Respiratory plasticity in response to changes in oxygen supply and demand

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Synopsis Aerobic organisms maintain O_2 homeostasis by responding to changes in O_2 supply and demand in both short and long time domains. In this review, we introduce several specific examples of respiratory plasticity induced by chronic changes in O_2 supply (environmental hypoxia or hyperoxia) and demand (exercise-induced and temperature-induced changes in aerobic metabolism). These studies reveal that plasticity occurs throughout the respiratory system, including modifications to the gas exchanger, respiratory pigments, respiratory muscles, and the neural control systems responsible for ventilating the gas exchanger. While some of these responses appear appropriate (e.g., increases in lung surface area, blood O_2 capacity, and pulmonary ventilation in hypoxia), other responses are potentially harmful (e.g., increased muscle fatigability). Thus, it may be difficult to predict whole-animal performance based on the plasticity of a single system. Moreover, plastic responses may differ quantitatively and qualitatively at different developmental stages. Much of the current research in this field is focused on identifying the cellular and molecular mechanisms underlying respiratory plasticity. These studies suggest that a few key molecules, such as hypoxia inducible factor (HIF) and erythropoietin, may be involved in the expression of diverse forms of plasticity within and across species. Studying the various ways in which animals respond to respiratory challenges will enable a better understanding of the integrative response to chronic changes in O_2 supply and demand.

Introduction

The effective transfer of O_2 from the environment to mitochondria is critical for aerobic organisms. Thus, rapid changes in O_2 supply or demand, as might accompany brief exposures to environmental hypoxia or arise from exercise, are typically met with acute cardiorespiratory reflexes that maintain tissue O_2 homeostasis. Chronic or repeated challenges, however, may elicit additional responses that further modify the respiratory phenotype, ostensibly in ways that improve and/or more efficiently regulate oxygenation of tissues. This is called *respiratory plasticity*, which has been defined as persistent changes based on prior experience that may involve structural and/or functional alterations (Mitchell and Johnson 2003). For example, chronic exposure to hypoxia alters the acute hypoxic ventilatory response as a

This paper summarizes one of the 22 symposia that constituted the "First International Congress of Respiratory Biology" held August 14–16, 2006, in Bonn, Germany.
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Integrative and Comparative Biology, volume 47, number 4, pp. 532–551
doi:10.1093/icb/icm070
Advanced Access publication July 23, 2007

result of multiple cellular/synaptic mechanisms at different sites in the respiratory control system (Powell et al. 1998). Respiratory plasticity is generally characterized by a relatively slow onset and by its persistence, at least transiently, after the stimulus is removed (Powell et al. 1998; Mitchell and Johnson 2003).

Although use of the term respiratory plasticity has become common only in recent years, scientific interest in respiratory adjustments following time spent at high altitude and after exercise training has a long history. [Although ''altitude'' strictly refers to height above a land surface, and ''elevation'' to height of a land surface above sea level, the term ''altitude'' in used throughout this article, even when referring to sites at high elevations, in order to conform to prevalent usage among researchers in this field]. Indeed, studies of respiratory plasticity following changes in O_2 supply and demand are pervasive in the scientific literature under such descriptors as training, learning, memory, acclimation, or acclimatization. ''Adaptation'' is another term used frequently for plasticity in the biomedical literature but it is important to distinguish true evolutionary adaptations in the genotype driven by natural selection from plasticity, which is a phenotypic change in an organism's physiology, morphology, or behavior. With this broader lens, even a cursory review of the literature reveals plasticity occurring at all levels of the O_2 cascade, including modifications of the gas exchanger, respiratory pigments, vasculature, and metabolically active tissues, as well as alterations in the muscles and neural control systems responsible for ventilating the gas exchanger.

In the following sections, we have assembled brief reports on respiratory plasticity reflecting both changes in O_2 supply (environmental hypoxia or hyperoxia) and O_2 demand (exercise-induced and temperature-induced increases in energy metabolism). These reports expand on symposium talks on this topic presented on the occasion of the *First International Congress of Respiratory Biology* held August 2006, and are chosen to exemplify plasticity at multiple levels of the respiratory system. We have focused primarily on plastic responses to chronic, continuous changes in O_2 supply and demand. Although the coverage is far from exhaustive, these reports illustrate the diversity in mechanisms of respiratory plasticity while highlighting recent advances and common themes in this rapidly growing field.

Growth, remodeling, and function of the mammalian lung following residence at high altitude (Hsia)

Native highlanders show larger lung capacities than do their lowland counterparts in a manner that transcends genetic background, from Tibetans who settled in the Himalayas about 25,000 years ago (Moore et al. 1992) and Peruvian Indians who settled in the Andes about 10,000 years ago (Frisancho 1969; Frisancho et al. 1975; Brody et al. 1977) to Caucasians who settled in Leadville, Colorado USA only a few generations ago (Remmers and Mithoefer 1969; DeGraff et al. 1970; Dempsey et al. 1971). Pulmonary gas exchange efficiency is also better in residents from high altitude (HA) who were born and raised at sea level (SL) and subsequently moved to HA as adults (Cerny et al. 1973). These human studies suggest that lung function is enhanced as a result of HA exposure but are unable to distinguish between structural and physiological sources of enhancement. Across the animal kingdom, ambient hypoxia is a potent primary stimulus for the growth of gas exchange organs (Bond 1960; Burri and Weibel 1971; Burggren and Mwalukoma 1983; Sekhon and Thurlbeck 1996a, 1996b). The ultimate dimension of the lungs is constrained by the size of the bony thorax, since growth and function of the lung and the rib cage are mechanically coupled. Indirect evidence suggests that the period of lung growth at HA may be prolonged due to delayed epiphyseal union of the ribs (Schutte et al. 1983), or lungs may continue to grow or remodel even after epiphyseal closure has occurred. In rats and mice, short-term exposure to ambient hypoxia for 3–4 weeks accelerates lung growth as evidenced by a larger lung volume, alveolar tissue volume, and alveolar surface area (Burri and Weibel 1971; Pearson and Pearson 1976; Sekhon and Thurlbeck 1996a, 1996b). The response appears to be maturity-dependent (Massaro et al. 1989; Blanco et al. 1991). In the perinatal period, even brief hypoxic exposure (10% O_2 for 1–2 h) blunts lung development at seven days of age (Massaro et al. 1989). Between 2 and 14 days, exposure to 13% O_2 mainly impairs alveolar formation. However, between 14 and 40 days, alveolar number increases more in hypoxic than in normoxic rats, suggesting accelerated alveolar proliferation (Blanco et al. 1991).

Only a few studies have examined the effect of longer HA exposure on lung growth and function. Weanling guinea pigs raised in extreme normobaric hypoxia (5100 m) (Lechner and Banchero 1980) showed initially accelerated alveolization above that

occurring in low altitude (LA) controls (1600 m). However, differences between groups became progressively less with longer exposure up to 14 weeks, raising the question of whether HA exposure in young animals actually extends the upper limit of lung size at maturity or merely accelerates the rate at which normal adult lung size is reached. To answer this question, we raised weanling guinea pigs at a more tolerable HA (3800 m) for 1, 3, or 6 months in comparison to their littermates raised at a LA (1250 m). Lung volume, alveolar tissue volume, and alveolar-capillary surface areas were higher in HA-raised animals at all time points. There was progressive acinar remodeling with a smaller alveolar duct volume and lower harmonic mean thickness of the diffusion barrier (Hsia et al. 2005). The anatomical changes were associated with a higher diffusing capacity of the lung for carbon monoxide (DL_{CO}) measured in the conscious spontaneously breathing animal at rest by a rebreathing technique (Yilmaz et al., unpublished data). We concluded that prolonged residence at HA during somatic maturation enhanced the growth and function of alveolae. In another study, young beagle dogs raised from 2 months of age to beyond maturity (16 months of age) at 3100 m (Johnson et al. 1985; Johnson 1994) showed a larger lung volume, DL_{CO}, alveolar tissue volume and surface area compared to control dogs raised at sea level. On the other hand, adult dogs kept at 3100 m for 3 years showed no change in these parameters. Thus, both small and large mammals exhibit maturity-dependent augmentation of lung dimensions in response to HA. The cumulative results from these different studies show that (1) moderate HA exposure accelerates developmental growth of the lung, (2) more extreme HA exposure exerts diminishing effect on stimulating developmental lung growth, and (3) HA exposure has no significant effect on adult lungs. Taken together, they support the interpretation that HA exposure augments ongoing postnatal lung development but does not constitute a sufficient signal, by itself, for the initiation of lung growth (Fig. 1).

