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The influence of chronic hypoxia upon chemoreception

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Abstract

Carotid body chemoreceptors are essential for time-dependent changes in ventilatory control during chronic hypoxia. Early theories of ventilatory acclimatization to hypoxia focused on time-dependent changes in known ventilatory stimuli, such as small changes in arterial pH that may play a significant role in some species. However, plasticity in the cellular and molecular mechanisms of carotid body chemoreception play a major role in ventilatory acclimatization to hypoxia in all species studied. Chronic hypoxia causes changes in (a) ion channels (potassium, sodium, calcium) to increase glomus cell excitability, and (b) neurotransmitters (dopamine, acetylcholine, ATP) and neuromodulators (endothelin-1) to increase carotid body afferent activity for a given P_{O_2} and optimize O_2-sensitivity. O_2-sensing heme-containing molecules in the carotid body have not been studied in chronic hypoxia. Plasticity in medullary respiratory centers processing carotid body afferent input also contributes to ventilatory acclimatization to hypoxia. It is not known if the same mechanisms occur in patients with chronic hypoxemia from lung disease or high altitude natives.

Keywords

carotid body; hypoxic ventilatory response; neural plasticity; ventilatory acclimatization

1. Introduction

Exposure to environmental hypoxia elicits chemoreflexes that increase ventilation and tend to reduce the effects of decreased inspired P_{O_2} on arterial P_{O_2}. With chronic hypoxia (hours to months), the increase in ventilation is greater than that found during acute exposure (minutes) to the same degree of hypoxia and this further minimizes the impact of the environmental challenge by raising arterial P_{O_2} (Weil, 1986). This time-dependent increase in ventilation with chronic hypoxia is known as ventilatory acclimatization to hypoxia (VAH) and involves plasticity in the chemoreflexes that control ventilation (Powell et al., 1998).

Most of what we know about the neurobiological mechanisms of the acute ventilatory response to hypoxia is based on studies of the carotid body and the same is true for plasticity in ventilatory chemoreflexes with chronic hypoxia. Using a variety of experimental preparations, from conscious large animals to *in vitro* co-cultures of chemoreceptor cells and afferent neurons, several laboratories have demonstrated physiologically significant increases in O_2-sensitivity of carotid bodies with chronic hypoxia (described below). Chronic hypoxia also induces

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plasticity in central respiratory centers that process afferent input from carotid bodies (Dwinell and Powell, 1999) and alters other aspects of the neural control of breathing, such as control of arterial P_{CO_2} (Weil, 1986;Smith et al., 2001). However, this paper focuses on plasticity in the carotid bodies with chronic sustained hypoxia and their effects on breathing. It also considers how important carotid body plasticity is relative to other mechanisms of VAH. The effects of chronic intermittent hypoxia on carotid bodies (Prabhakar, 2001) are not covered here.

2. Carotid body chemoreceptors

Several excellent reviews are available on carotid body structure and function (Fidone and Gonzalez, 1986;Gonzalez et al., 1994;Gonzalez et al., 1995). The carotid body is a complex sensory organ that is richly perfused by arterial blood and innervated by a branch of the glossopharyngeal nerve (i.e. carotid sinus nerve), which makes it ideally suited for monitoring arterial blood gases and pH. It also receives sympathetic innervation which may control blood flow and other functions. Carotid bodies are composed of two main cell types called glomus cells and sustentacular cells. Glomus cells, also known as Type I cells, are of neural crest origin and are generally believed to be the primary chemoreceptors. Sustentacular cells, or Type II cells, are similar to glial cells but their function in the carotid body is not known. In response to changes in arterial P_{O_2}, P_{CO_2} and pH, glomus cells presumably release an excitatory neurotransmitter to depolarize carotid sinus nerve afferent terminals. This sends action potentials to respiratory centers in the CNS and stimulates ventilation. Hence, the first question to answer for understanding plasticity of the carotid body ventilatory reflex during chronic hypoxia is how the physiological stimuli for arterial chemoreceptors change under such conditions.

