the pulmonary vascular resistance by (a) producing pulmonary vasodilatation from activation of soluble guanylate cyclase (sGC) and potassium channels (Ndisang and Wang, 2003; Williams et al., 2004; Wilkinson and Kemp, 2011) and (b) decreasing the cardiovascular remodeling and smooth muscle cell proliferation in the pulmonary vasculature induced by hypoxia (Kourembanas, 2002; Vitali et al., 2005; Kourembanas, 2011). Modifying the remodeling seems to be the more effective mechanism for carbon monoxide, rather than enhancing the vasodilatation.

The South American *Camelidae* include domestic and wild species. Among the domestic species are the llama (*L. glama*) and the alpaca (*Lama pacos*) and the wild examples include the vicuña (*Vicugna vicugna*) and the guanaco (*Lama guanicoe*). The *camelidae*, and in particular the llama, have lived in the *Alto Andino* for at least 2-million years, allowing the species to adapt to this hypobaric environment (Stanley et al., 1994). The llama has developed several adaptations to allow it to efficiently withstand the chronic hypobaric hypoxia of life at high altitude (Harris et al., 1982; Benavides et al., 1989; Llanos et al., 2003, 2007, 2011; Ebensperger et al., 2005). Among these mechanisms is the HO–CO system, which is enhanced during the neonatal period protecting its pulmonary vasculature against the deleterious effects of chronic hypoxia (Herrera et al., 2008b). Several studies demonstrate this protective role of the HO–CO system. Firstly, neonatal llamas whose conception, gestation, delivery and neonatal period took place at high altitude (HHH; Caquena 4600 m and Putre 3600 m) showed an enhanced pulmonary HO-1 protein expression and carbon monoxide production by the pulmonary vessels compared to low altitude llama neonates (LLL; Santiago 580 m), while having similar mean pulmonary arterial pressure, pulmonary vascular resistance, pulmonary eNOS protein expression, pulmonary NO production pulmonary sGC protein expression and small pulmonary artery wall thickness (Fig. 1; Fig. 6). The increased HO–CO function at high altitude is likely to be one mechanism that ameliorates inappropriate pulmonary vasoconstriction and pulmonary artery remodeling, maintaining normal pulmonary vascular resistance and pulmonary arterial pressure in spite of the chronic hypoxia. Secondly, carbon monoxide can elicit vasodilatation without intact endothelial function and this may be critical in the chronically hypoxic fetus and newborn where endothelial dysfunction is frequent (Motterlini and Otterbein, 2010). Thirdly, administration of inhaled CO to chronically hypoxic rats reversed right heart size and pulmonary artery pressure (Zuckerbraun et al., 2006). Fourthly, inhaled CO, 50 ppm for 21 days, prevented pulmonary hypertension in chronically hypoxic rats (Dubuis et al., 2005). Fifthly, we studied whether CO inhaled acutely might produce similar actions to CO administered chronically on pulmonary vascular resistance, utilizing adult sheep instead of adult rats. We studied the effect of inhaled CO (40 ppm) on pulmonary vascular resistance observed during acute hypoxemia in non-pregnant female adult sheep. These ewes showed a modest but significant decrease in the elevated pulmonary vascular resistance during acute hypoxemia with CO (Nachar et al., 2001). This is similar to the effect of chronic administration of CO reversing pulmonary hypertension in chronic hypoxia in adult rats (Zuckerbraun et al., 2006; Dubuis et al., 2005). This is in contrast to the findings of Grover et al. (2000), who found that inhaled CO administered by a mechanical ventilator to late gestation fetal lambs did not change lung blood flow or pulmonary vascular resistance.

	HHH	LLL
mPAP & PVR	+	+
HO-1 protein expression	++	+
CO production	+++	++
eNOS protein expression	+	+
NO production	+	+
sGC protein expression	+	+
Pulmonary artery wall thickness	+	+

Fig. 1. Comparative importance of properties of pulmonary circulation and tissues from newborn llamas whose conception, gestation, delivery and neonatal period took place at high altitude (HHH) and at low altitude (LLL).

Taking all these data together we propose that decreased function the in HO–CO system in the neonatal sheep could be one of the mechanisms causing increased vascular resistance in the lung and pulmonary hypertension. In contrast, the increased function the in HO–CO system in the neonatal llama could be one of the mechanisms causing decreased vascular resistance in the lung and pulmonary normotension.

3. HO–CO function at high altitude in sheep neonates

High altitude newborn sheep, in contrast to neonatal llamas, show a markedly impaired function of the HO–CO system, overt pulmonary hypertension and augmented pulmonary vascular resistance (HHH) (Figs. 2–4 and 6; Herrera et al., 2007, 2008b). Similarly, newborn lambs in which the gestation took place partially