In order to separate the reversible from the irreversible components of acclimatization to HA, we raised foxhounds from 2.5 to 7.5 months of age at moderate HA (3800 m) (McDonough et al. 2006; Hsia et al. 2007). Resting lung function was measured 1 month after return to SL before animals reached somatic maturity. Then the animals were trained to exercise on a treadmill and lung function was measured during exercise one to 2 years later. In HA-exposed animals compared to control littermates raised simultaneously at SL,

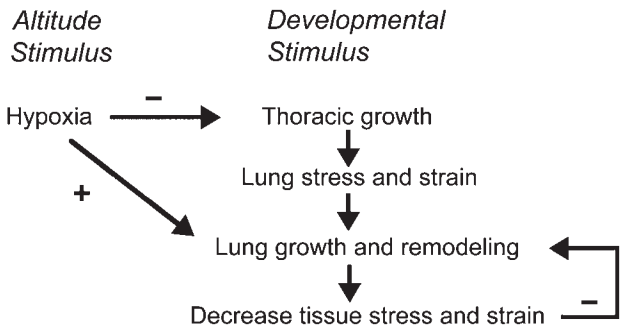

Fig. 1 Hypoxia interacts with developmental signals of lung growth. During development, the enlarging bony thorax exerts mechanical stress and strain on lung tissue, activating a cascade of cellular events associated with lung growth, which in turn reduces tissue stress and strain. This interaction continues until somatic maturity when the epiphyses close and maximal thoracic and lung sizes are matched. Exposure to high altitude exerts opposing effects on thoracic growth and lung growth. Exposure to moderate high altitude accelerates alveolar tissue growth. Above a critical altitude, severe hypoxia blunts thoracic growth and offsets the benefit derived from stimulation of lung growth since thoracic dimension determines the upper limit of lung size.

lung volume at a given transpulmonary pressure remained elevated 1 month and 2 years after return to SL. Maximal oxygen uptake was similar between groups when measured 1 year after return to SL, but peak arterial pH was higher and blood lactate concentration lower in HA-raised animals while breathing a hypoxic gas mixture. Exercise DL_{CO} and lung diffusing capacity for oxygen assessed by independent methods remained elevated in HA-exposed animals, attributed to a higher membrane diffusing capacity, and higher blood volume of pulmonary capillaries. Ventilation–perfusion matching was unchanged. Thus, metabolic efficiency during hypoxic exercise and alveolar diffusive gas transport were persistently enhanced in HA-exposed animals even after reacclimatization to SL.

Unexpectedly, hematologic volumes (blood, erythrocyte, and plasma) remained elevated in HA-exposed animals up to 2 years after return to SL despite normalization of systemic hematocrit. Two explanations for these observations are possible: (1) Exercise training causes a modest increase in blood volume (Mackintosh et al. 1983; Heinicke et al. 2001) and may have prevented the regression of HA-induced increase in blood volume (Schmidt et al. 2002), and (2) HA residence permanently enlarges total body microvascular blood volume and possibly a larger splenic blood reservoir. In aerobic species, a large spleen stores up to 30% of total body erythrocytes and about 10% of total body blood volume at a hematocrit of 80–90% (Barcroft and Poole 1927; Barcroft and Stephens 1927;

Guntheroth et al. 1967; Cabanac et al. 1997). Upon exercise or hypoxic exposure, splenic contraction releases the stored erythrocytes (Vatner et al. 1974), thereby increasing circulating blood volume and hematocrit, which in turn augments convective as well as diffusive O_2 transport. Because splenic autotransfusion occurs transiently in response to O_2 demand, resting hematocrit remains normal despite a higher total erythrocyte volume, and pulmonary and systemic arterial hypertension does not develop. Splenectomy in thoroughbred horses and dogs eliminates exercise-induced polycythemia and significantly impairs convective and diffusive O_2 transport in the lung and the periphery (Wagner et al. 1995; Dane et al. 2006). Thus, the splenic erythrocyte reservoir effectively facilitates oxygen transport. In dogs and other species with a high aerobic capacity, dynamic splenic release of erythrocytes is a potential compensatory mechanism for increasing oxygen transport at HA, while minimizing the adverse systemic effects associated with chronic polycythemia.

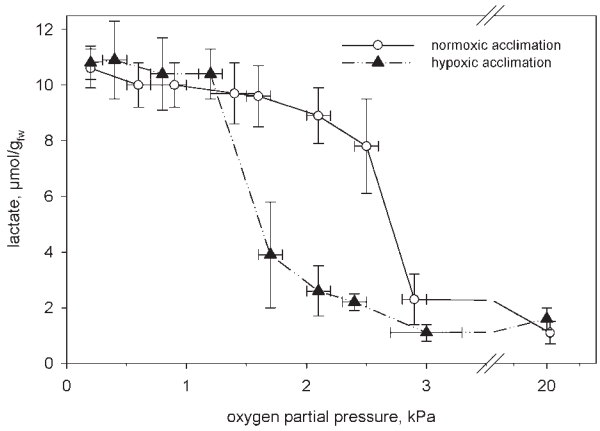

Fig. 2 Acclimation to conditions of low oxygen shifts the threshold for anaerobic energy metabolism in *Daphnia magna*. Animals raised at normoxic conditions ($Po_2 = 21$ kPa, hemoglobin: 131 ± 65 µmol heme/l hemolymph) and counterparts from hypoxic conditions ($Po_2 = 3$ kPa, Hb: 519 ± 113 µmol heme/l hemolymph) were exposed to different low oxygen partial pressures for 3 h. L-lactate concentration indicated the intensity of ATP production by anaerobic glycolysis ($n = 3$). Thus, the benefit of enhanced oxygen transport capacity was a reduction of the critical oxygen limit (*Pc*).

Hemoglobin induction extends the limits of oxygen concentration and temperature in *Daphnia* (Zeis, Maurer, Paul)

Small planktonic animals like *Daphnia* experience high fluctuations of environmental oxygen content and temperature. Changes in both abiotic factors can impede oxygenation of tissues. While this is obvious in the case of hypoxia, it is a result of an increasing mismatch between reduced oxygen solubility and enhanced oxygen demand of these poikilothermic animals at higher temperatures. This gap can be closed by increased ventilation and perfusion transiently. Persistent impairment of tissue oxygenation in *Daphnia magna*, however, is answered by an increase in oxygen transport capacity that restores cellular oxygen homeostasis. Differential expression of a set of seven hemoglobin subunits enhances both concentration and oxygen affinity of the respiratory protein (Kobayashi and Yamagata 2000; Zeis et al. 2003; Paul et al. 2004). Different di-domain orthologs of *D. magna* hemoglobin can be combined to native aggregates of 16 subunits shifting the P_{50} of half-maximal oxygenation from 1.0 ± 0.1 kPa (acclimation to normoxia at 20°C) to 0.5 ± 0.1 kPa (acclimation to hypoxia at 20°C) or 0.8 ± 0.1 kPa (acclimation to 30°C at normoxia) (Zeis et al. 2003; Lamkemeyer et al. 2003, 2005, 2006). Oxygen-sensitive induction of hemoglobin orthologs is known to be regulated by HIF (hypoxia-inducible factor), which is translocated into the nucleus after dimerization when low oxygen supply prevents its degradation, thus binding to hypoxia-responsive elements in the intergenic regions of the *Daphnia* hemoglobin cluster (Kobayashi and Yamagata 2000; Gorr et al. 2004).

In an integrative approach, the consequences of the observed plasticity of hemoglobin quantity and quality were assessed. The limits of oxygen concentration and temperature allowing for aerobic metabolism were characterized by the onset of L-lactate accumulation. These limits were shifted by long-term acclimation; the critical oxygen partial pressure was lowered in animals from hypoxic acclimation with their high hemoglobin concentration and affinity (Fig. 2). During the experiments, animals were exposed for three hours to water equilibrated to several defined mixtures of air and nitrogen (using gas mixing pumps from Wösthoff, Krefeld, Germany). Extract preparation and determination of L-lactate concentration were performed as described elsewhere (Zeis et al. 2004). While the critical partial pressure of oxygen was reached below a Po_2 of 2.7 kPa for normoxia-raised animals with a hemoglobin content of 131 ± 65 µmol heme/l hemolymph, this threshold was lowered to 1.6 kPa in animals from hypoxic acclimation containing hemoglobin at a concentration of 519 ± 113 µmol heme/l hemolymph. Thus, the enhanced oxygen transport

capacity still allowed for aerobic energy metabolism at oxygen conditions lowered by about 1.1 kPa.