2.1 Chemoreceptor stimuli in chronic hypoxia

Early theories and experiments for ventilatory acclimatization to hypoxia focused on potential changes in the known stimuli for chemoreceptors and assumed sensory transduction was unchanged. For example, one of the original theories for ventilatory acclimatization to hypoxia focused on changes in the pH of cerebrospinal fluid (CSF pH) as a stimulus to central CO_2-sensitive chemoreceptors. While the change in CO_2 regulation in chronic hypoxia remains unexplained, it is now generally accepted that time-dependent changes in CSF pH do not explain ventilatory acclimatization (Forster and Dempsey, 1981). Failure of the CSF pH theories to explain ventilatory acclimatization to hypoxia lead to the idea of neural plasticity, i.e. central nervous system (CNS) processing of afferent information was enhanced by chronic hypoxia (Forster et al., 1974). Today we know that plasticity occurs in both the arterial chemoreceptors and the CNS, as detailed later in this paper.

In general, carotid body stimulation is predicted to decrease during chronic hypoxia. The time dependent increase in ventilation during acclimatization acts to decrease P_{CO_2} further and raise P_{O_2} back towards control levels (Rahn and Otis, 1949). On average, arterial pH does not change with chronic hypoxia relative to the value measured during acute hypoxia (Dempsey and Forster, 1982). In other words, metabolic compensation is complete for the time-dependent decrease in Pa_{CO_2} with increased ventilation during acclimatization to hypoxia. Therefore in general, none of the arterial stimuli for carotid bodies are increasing so they cannot explain time-dependent increases in ventilation. However metabolic compensation is more effective in some experimental animals, for example decreasing pHa by 0.05 units relative to the acute hypoxic value in chronically hypoxic rats (Olson, Jr. and Dempsey, 1978) and cats (Barnard et al., 1987). The consequences of this are considered at the end of the next section.

2.2. Afferent activity from carotid bodies during chronic hypoxia

Chronic hypoxia has been shown to increase O_2-sensitivity in the carotid body and increase the frequency of action potentials in the carotid sinus nerve for a given P_{O_2} in several different experimental preparations. Continuous recording of the carotid sinus nerve in goats for 5 hrs of hypoxia show steady increases in the frequency of action potentials (Nielsen et al., 1988) while this does not occur with 4 hrs exposure to hypercapnia. Single unit recordings from cats exposed to 28 days of hypoxia (Barnard et al., 1987), as well as whole carotid sinus nerve recordings from cats exposed to 48 hours of hypoxia (Vizek et al., 1987), also show increased O_2-sensitivity. *In vitro* carotid body preparations from rats acclimatized to hypoxia from 3 to 16 days show increased O_2-sensitivity too (Chen et al., 2002b). All of these studies find about a 100% increase in the frequency of action potentials in acute hypoxia following chronic hypoxia.

Chronic hypoxia does not affect carotid body sensitivity to pH (Barnard et al., 1987) but in some animals there may be a small increase in pH stimulation of carotid bodies (see above). Data from normoxic cats can be used to predict that a 0.05 drop in pH (~5 nmol/L) would increase action potential frequency in the carotid sinus nerve by only 10% (Lahiri and DeLaney, 1975). However, the same study shows that such a change in pHa and carotid body activity could almost double ventilation, which is as great as the effect of decreasing Pa_{O_2} from 95 to 40 Torr (Fig. 9 in (Lahiri and DeLaney, 1975). Hence the role of a decrease in arterial pH could be considerable in ventilatory acclimatization to hypoxia but experiments to quantify this effect have not been done. In any case, the predicted effects of such small changes in pHa on carotid body afferent activity is small compared to the effect of chronic hypoxia described above.