Analogous studies with animals acclimated to different temperatures showed a shift of the critical temperature leading to lactate accumulation (Zeis et al. 2004). While *D. magna* from cold acclimation (10°C) with a hemoglobin content of 75 ± 26 µmol heme/l hemolymph had to rely on anaerobic metabolism at 35°C ambient temperature, animals from 20°C or 30°C acclimation did not accumulate lactate under these conditions. Animals from 20°C acclimation (131 ± 65 µmol heme/l hemolymph Hb) showed lactate formation at 37°C, while in warm acclimated (30°C) animals (209 ± 51 µmol heme/l hemolymph Hb) only a minor increase of this anaerobic metabolite could be measured at that temperature. Thus increased oxygen transport capacity by hemoglobin induction as a result of thermal acclimation extends the aerobic "working range" on the temperature scale.

Parallel studies on the swimming activity of *D. magna* raised at different temperatures showed that lactate formation beyond the critical thermal limit was not the result of exhaustive muscular work since animals reduced their motility at higher temperatures. Furthermore, these studies indicate that the thermal optimum was shifted by temperature acclimation as animals showed maximal swimming activity at their acclimation temperature (Zeis et al. 2004). Finally, the preference of temperature or oxygen conditions offered in a linear gradient was influenced by the acclimation conditions of *D. magna* (Lamkemeyer et al. 2003; Zeis et al. 2005). While animals from 10°C and 20°C acclimation chose temperatures near these values, animals raised at 30°C showed a preference for a temperature of 21.4°C. These alterations of the animals' behavior can be discussed with respect to the observed plasticity at the biochemical level. Despite enhanced capacity for oxygen transport in warm-acclimated daphnids, animals from 30°C acclimation reduced their metabolism by choosing water of lower temperature. Analogous experiments in an oxygen gradient revealed euryoxic behavior of animals from moderate oxygen acclimation; the individuals spread widely throughout the offered oxygen gradient. Animals from both extremes of oxygen acclimation showed stenoxic behavior; animals from hypoxia (Po_2: 2 kPa) crowded at the low end of the gradient and animals from hyperoxic conditions (Po_2: 73 kPa) stayed at the oxygen-rich end.

In conclusion, it seems that the characteristics of the hemoglobin present in the hemolymph play an important role in the oxygen concentration and temperature ranges optimal for physiological performance in *Daphnia*. The degree of oxygenation of the tissues limits the tolerance for stressful temperatures (Pörtner 2002). The window of optimal performance can be extended by hemoglobin induction, restoring cellular oxygen supply. Thus, hemoglobin can be considered as an element in the homeostatic response, the slow, stressor-specific defense following a faster, but unspecific, universal stress response (Kültz 2005). Comparing different *Daphnia* species in terms of hemoglobin induction as a function of oxygen and temperature may provide an estimate of their acclimation potential for both factors.

Respiratory muscle plasticity following chronic hypoxia (Bradford, McGuire, O'Halloran)

Little is known about the effects of chronic hypoxia on the structure and contractile function of respiratory muscles or, indeed, other skeletal muscles. This is surprising considering how commonplace exposure to chronic hypoxia is in humans (e.g., with exposure to high altitude, sleep apnea, and respiratory disease in general). Patients with chronic obstructive pulmonary disease have a reduction in respiratory and limb muscle force and endurance (Zattara-Hartmann et al. 1995), perhaps because of chronic hypoxia. The results from studies of muscle contraction and fatigue in humans exposed to high altitude have been inconsistent (Garner et al. 1990; Fulco et al. 1994; Kayser et al. 1994; Caquelard et al. 2000), but there is good evidence that chronic exposure to high altitude leads to a decrease in mitochondrial function and aerobic metabolism (Green et al. 1989; Hoppeler et al. 2003). The functional implications of this are unclear, however, because whilst chronic continuous hypoxia was shown to reduce citrate synthase activity (indicating reduced aerobic capacity) in rats, maximal oxygen consumption was nevertheless increased, probably due to other plastic responses (Abdelmalki et al. 1996). It should be noted, however, that patients with chronic obstructive pulmonary disease and chronic hypoxia also have reduced aerobic metabolism both in respiratory muscles and in limb muscles (Gertz et al. 1977) and have a higher proportion of fast fibers in limb muscles (Hildebrand et al. 1991) that is accompanied by increased muscle fatigability (Zattara-Hartmann et al. 1995). The reduction in cellular aerobic capacity caused by systemic hypoxia is in contrast to the increase in capacity associated with the local hypoxia of exercise training

(Hoppeler and Flück 2003), and it is also in contrast to the improved performance caused by training in normoxia but being hypoxic for the remainder of the time (see ''Effect of 'living high—training low' on sea level performance'').

The effects of chronic continuous hypoxia on muscle structure are also controversial. In humans, there is evidence that the fiber type of limb muscles is unaffected by altitude exposure (Green et al. 1989). In rats, however, limb muscles generally showed a transition from slow to fast phenotype (Sillau et al. 1977; Itoh et al. 1990; Bigard et al. 1991; Ishihara et al. 1995), although Shiota et al. (2004) observed the opposite. Remarkably, there have been very few studies of the effects of chronic, continuous hypoxia on the function of isolated muscle and the results that are available are equivocal. Itoh et al. (1990) reported that chronic hypobaric hypoxia in rats resulted in a reduction in fatigue in the *extensor digitorum longus* but *soleus* fatigue was unaffected. Shiota et al. (2004) found that chronic hypobaric hypoxia had no effect on muscle fatigue in muscles of the diaphragm or limb and we found that hypobaric hypoxia caused an increase in muscle fatigue in the upper airway of rats (El-Khoury et al. 2003).

The effects of chronic intermittent rather than continuous hypoxia have particular relevance to sleep apnea in humans. In this condition, periods of hypoxia and normoxia occur during sleep as a result of periodic apneas. The usual cause of this is collapse of the upper airway as a result of an imbalance between diaphragm-generated collapsing pressures and airway-stabilizing muscle contraction in the upper airway. The upper airway muscles in this condition are abnormal and generally show a fiber-type transition from slow to fast (Series et al. 1995, 1996). Similar findings have been reported for the English bulldog, which is an animal model of sleep apnea (Petrof et al. 1994). This effect has been ascribed to the greater loads and intensity of activation encountered by these muscles, especially since the limb muscles in the English bulldog (which would not experience these changes) were found to be normal (Petrof et al. 1994). However, since there is evidence that chronic continuous hypoxia causes transition from slow to fast fiber-type, and decreases aerobic capacity and increases fatigue, we hypothesized that the slow-to-fast transition in sleep apnea was caused by chronic intermittent hypoxia and that this would be reflected by changes in contractile function. We used a rat model of sleep apnea that we have developed over the past 10 years in which animals breathe rapidly-changing normoxic and hypoxic/asphyxic gas mixtures to induce oscillations in blood gases similar to those occurring in human sleep apnea. This model demonstrates plasticity in a number of systems, developing a variety of pathophysiological changes similar to human sleep apnea such as increased hematocrit (McGuire and Bradford 1999, 2001), systemic and pulmonary arterial pressure (McGuire and Bradford 2001), blood coaguability (Dunleavy et al. 2005a) and arrhythmogenesis (Dunleavy et al. 2005b) as well as altered central respiratory control (O'Halloran et al. 2002). We found that chronic intermittent hypoxia and asphyxia caused only minor changes in the structure of respiratory muscles and limb muscles but, in general, increased fatigability in muscles of the diaphragm, upper airway and limbs (McGuire et al. 2002a, 2002b, 2003). Clanton et al. (2001), on the other hand, found no effect of chronic intermittent hypoxia on diaphragm fatigability or fiber-type although tolerance to anoxia was improved. More recently, broadly similar results to ours were obtained by Pae et al. (2005) who found that intermittent hypoxia increased muscle fatigability in the upper airway and caused a slow-to-fast fiber-type transition in upper airway muscles but not in the diaphragm.

In conclusion, increased fatigability may be a feature of both chronic continuous and intermittent hypoxia and may exacerbate conditions such as sleep apnea and respiratory disease in general. Plasticity of respiratory muscles following chronic continuous and chronic intermittent hypoxia may be due to the generation of reactive oxygen species. Recent preliminary data from our group indicate that antioxidant treatment can completely block the effects of chronic hypoxia on muscle fatigue. Thus, oxidant stress, which has been implicated in sleep apnea and chronic obstructive pulmonary disease, may contribute to dysfunction of respiratory muscles following chronic hypoxia.

Erythropoietin in the neural control of hypoxic ventilation (Soliz, Gassmann)

When mammals face hypoxic or hypoxemic conditions, pulmonary ventilation and arterial oxygen content are elevated by two complementary systems, (1) the neural respiratory response (elaborated through the central and peripheral nervous system) that leads to increased minute ventilation thereby increasing tissue oxygenation, and (2) renal-derived erythropoietin (Epo) that stimulates maturation of erythroid progenitor cells (erythropoiesis) and elevates, in turn, the blood's capacity for

oxygen transport. Despite the fact that both regulatory mechanisms are responsible for increasing oxygen availability, no interaction between these systems has been described so far. Recently, we reported that these systems are tightly interconnected (Soliz et al. 2005).

Since classically it is recognized that Epo is mainly produced by fetal hepatocytes and by interstitial fibroblasts in the adult kidney, in recent years we, and others, have demonstrated that Epo and its receptor (EpoR) are functionally expressed in a variety of tissues, including glial cells and neurons of mice, monkeys, and humans, thus showing that Epo could have a much broader field of action than was originally recognized (Digicaylioglu et al. 1995; Marti et al., 1996; Bernaudin et al. 1999; Siren and Ehrenreich 2001; Gassmann et al. 2003). In addition, it was found that the amount of brain-derived Epo dramatically increases after hypoxic stimulation (Digicaylioglu et al. 1995; Marti et al. 1996), thereby implying that Epo plays a neuroprotective role. In fact, many subsequent studies proved that Epo exerts a protective function in several stroke models (Gassmann et al. 2003; Ghezzi and Brines 2004; Marti 2004), against light-induced (but not inherited) retinal degeneration (Grimm et al. 2004; Kilic et al. 2005a, 2005b), and against mechanical (Gorio et al. 2002) and ischemic (Celik et al. 2004) injury of the spinal cord. Despite the increasing data on the nonerythropoietic impact of Epo, however, little is known regarding Epo's physiological functions in the brain. This is surprising considering the extent of expression of Epo and its receptor in glial and neuronal cells. In addition, no studies have addressed the potential effects of Epo on the systemic control of oxygen homeostasis.

The mechanism by which Epo exerts the functions described earlier includes several pathways for control of oxygen homeostasis. Epo has been recognized to be involved in the upregulation of oxygen free radical scavenger enzymes, activation of antiapoptotic and anticytotoxic factors and activation of voltage-gated calcium channels (Digicaylioglu and Lipton 2001; Grimm et al. 2002; Gassmann et al. 2003). In view of the fact that most of these processes are directly or indirectly involved in acclimatization to hypoxia and the associated morphological and neurochemical changes in the neural respiratory network, we propose the following: when acutely exposed to hypoxia, the organism's first adaptive response is an increase in ventilation. In this process, Epo-based enhancement of erythropoiesis does not have an immediate but rather a delayed protective one. Considering that Epo and its receptor are present in neural tissue, however, we propose that Epo directly influences the respiratory centers in the brain and/or the carotid bodies by enhancing the hypoxic ventilatory response (HVR). In turn, this would allow fast acclimatization to reduced environmental oxygen. To test this hypothesis, we used a transgenic mouse line (tg21) constitutively overexpressing human Epo in brain only (Wiessner et al. 2001). This transgenic mouse model allows us to test the impact of elevated brain Epo (respiratory center) omitting the influence of elevated plasma Epo that would probably interfere with the carotid body.

Upon exposure to severe hypoxia, male tg21 mice showed a greater ventilatory response to severe acute hypoxia and, moreover, greater ventilatory acclimatization to chronic hypoxic exposure (3 days at 10% O_2; Fig. 3A and B). Furthermore, following bilateral transection of carotid sinus nerves (chemodenervation) that uncouples the brain from the carotid body, tg21 mice adapted their ventilation to acute severe hypoxia, while chemodenervated wild type (WT) animals developed a life-threatening apnea (Fig. 3C). As tg21 mice express human Epo only in brain without increasing their Epo plasma level, these results imply that cerebral Epo modulates ventilation. In support of this, immunohistochemical analysis revealed that Epo receptor (EpoR) is expressed in the main respiratory centers of the brainstem; these include Neurokinin-1receptor (NK-1R) immunopositive neurons in the pre-Bötzinger complex, that are involved in generation of the respiratory pattern, and the *nucleus tractus solitarius* (NTS) that relays input from peripheral chemoreceptors to the central respiratory areas, thereby increasing ventilation upon hypoxic exposure (Fig. 3D).

Carotid bodies are sensory organs whose stimulation by hypoxia activates a chemoreflex pathway (Pardal and Lopez-Barneo 2002). These organs are capable of detecting changes in arterial blood oxygen and they initiate reflexes that are important for maintaining homeostasis during hypoxemia (Prabhakar 2003). The sensory information is relayed to brainstem neurons that modulate compensatory ventilatory adjustments. On account of nonerythroid cells displaying neuronal characteristics and carrying the EpoR—such as PC12 cells—showing increased intracellular Ca^{2+} concentration, dopamine release, tyrosine hydroxylase (TH) activity, and membrane depolarization upon exposure to Epo (Assandri et al. 1999; Koshimura et al. 1999; Yamamoto et al. 2000; Tanaka et al. 2001), we suspected that blood plasma Epo participates in the regulation of ventilation. As PC12 cells mimic carotid body type I cells

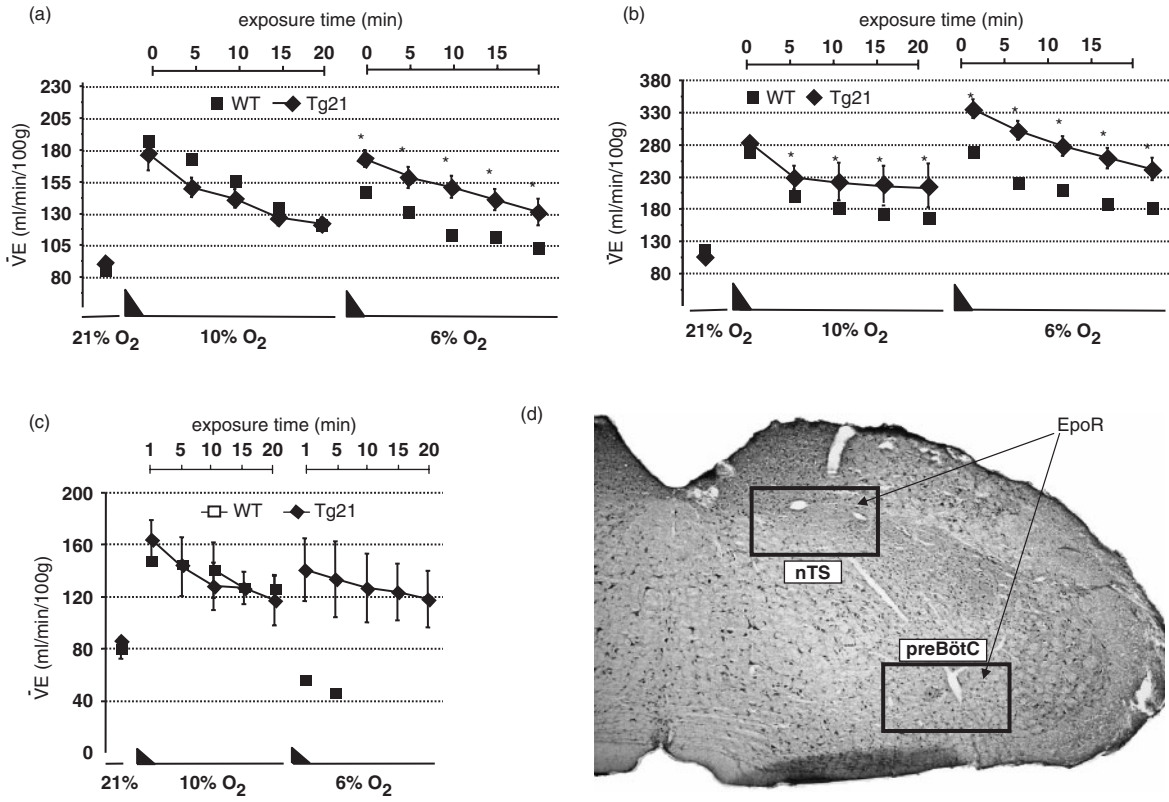

Fig. 3 The Tg21 mouse line overexpressing Epo in brain shows HVR to acute (**A**) and after chronic (**B**) hypoxia. Severe hypoxia (6% O_2) produces pronounced respiratory depression in chemodenervated WT but not in tg21 chemodenervated mice (**C**). The gradual reduction of the fraction of inspired O_2 from normoxia to hypoxia is represented by the black triangles. Epo receptor (EpoR) is present in respiratory areas of the brainstem, including the *nucleus tractus solitarius* (NTS) and the pre-Bötzinger Complex (pre-BötC) (**D**). Figure modified from Soliz et al. (2005).