3. Cellular mechanisms of chemoreception

Functional plasticity involves the molecular mechanisms of chemoreception, ion channels and neurotransmitters in the carotid body (recently reviewed by (Bisgard, 2000) and (Dinger et al., 2003)). There are important histological changes in carotid bodies during chronic hypoxia, such as glomus cell hypertrophy and decreased covering of glomus cells by sustentacular cells (Kusakabe et al., 1993). The latter change increases the potential area available for gap junction connections between glomus cells, which have been shown to enhance glomus cell sensitivity (Eyzaguirre and Abudara, 1999). However, these morphological changes generally take much longer than the increased O_2-sensitivity of the carotid body described above (days versus hours) so they do not appear necessary for functional plasticity in chemoreception.

3.1 Molecular chemoreceptors

Studies on isolated carotid bodies *in vitro* and isolated glomus cells in culture indicate that the glomus cells contain the primary O_2-sensing mechanism in carotid bodies (Fidone and Gonzalez, 1986;Gonzalez et al., 1994). The metabolic hypothesis of chemoreception proposes specialized forms of heme-containing molecules, which are generally involved in oxygen metabolism, are the primary sensors of hypoxia (Prabhakar, 2006). An early version of the metabolic hypotheses was based on a low-O_2 affinity cytochrome a3 found in carotid bodies and presumably in the mitochondria (Mills and Jöbsis, 1972). More recent experiments show mitochondria in carotid bodies depolarize at O_2 tensions much higher than those causing changes in other cell types, although this may represent modulation by factors such as nitric oxide (NO) instead of unique heme molecules (Donnelly and Carroll, 2005). Experimental evidence also exists for NADPH oxidase and heme oxygenase 2 (HO-2) being involved in O_2-sensing (Prabhakar and Jacono, 2005).

Several links between mitochondrial function and glomus cell depolarization and excitatory neurotransmitter release have been proposed. However, ATP levels, as indicators of cellular

energy state do not correlate well with glomus cell activation (Donnelly and Carroll, 2005). Release of mitochondrial calcium stores in carotid bodies was hypothesized to be a key step in O_2-transduction (Duchen and Biscoe, 1992), although this may not be simply increasing intracellular calcium in glomus cells to promote excitatory neurotransmitter release. Donnelly and Carroll (Donnelly and Carroll, 2005) reviewed the literature and conclude that "the calcium buffering function of mitochondria through the mitochondrial potential and the ability of the mitochondrial redox state to modulate other cell processes through generation of reactive oxygen intermediates" may be important for O_2 sensing.

There is relatively little information on the effects of chronic hypoxia on these putative molecular mechanisms of O_2-sensing in the carotid body during chronic hypoxia. However, recent work on the pulmonary circulation has demonstrated a role for NADPH oxidase in the pathogenesis of pulmonary hypertension during chronic hypoxia (Liu et al., 2005). Mice without a subunit protein of the superoxide producing NADPH oxidase ($gp91^{phox}$) have significantly reduced levels of superoxide production and reduced vasoconstrictor responses to endothelin-1 in chronic hypoxia compared to wild type mice. Under normal conditions, $gp91^{phox}$ is not necessary for O_2-sensing by carotid bodies (Roy et al., 2000) but NADPH oxidase may play an important role in plasticity during chronic hypoxia when it produces more superoxide that could enhance the effects of other neuromodulators. As discussed later, endothelin-1 appears to be involved in increased carotid body O_2-sensitivity with chronic hypoxia.

3.2 Ion channels

Glomus cells have ion channels that are both sensitive and not sensitive to hypoxia. However, these ion channels interact, for example through voltage or calcium dependency, to determine glomus cell excitability and the response to acute hypoxia. Chronic hypoxia has been shown to affect both kinds of ion channels with the net result being increased excitability.