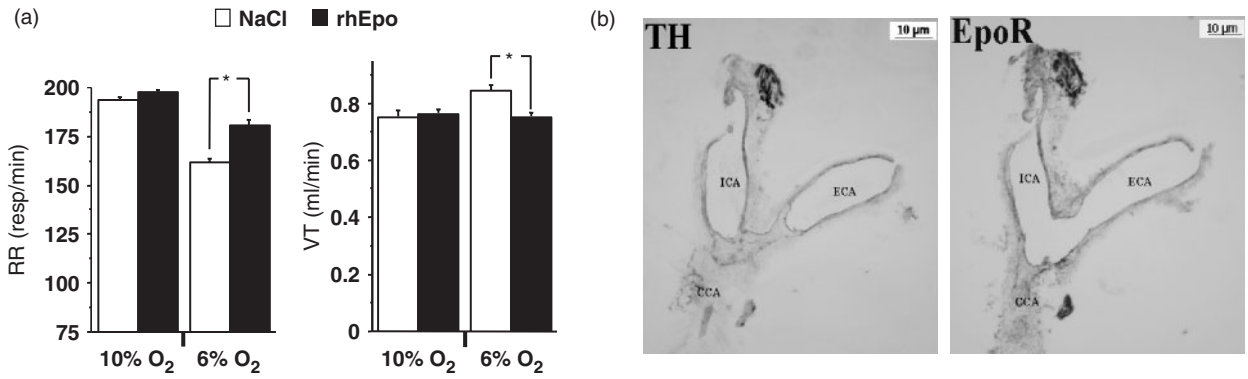

Fig. 4 Intravenous injection of recombinant human Epo (2000 U kg^{-1}) significantly increases the hypoxic respiratory rate in WT mice (**A**) Carotid bodies are immunopositive for both tyrosine hydroxylase (TH) and EpoR (**B**) Abbreviations: ICA, ECA, CCA = internal, external and common carotid artery, respectively. Figure modified from Soliz et al. (2005).

(Tanaka et al. 2001), we hypothesized that these peripheral chemoreceptors were also activated by Epo. To address this question, we measured HVR in WT mice upon injection of 2000 U/kg rhEpo or 0.9% NaCl solution. Epo-injected animals showed higher respiratory frequency but lower tidal volume than did saline injected controls under severe hypoxia (6% O_2; Fig 4A). These data suggest that Epo has an impact on carotid body cells, most probably by binding to the EpoR. Thus, to determine whether EpoR is present in the carotid body, we performed immunostaining in serial lateral sections

from the carotid bifurcation. TH staining was used to identify the glomus cells on one section, and EpoR staining was performed on the subsequent one. We found a dense staining of EpoR in the carotid body, apparently localized within islets of chemosensitive cells (Fig. 4B). These data imply that peripheral chemoreceptors can be activated by plasma Epo upon its binding to EpoR.

In summary, our results suggest that high Epo levels in respiratory neurons of the brainstem increase the ventilatory response to hypoxia. Furthermore, we propose a cross-talk between plasma Epo and the neural respiratory system in which the carotid bodies are activated upon the binding of circulating Epo to its receptor. These data provide evidence that Epo has a crucial role in the fine-tuning of oxygen homeostasis and might be implicated in respiratory disorders, including those occurring at high altitude.

Plasticity in the central nervous system (CNS) during acclimatization to hypoxia (Powell, Reid, Kim, Wilkinson, Fu)

Ventilatory acclimatization to hypoxia (VAH) represents an important form of plasticity when animals are faced with chronic decreases in oxygen supply, for example during acclimatization to HA. There are two components to VAH and both serve to increase ventilation and arterial partial pressure of O_2 (P_{O_2}), thereby reducing the impact of environmental hypoxia on the organism. First, there is an increase in the ventilatory sensitivity to hypoxia seen as an increase in the slope of the acute HVR (Aaron and Powell 1993). This involves time-dependent increases in both (1) O_2-sensitivity of carotid body chemoreceptors (Smith et al. 2001) and (2) the gain of the HVR (i.e., the magnitude of ventilatory motor output for a given afferent input from arterial chemoreceptors) (Dwinell and Powell 1999). Second, there is a time-dependent increase in ventilation and this is associated with a change in arterial P_{CO_2} (Pa_{CO_2}) regulation (Weil, 1986). Pa_{CO_2} decreases with acute HVR, but it decreases further with chronic hypoxia. When normoxia is restored, the decrease in carotid body stimulation results in a decrease in ventilation and an increase in Pa_{CO_2}, but Pa_{CO_2} remains less than the control value and ventilation is greater than in controls.

The mechanisms of plasticity that increase O_2-senstivity at the carotid bodies are still under study but they involve ion channels and neurotransmitters or modulators (Powell 2007). The mechanisms of plasticity causing changes in the CNS gain of the HVR or increased ventilatory drive/altered Pa_{CO_2} regulation are unknown (Powell et al. 2000). We hypothesize that plasticity within a single respiratory nucleus in the CNS explains both of these aspects of VAH. The NTS is the site of the primary synapse from the chemoreceptors of the carotid body (Housley et al. 1987) and it is also a site of central CO_2-sensitivity. While central CO_2-sensitivity was initially localized to the ventral surface of the medulla, we now know that there are multiple CO_2-sensitive sites in the brainstem, including the NTS (Coates et al. 1993). The significance of multiple chemosensitive sites is unknown but different sites may serve unique functions under different conditions (Feldman et al. 2003). We hypothesize that chemoreceptors in the NTS respond differently than do central chemoreceptors at other sites following chronic hypoxia. The rationale for this theory is that the NTS receives a tonic increase in sensory input from arterial chemoreceptors during chronic hypoxia that does not necessarily occur in other chemosensitive areas of the brainstem.

We tested the hypothesis that plasticity in glutamate neurotransmission in the NTS explains the enhanced ventilatory motor output for a given afferent input from the arterial chemoreceptors after chronic hypoxia. The rationale for this is that glutamate is the primary excitatory neurotransmitter in the central reflex pathway for the HVR. Others have shown a decrease or elimination of the HVR with microinjection of glutamate receptor blockers, including both NMDA and non-NMDA antagonists, in the NTS of experimental animals (Vardhan et al. 1993; Ohtake et al. 1998). We measured the HVR in awake rats using whole body, barometric pressure plethysmography (Aaron and Powell 1993) with bilateral microinjection (50 nl) of artificial cerebrospinal fluid (aCSF) or a cocktail of MK-801 (2.5 nmole NMDA receptor antagonist) plus DNQX (1 nmole non-NMDA receptor antagonist). We studied both normoxic control rats ($n = 4$) and chronically hypoxic rats that were acclimatized to inspired $P_{O_2} = 70$ Torr (9.3 kPa) in a hypobaric chamber for 7 days ($n = 8$). Figure 5 shows that microinjecting the cocktail of glutamate receptor antagonists in the NTS reversed the effect of chronic hypoxia on ventilation in both normoxia and hypoxia. Hence, the results are consistent with our hypothesis that plasticity in glutamatergic neurotransmission in the NTS is involved in both the increased hypoxic sensitivity and the increased ventilatory drive in normoxia. Further studies are necessary to determine the role of different glutamate receptor types (e.g., NMDA, AMPA) in different aspects of VAH

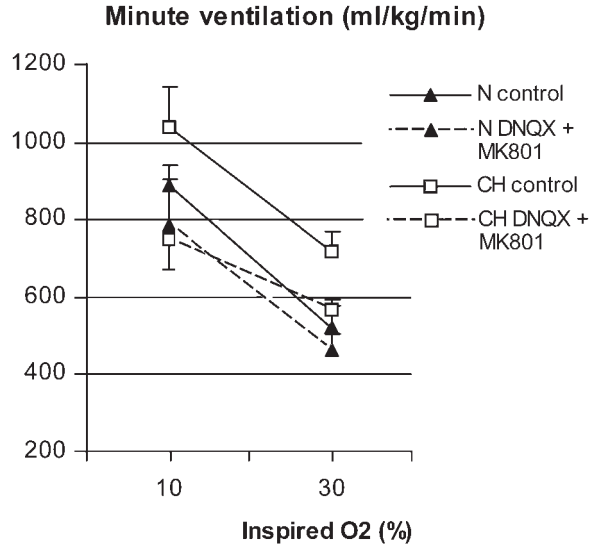

Fig. 5 Effect of microinjecting artificial CSF (control) or drug (MK801 + DNQX) into the NTS on the HVR of normoxic control rats (N) and chronically hypoxic rats (CH). Drug significantly decreases ventilation in normoxia and hypoxia in CH rats but not in N rats, and it effectively reverses ventilatory acclimatization to hypoxia.

and the cellular and molecular mechanisms of plasticity (e.g., receptor upregulation, increased neurotransmitter release).

To test the role of central chemoreceptors in the NTS in VAH, we made substance P-saporin (SP-sap) lesions. With this method, saporin is conjugated to a neurotransmitter that binds to a receptor. When the receptor is internalized during normal recycling, saporin enters the cell also and inactivates the ribosome, resulting in cell death. There is a strong correlation between central chemosensitive sites and the NK1R that binds SP, although the precise role of SP in central chemoreception is not known. SP-sap microinjections in the retrotrapezoid nucleus (RTN), which is an important site of central CO_2-sensitivity, have been shown to decrease the normal hypercapnic ventilatory response (Nattie and Li 2002). The remaining ventilatory response to CO_2 apparently results from other central chemoreceptors and arterial chemoreceptors. In preliminary studies, we made bilateral microinjections of SP-sap (200 nl) in the caudal NTS and measured the ventilatory response to CO_2 in awake rats. Compared to control rats that had saporin only microinjected ($n = 9$), the SP-sap rats ($n = 14$) showed no difference in ventilatory response under normoxic conditions. However, after the rats acclimatized to chronic hypoxia ($P_{I_{O_2}} = 70$ Torr for 7 days), the ventilatory response to 7% CO_2 decreased with SP-sap, although this was not statistically significant. Further, experiments are necessary to determine if variation in the extent of the lesions explain variations in the decreased response after chronic hypoxia. We also need to determine if differences in the effect of the lesions with chronic hypoxia are unique to NTS central chemoreceptors, as we hypothesize, or are similar in other central chemosensitive sites such as the RTN.

Our hypothesis that the NTS plays a unique role in VAH suggests that chronic increases in neural activity from carotid body afferent input may induce plasticity. However, decreased O_2 levels might also directly lead to plasticity, for example through changes in gene expression. To test this hypothesis, we are using transgenic mice with the gene for hypoxia inducible factor-1α (HIF-1α) selectively deleted from the CNS using the Cre-loxP strategy. Randall Johnson (University of California, San Diego, CA, USA) provided mice in which the E2 allele HIF-1α gene was flagged with loxP (Ryan et al. 2000). Ioannis Dragatsis (University of Tennessee) provided mice in which Cre-recombinase expression was linked to the promoter for a calcium/calmodulin-dependent protein kinase (CaMKIIα) (Dragatisis and Zeitlin 2000). CaMKIIα is only expressed in the CNS so when these two mice are bred, the double transgenic mouse has Cre-recombinase expression only in the CNS, which removes the genetic material for HIF-1α between the loxP sites. Previous studies had shown that HIF-1α in the carotid bodies is important for VAH (Kline et al. 2002), so it was essential to isolate our gene deletion to the brain in order to test the role of HIF-1α in the CNS for VAH. We compared the HVR measured with barometric pressure plethysmography (Szewczak and Powell 2003) in the single transgenic loxP mouse ($n = 10$), which has a normal HIF-1α response to hypoxia, to that in the double transgenic mouse ($n = 10$). In normoxic control mice, the HVR was the same. After acclimatization to hypoxia (inspired $P_{O_2} = 70$ Torr for 7 days), however, the HVR increased significantly less in the CNS-HIF-1α knockout mouse. Hence, hypoxia *per se* appears to be important for VAH, although these results do not rule out possible effects from increased neural activity also. Further studies are necessary to test for neural effects and to localize the effects of HIF-1α, which we hypothesized to be in the NTS.

In conclusion, results to date support our hypothesis that the NTS plays a critical role for plasticity of both the HVR and ventilatory drive with chronic hypoxia. The NTS is unique in receiving both direct afferent input from peripheral chemoreceptors and containing central chemoreceptors.

Plasticity in the NTS that contributes to ventilatory acclimatization to hypoxia involves glutamatergic neurotransmission and may involve changes in CO_2-sensitive chemoreceptors. Further studies are needed to determine if changes in central CO_2-sensitivity are different in the NTS versus other chemosensitive sites. Further study is also necessary to determine the specific sites in the CNS at which HIF-1α contributes to ventilatory acclimatization. Finally, more experiments are needed to determine if the tonic increase of afferent input from the carotid body during chronic hypoxia has any effects on the NTS that are independent of decreased P_{O_2}.

O_2 supply and development of the carotid body (Bavis, Miller, Fergusson)

Birth imposes a dramatic change in O_2 supply in mammals, with arterial P_{O_2} normally rising to between 3-fold and 4-fold above *in utero* levels. Associated with this change in O_2 availability is a well-documented resetting of O_2 sensitivity of the carotid body. Although the carotid body exhibits little response to acute hypoxia at birth, O_2 sensitivity gradually increases during the postnatal period (Carroll and Kim 2005; Donnelly 2005), with postnatal maturation occurring over the first 2 weeks in rats (Kholwadwala and Donnelly 1992; Sterni et al. 1999). This resetting matches the dynamic range of the carotid body to the higher postnatal P_{O_2} levels and contributes to a concomitant increase in the acute HVR of the animal, although CNS maturation is also critical to postnatal changes in the HVR (Bissonnette 2000).

Resetting of the carotid body appears to be initiated by the rising P_{O_2} at birth rather than by developmental age *per se*. Blanco et al. (1988) ventilated the lungs of late-gestation fetal sheep *in utero* to maintain blood gases normoxic or hyperoxic relative to normal fetal arterial P_{O_2}. Sheep receiving hyperoxic ventilation (>24 h) exhibited increased carotid body sensitivity to hypoxia, suggesting premature resetting. Similarly, O_2-initiated resetting may explain the well developed peripheral chemosensitivity exhibited in preterm infants as early as 28 weeks postconception (Rigatto et al. 1975; Carroll and Kim 2005). Just as raising O_2 levels hastens resetting of the carotid body, chronic hypoxia appears to delay this process. For example, kittens raised in 13–15% O_2 from birth exhibit dramatically reduced chemoreceptor function as assessed by a reduced ventilatory response to brief (two-breath) hypoxic challenges (Hanson et al. 1989a) and diminished responses by the carotid sinus nerve (Hanson et al. 1989b). Similar results have been obtained using brief hyperoxic exposures to test peripheral chemosensitivity in young sheep, rats, and humans hypoxic from birth (Hertzberg et al. 1992; Sladek et al. 1993; Katz-Salamon et al. 1996), and from recordings of the carotid sinus nerve in chronically hypoxic rat pups (Landauer et al. 1995). Delayed maturation of O_2-sensing mechanisms in type I cells of the carotid body contribute to these effects of postnatal hypoxia (Sterni et al. 1999), although additional plasticity at other levels of the chemoafferent pathway cannot be ruled out. While it is widely accepted that perinatal O_2 availability influences the timing of carotid body maturation, to what extent do perinatal O_2 levels affect functioning of the carotid body in the mature animal?

Hypoxia during early life alters the HVR into adulthood; however, the role of the carotid body in this plasticity is unclear. For example, Peyronnet et al. (2000) observed that dopamine content of the carotid body was reduced at 1, 3, and 9 weeks of age in rats exposed to 10% O_2 prenatally (E5–E20), but the functional significance of these changes is difficult to interpret since HVR was either enhanced (3 weeks) or attenuated (9 weeks) depending on the age at which the rats were studied. Chronic hypoxia in the early postnatal period also induces long-lasting changes in the HVR in rats (Okubo and Mortola 1990; Bavis et al. 2004) and sheep (Sladek et al. 1993), and may explain the reduced HVR in humans raised at altitude (Lahiri 1981). Interestingly, O_2 sensitivity of the carotid body normalizes within a few days following neonatal hypoxia in rats (Hertzberg et al. 1992; Sterni et al. 1999), suggesting that the carotid body is not responsible for the observed blunting of HVR. Indeed, responses of the phrenic nerve to hypoxia are also normal following neonatal hypoxia (Bavis et al. 2004). Thus, persistent effects of postnatal hypoxia on the HVR appear to be mediated by factors other than chemosensitivity, perhaps through effects on respiratory mechanics or associated control loops (Bavis 2005).