O_2-sensitive potassium (K^+) channels in glomus cells include (a) TASK-like background channels, which also are sensitive to pH and determine resting potentials, and (b) voltage gated K^+ channels (Kv) that decrease conductance at very modest levels of hypoxia (P_{O_2} = 80 Torr), which is consistent with chemoreceptor function (Patel and Honore, 2001). Chronic hypoxia is reported to decrease K^+ channel density in glomus cells from chronically hypoxic neonatal rats and this would increase excitability (Hempleman, 1995). Chronic hypoxia is reported to increase expression and current density of Ca^{2+} activated K^+ channels (maxi-K channels) in a HEK293 cell culture system (Hartness et al., 2003). However, it is difficult to extrapolate between organs or model cell systems because regulatory proteins required for O_2-sensitivity and Kv subunits are differentially expressed in different cell types. (Patel and Honore, 2001).

Although sodium (Na^+) channels are expressed at relatively low levels in glomus cells (e.g. 10% of the level found in the squid giant axon), they are important for glomus cell membrane and action potentials (Gonzalez et al., 1994). Na^+ channel density increases in glomus cells cultured in chronic hypoxia, which could contribute to increased excitability of glomus cells after stimulation by hypoxia (Stea et al., 1992). (Hempleman, 1995) found increased Na+ currents in glomus cells from chronically hypoxic neonatal rat pups, which could increase excitability given the decrease he found in K^+ channel density in the same preparation (see above).

(Hempleman, 1996) also found increased calcium channel density in glomus cells from chronically hypoxic neonatal rats. This was primarily the L-type Ca^{2+} channel, which is enhanced by hypoxia more than other types of Ca^{2+} channels found in glomus cells, i.e. P/Q, N and R or resistant types (Prabhakar and Jacono, 2005).

There is also some evidence that the afferent nerve endings of the carotid sinus nerve have O_2-sensitive ion channels. Donnelly recently showed that a persistent Na^+ current (I_{NaP}), which is sensitive to riluzole and tetrodotoxin and present only on afferent nerves, plays a critical role in carotid body O_2-sensitivity (Faustino and Donnelly, 2006). Chemosensitivity in the afferent nerve endings is an attractive hypothesis that might explain experimental results such as the recovery of chemosensitivity in the carotid sinus nerve after removal of the carotid body (Mitchell et al., 1972), and the large variation in effects of a given neurotransmitter or neuromodulators on chemoreception between species (see below). This hypothesis deserves further study because it has been difficult to experimentally test given the challenges in making neural recordings from the fine nerve endings in the carotid body (Hayashida et al., 1980). However, at this time the mechanism is only hypothetical and any role for afferent nerve chemosensitivity in plasticity of carotid body function during chronic hypoxia remains hypothetical.

3.3. Neurochemicals

Dopamine (DA) is one of the most abundant neurotransmitters in the carotid body and it has been studied extensively, yet its function still is not completely understood. DA is synthesized by tyrosine hydroxylase (TH) in glomus cells and it is released from glomus cells by physiological chemoreceptor stimuli (Gonzalez et al., 1994). Despite such biochemical evidence for DA being an excitatory neurotransmitter in carotid bodies, pharmacological and physiological studies indicate that DA is primarily inhibitory (Fidone et al., 1990). D_2 dopamine receptors (D_2-R) are present on both afferent nerve terminals and glomus cells and these are generally inhibitory. The physiological role of DA in the carotid body appears to be primarily an auto-regulatory mechanism that limits glomus cell depolarization during chemoreceptor stimulation.

Given the inhibitory effects of DA in the carotid body, it was hypothesized that decreased DA effects may explain increased O_2-sensitivity in chronically hypoxic carotid bodies (Bisgard, 2000). However, such dis-inhibition would have to overcome effects of increased DA levels in the carotid body with chronic hypoxia, which results from effects of hypoxic inducible transcription factor (HIF-1α) on TH (see below). Experiments on cats exposed to 2 days of chronic hypoxia showed that D_2-R antagonists completely reversed the time-dependent increase in ventilatory O_2-sensitivity that occurred during VAH (Tatsumi et al., 1995). This supported the idea that decreased inhibition by DA at D_2-R explains VAH. However, subsequent studies in rats showed that while decreased inhibition by DA in the carotid body occurred after 2 days of chronic hypoxia, the inhibitory effects of DA were restored after 7 days of chronic hypoxia (Huey et al., 2000). Hence, it appears that the primary function of DA in the carotid, and the physiological significance of changing DA and D_2-R during chronic hypoxia, is to maintain normal O_2-sensitivity in the face of long-term changes of stimulation and base-line activity levels. Re-establishing control levels of sensitivity after exposure to chronic perturbations appears to be a general principle of sensory systems (Turrigiano, 1999). Also, it is consistent with the proposal that a “push-pull” mechanism between excitatory and inhibitory neurotransmitters in carotid bodies may be more important in chronic hypoxia compared to “normal” conditions (Prabhakar, 2006).

Acetylcholine (ACh) has been identified as a classical excitatory neurotransmitter in the carotid body and may play a role in increased O_2-sensitivity with chronic hypoxia (reviewed by (Bisgard, 2000;Kim et al., 2004;Prabhakar and Jacono, 2005). Nicotinic cholinergic receptors on carotid body afferent nerves are increased by chronic hypoxia and the excitatory effect of exogenous ACh on the carotid body is enhanced by chronic hypoxia. However, nicotinic receptor antagonists do not reverse the enhanced response to acute hypoxia after acclimatization suggesting another transmitter that is co-released with ACh may be important.

ATP, which is co-released with ACh from glomus cells, has been shown recently to make an important contribution to increased O_2-sensitivity of carotid bodies after chronic hypoxia (He et al., 2006). Blocking the $P2X_2$ purinoreceptors decreased the neural response to acute hypoxia in carotid bodies from both normoxic control and chronically hypoxic rats. Also, the absolute decrease in neural activity was greater in chronically hypoxic preparations consistent with ATP contributing to enhanced O_2-sensitivity. However, $P2X_2$ blockaded did not completely eliminate the response to hypoxia in either condition indicating that ATP acts in concert with other excitatory mechanisms in the carotid body. This could be an excitatory neurotransmitter, such as ACh discussed above, or perhaps enhanced electrical coupling between glomus cells and afferent nerve terminals considering that chronic hypoxia up-regulated gap junction proteins (connexin-43) in carotid bodies (Chen et al., 2002a).

Endothelin 1 (ET-1) has the strongest evidence for explaining increased O_2-sensitivity in carotid bodies with chronic hypoxia. ET-1 is a vasoactive peptide that increases with chronic hypoxia in O_2-sensitive tissues such as the pulmonary artery (Li et al., 1994) and carotid bodies (Chen et al., 2002b). ET-1 alone does not excite carotid bodies but it does enhance the response to hypoxia and this effect is mediated by ET_A receptors on glomus cells (Chen et al., 2000). Chronic hypoxia increases the expression of ET-1 and ET_A receptors in glomus cells and blocking ET_A receptors reverses the increased hypoxic sensitivity observed in chronic hypoxia, returning it to control levels (Chen et al., 2002b). The effect of ET-1 to enhance hypoxic sensitivity of glomus cells involves increased calcium currents that could result from ion channel modulation by signals from ETA receptors (Chen et al., 2002c).

Other neurotransmitters present in the carotid body and postulated to be important in chemoreception, namely norepinephrine and serotonin, have not been shown to play an important role in acclimatization of the carotid body to date (reviewed by (Bisgard, 2000). Changes in NO in the carotid body during chronic hypoxia are predicted to inhibit O_2-sensitivity (reviewed by (Bisgard, 2000). However, NO might be a modulator that optimizes the ability of the carotid body to respond to changes in O_2 level at different chronic levels of O_2, similar to DA discussed above.