In contrast to the effects of hypoxia, perinatal hyperoxia has clear effects on the function of the carotid body that persist into adulthood (Ling et al. 1997; Bavis 2005). Rats exposed to 30–60% O_2 for the first 1–4 postnatal weeks exhibit blunted HVR as adults (Ling et al. 1996; Bavis et al. 2003, 2007), and this reflects impaired functional development of the carotid body and its chemoafferent neurons (Ling et al. 1997; Bavis 2005). Following perinatal hyperoxia, carotid bodies are 40–75% smaller and there

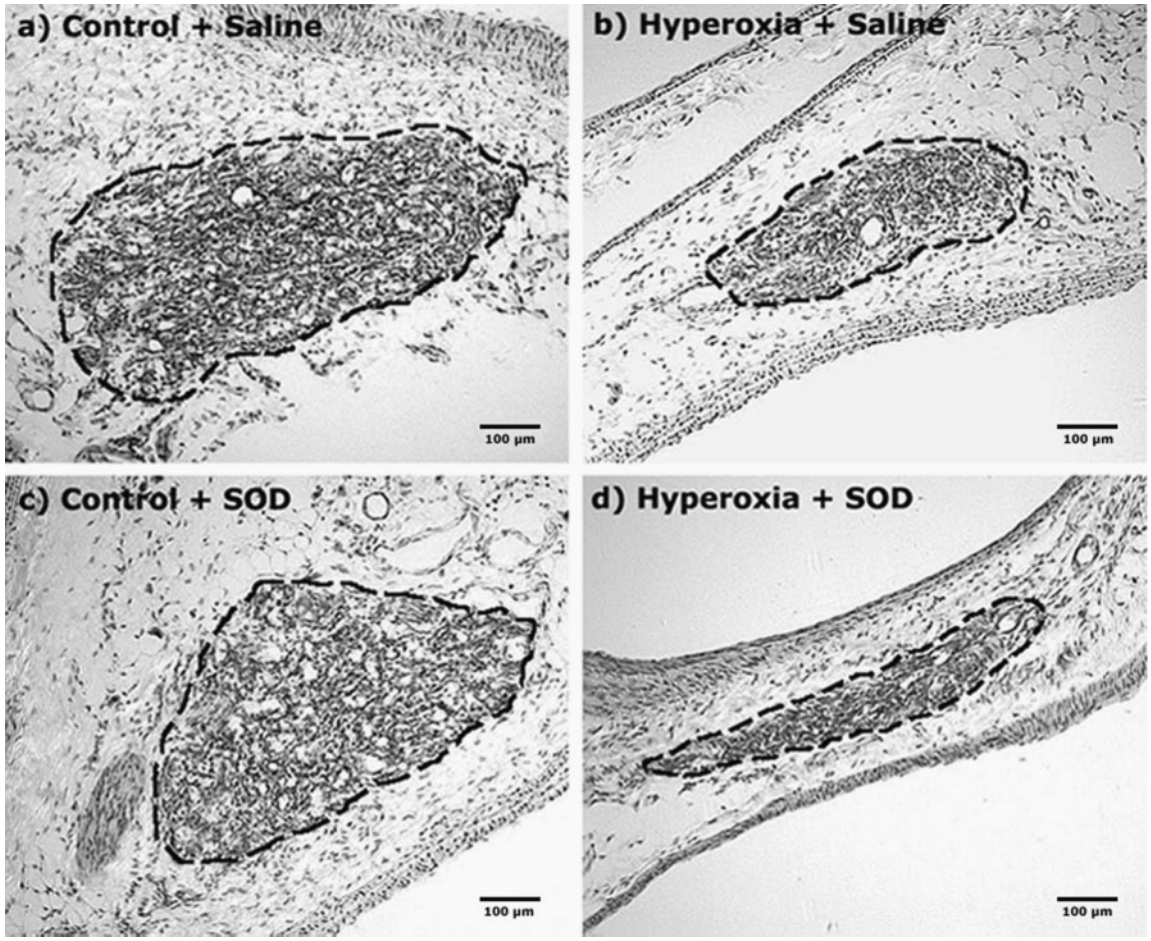

Fig. 6 Representative sections through the carotid body of adult rats exposed to hyperoxia (60% O_2) for the first two postnatal weeks, with or without daily treatment with a superoxide dismutase (SOD) mimetic, manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride; control animals were raised under normoxic conditions. Estimated volumes of the carotid body ($\times 10^7\ \mu m^3$) were: Control + Saline, 4.5 ± 0.4, Control + SOD mimetic, 4.3 ± 0.7; Hyperoxia + Saline, 1.5 ± 0.1; Hyperoxia + SOD, 1.7 ± 0.1. Values are mean ± SEM and $n = 7$ for each group. Antioxidant treatment had no effect on volume of the carotid body (SOD and SOD × hyperoxia interaction, both $P > 0.05$; two-way ANOVA).

are reductions in the numbers of unmyleninated neurons in the carotid sinus nerve and in the numbers of tyrosine hydroxylase-positive cells in the petrosal ganglion (Erickson et al. 1998; Fuller et al. 2002; Bisgard et al. 2003; Prieto-Lloret et al. 2004). As a result of these morphological changes, and potentially changes in O_2-sensitivity of the surviving cells (Kim et al. 2003; Donnelly et al. 2005), responses of the carotid body to hypoxia are markedly reduced in the adult animal (Ling et al. 1997; Fuller et al. 2002; Bisgard et al. 2003; Prieto-Lloret et al. 2004; Donnelly et al. 2005).

We recently asked whether this chronic effect was mediated by overproduction of reactive oxygen species (ROS) during the hyperoxic exposure. Rat pups were given daily injections of a powerful antioxidant [manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride; 5–10 mg/kg, i.p.] throughout a 2-week exposure to 60% O_2. In adults, volume of the carotid body was estimated for these rats based on the area of serial sections (12 µm thickness) through the organ as described elsewhere (Bisgard et al. 2003). Volumes of the carotid body were significantly reduced in rats exposed to perinatal hyperoxia regardless of antioxidant treatment (Fig. 6); similarly, HVR was not improved by the antioxidant treatment (Bavis et al., unpublished data). These data do not support a causal role for ROS-mediated toxicity in this plasticity. Given that activity of the carotid body decreases as P_{O_2} increases, an alternative explanation is that prolonged hyperoxia inhibits normal, activity-dependent development in the carotid body. Consistent with this hypothesis, increased CO_2 levels during

hyperoxic exposure reduces subsequent impairment of the adult HVR, possibly by enhancing carotid body activity during the critical developmental period (Bavis et al. 2007).

In conclusion, current evidence suggests that changing availability of O_2 is an important regulator of normal development of the carotid body. However, abnormal O_2 levels during the perinatal period may have long-lasting, and potentially harmful, effects on carotid body function. Interestingly, the effects of hypoxia and hyperoxia are not simply opposite one another, suggesting that O_2 influences development of the carotid body through multiple pathways.

Effect of "living high–training low" on sea level performance (Peltonen, Tikkanen, Rusko)

It is well established that adequate acclimatization and physical training at altitude (living high–training high, LHTH) improves performance *at altitude* (Fulco et al. 2000). It has also been hypothesized that hypoxia may increase training stimulus, magnify the effects of endurance training, and lead to improved performance at sea level. However, the scientific literature on LHTH for performance at sea level is equivocal and it is questionable whether living and training at altitude also improves performance at sea level, more than training at sea level itself. The reasons for not having a positive effect of LHTH include: (1) the acclimatization effect may have been insufficient to stimulate an increase in red cell mass/hemoglobin mass; (2) the training intensity at altitude may have been compromised; and (3) enhanced stress with possible symptoms of overtraining and an increased frequency of infections (Rusko et al. 2004). Thus, reductions in power output while exercising at altitude as well as various physiological mechanisms may lead to relative deconditioning and offset potential beneficial changes resulting from altitude acclimatization. To avoid potentially harmful effects of both living high and training high on performance at sea level a solution of interrupting hypoxic exposure by living at altitude but training at lower altitude/sea level (living high–training low, LHTL) may allow both the potentially advantageous changes associated with acclimatization to develop and the opportunity to train without reducing power output. Recently, the suggested mechanisms responsible for expected improvement in endurance capacity with LHTL have been a subject of vivid debate (Gore and Hopkins 2005; Levine and Stray-Gundersen 2005). LHTL has been shown to be erythropoietic even in elite athletes and lead to increase in erythrocyte volume/red cell mass/hemoglobin mass, maximal oxygen uptake, and performance in endurance sport (Levine and Stray-Gundersen 1997; Rusko et al. 1999; Levine and Stray-Gundersen 2005; Wehrlin et al. 2006). Increased erythrocyte volume/red cell mass/hemoglobin mass, however, has not been found in other studies after LHTL (Ashenden et al. 1999; Hahn et al. 2001). Instead, improvements in efficiency, muscle buffer capacity, and ventilatory chemosensitivity (Gore et al. 2001; Townsend et al. 2002; Gore and Hopkins 2005) have been suggested as explanations for potential beneficial mechanisms of LHTL. A plausible explanation for this discrepancy is that the minimum dose to attain a hematological acclimatization effect is >12 h a day for at least 3 weeks at an altitude or a simulated altitude of 2100–2500 m (Rusko et al. 2004). Furthermore, iron deficiency and infection may blunt hematological responses (Levine and Stray-Gundersen 2005) and the benefits of LHTL may be associated with the athlete's genotype for angiotensin converting enzyme (Patel et al. 2003; Hinckson and Hopkins 2005).

In conclusion, presently there is no general agreement on potential beneficial mechanisms of LHTL on performance at sea level as both hematological and nonhematological explanations have been offered. To the best of our knowledge, however, LHTL has never been shown to be detrimental for endurance performance in comparison with living and training at sea level.

Discussion

The preceding sections highlight only a few of the many examples of respiratory plasticity following changes in O_2 supply and demand that have been described in the literature. This brief review illustrates, however, that plasticity occurs throughout the respiratory system. In response to chronic environmental hypoxia, for example, adult animals can respond by increasing blood O_2 carrying capacity (hemoglobin concentration, O_2 affinity) and HVR (carotid body and CNS), as well as by changes in the function of respiratory and nonrespiratory muscles; additional plasticity at the gas exchanger may occur during development. The occurrence of plasticity in multiple tissues emphasizes the need for integrative approaches when studying animals' responses to chronic changes in the availability of O_2. In many cases, plasticity at multiple sites will work together in maintaining O_2 homeostasis, as exemplified

by additive effects at the carotid body and NTS in ventilatory acclimatization. However, plastic responses may have different time domains or different threshold stimuli, making it difficult to predict the extent of these interactions. As discussed earlier, this complexity may contribute to conflicting reports on the benefits and underlying mechanisms of LHTL strategies for improving exercise performance.

Moreover, not all respiratory plasticity is adaptive. For example, it is difficult to assign positive benefit to increased fatigability in muscle following chronic sustained or chronic intermittent hypoxia, or to attenuated carotid chemoreflexes following perinatal hyperoxia. In some instances, chronic hypoxia or hyperoxia may harm aerobic tissues directly (e.g., cellular mortality), thereby impairing respiratory function; plasticity may be unavoidable in these cases. In other instances, paradoxical responses may occur when normal mechanisms of plasticity are induced by respiratory conditions not anticipated by evolutionary history, such as those imposed by disease, environmental toxins, or even medical interventions (e.g., O_2 therapy)—having the capacity for adaptive plasticity may increase vulnerability to novel environmental stimuli. Thus, whole animal performance could reflect the simultaneous expression of adaptive, maladaptive, and functionally neutral plastic responses, and teasing apart these effects is an important direction for research. Similarly, little is known about trade-offs that potentially erode the benefits of, or constrain the expression of, respiratory plasticity. Examples may include energetic costs of maintaining larger gas exchangers or increased activity of respiratory muscles, acid–base disturbances associated with increased ventilation, or increased viscosity of hemoglobin-rich blood. Understanding these trade-offs may provide insight into the evolution of respiratory plasticity versus alternative, genetically fixed traits. In fact, the evolution of respiratory plasticity is an important question that is ripe for more study.

One factor that contributes to the characteristic time-lag between the onset of a stimulus and the expression of plasticity is the time required for the synthesis of new proteins through transcriptional and/or translational regulation. Within muscles and neural control systems, gene expression can be mediated by activity-dependent changes in intracellular Ca^{2+} (Moody and Bosma 2005). However, much of the recent interest in the molecular basis of respiratory plasticity has focused on O_2-mediated and ROS-mediated activity of transcription factors such as HIF, activator protein-1 (AP-1), and nuclear factor-κB (NF-κB) (Michiels et al. 2002; Prabhakar and Jacono 2005; Chandel and Budinger 2007). HIF transcriptionally regulates the expression of hundreds of proteins, so it is not surprising that this molecule is directly implicated in several of the responses to chronic hypoxia discussed here, including hemoglobin expression in *Daphnia* and erythropoiesis and NTS components of ventilatory acclimatization in mammals. Additional links are likely given the newly appreciated role of Epo, itself regulated by HIF, in peripheral chemoreceptors and respiratory control regions of the CNS. These observations reveal common molecular mechanisms underlying diverse forms of respiratory plasticity. While the examples discussed in this article have been heavily biased toward mammals, it is important to recognize that respiratory plasticity is equally prevalent in invertebrates and nonmammailian vertebrates and may share highly conserved molecular pathways. Indeed, invertebrate models have been critical to understanding the role of O_2-sensitive transcription factors and the functional significance of the genes they regulate (Gorr et al. 2006).

Another theme that emerges from the brief reports given in the preceding sections is that age influences the expression of respiratory plasticity, with young animals often exhibiting either unique or longer-lasting plasticity than their mature counterparts. This adds essentially a third dimension to the organism's ability to adapt, which includes genetic adaptation, physiological acclimatization, and development, and all of these may interact. Developmental plasticity (Carroll 2003) is exemplified by age-dependent effects of altitude on lung growth and diffusing capacity as well as the effects of both neonatal hypoxia and hyperoxia on development of the HVR; in each case, equivalent exposures in adults do not elicit similar plasticity (Johnson et al. 1985; Okubo and Mortola 1990; Ling et al. 1996). The events defining the critical developmental periods during which animals are susceptible to this plasticity are poorly understood and remain an exciting area of research. Oxygen levels directly, or indirectly through ROS production, regulate activity of critical transcription factors, as discussed earlier, and influence cellular metabolism, senescence, and death (Chandel and Budinger 2007), and any of these mechanisms could potentially alter developmental trajectories. Interestingly, plasticity in the functional morphology of both the lung and carotid body may be linked to oxygen's influence on tissue growth during development—hypoxia accelerates lung growth

(see "Growth, remodeling, and function of the mammalian lung following high altitude residence" Section earlier), whereas hyperoxia inhibits cellular proliferation in the carotid body (Wang and Bisgard 2005). If there is a limited window for somatic growth, altered growth rates may profoundly influence the amount of functional tissue produced, and these structural changes may not be reversible once somatic growth has ceased.

Both the similarities and the differences among models of respiratory plasticity, as illustrated in this review, are instructive. Studying the diverse ways in which animals respond to respiratory challenges and being mindful of complementary, or even counterproductive, responses at various levels of the respiratory system will enable a better understanding of integrative response to chronic changes in O_2 supply and demand.

Acknowledgments

The authors would like to thank Dr Steven F. Perry and the rest of the organizing committee for making the *First International Congress of Respiratory Biology* a great success. The work described in this review was made possible by support from many funding agencies. R.W.B., E.K.F., and B.M.M. were supported by National Institutes of Health grant P20 RR-016463 from the INBRE Program of the National Center for Research Resources. A.B. was supported by the Health Research Board (Ireland), by a Marie Curie Fellowship of the European Community Programme IHP (contract number HPMD-CT-2000-00041) and by the Research Committee of the Royal College of Surgeons in Ireland. C.C.W.H. was supported by National Heart, Lung and Blood Institute grants RO1 HL-40070, HL-54060, HL-45716, and HL-62873. J.E.P. was supported by the Ministry of Education (Finland). F.L.P. was supported by National Heart, Lung and Blood Institute grant R01 HL-081823. J.S. and M.G. were supported by grants from RoFAR and the Swiss National Science Foundation.

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