3.4 Hypoxic sensitive gene expression

Many of the cellular changes in carotid body chemoreception during chronic hypoxia result from changes in gene expression. A myriad of transcription factors are sensitive to oxygen (Cummins and Taylor, 2005) but only a few have been studied in the carotid body (e.g. CREB, AP-1, p53). Hypoxic inducible factor 1a (HIF-1α) has been studied the most in the carotid body (Kline et al., 2002;Wilson et al., 2005). HIF-1α is increased by chronic hypoxia in brainstem nuclei important for the ventilatory control (Soulage et al., 2004;Soliz et al., 2005) suggesting it may play a role in CNS, as well as carotid body, plasticity in chronic hypoxia. It is important to note that plasticity in carotid bodies or the CNS need not necessarily result from hypoxic sensitive gene expression because the tonic changes in activity levels with chronic hypoxic stimulation could also induce gene expression.

4. Plasticity in the carotid body ventilatory reflex

Hypoxic stimulation of carotid body chemoreceptors leads to a powerful reflex increase in ventilation known as the hypoxic ventilatory response (HVR). Given the enhanced O_2-sensitivity in carotid chemoreceptors during chronic hypoxia described above, it is not surprising that hours to months cause changes in the control of breathing (Weil, 1986;Smith et al., 2001).

4.1 Chronic hypoxia increases the HVR

The increased HVR observed in hours to months of chronic hypoxia is opposite the decreased, or blunted, HVR seen in high altitude natives (see below). There has been some disagreement about the effects of chronic hypoxia on the HVR in both human and animal studies but it appears this can be explained by changes in CO_2 levels during the acute hypoxic challenges used to measure the HVR. If the HVR is tested in isocapnia, so Pa_{CO_2} not allowed to fall with hyperventilation during acute hypoxia, then the HVR is increased by chronic hypoxia (Powell et al., 2000a). The quantitative roles of different mechanisms in explaining the increased HVR in chronic hypoxia remain to be established, however. There may be some effects from small changes in arterial pH (see above) but the major determinants appear to be plasticity in carotid bodies and the CNS.

4.2 Carotid body denervation experiments

Carotid body denervation experiments have established a definitive role for arterial chemoreceptors in ventilatory acclimatization to hypoxia. Bilateral section of the carotid sinus nerve or excision of the carotid bodies causes hypoventilation and increased Pa_{CO_2} normoxia, and eliminates or significantly reduces the acute ventilatory response to hypoxia or carotid body stimuli, e.g. sodium cyanide (Forster, 2003). Carotid body denervation in cats, dogs, goats, ponies, rabbits, rats and sheep eliminates the time-dependent increases in ventilation normally observed in chronic hypoxia (Bouverot et al., 1973;Vizek et al., 1987;Olson, Jr. et al., 1988;Smith et al., 2001).

It is also relevant that ventilatory acclimatization to hypoxia does not occur in animals that exhibit some recovery of their acute HVR long after carotid body denervation (Forster, 2003). Plasticity in the HVR following carotid body denervation has been observed in several species but it is not due to reinnervation. Hypothesized mechanisms include upregulation of alternate sensory pathways (e.g. serotonin-mediated aortic body chemoreception) or upregulation of the efferent limb of a reflex (e.g. serotonin-mediated facilitation of phrenic motor neurons). However, even after the acute HVR and normoxic Pa_{CO_2} levels have returned towards normal in dogs, goats, ponies, and rabbits with denervated carotid bodies, acclimatization to chronic hypoxia remains attenuated or even eliminated. This is not surprising considering the plasticity in O_2-chemoreception in carotid bodies described above. However, it cannot be used to quantify the role of peripheral (carotid body) versus central (CNS) plasticity in normal acclimatization to hypoxia (Dempsey and Forster, 1982). For example, CNS plasticity may not occur in carotid body denervated animals because the tonic increase in afferent input in chronic hypoxia would not occur.

4.3 Plasticity in carotid body

Increased O_2-sensitivity of the carotid body with chronic hypoxia is a major determinant of the increased HVR with chronic hypoxia. This was demonstrated by Bisgard and his colleagues using goats with the carotid bodies isolated from the systemic circulation. This preparation allows P_{O_2} or P_{CO_2} in blood perfusing the carotid body to be changed independently from systemic arterial P_{O_2}or P_{CO_2} for up to eight hours. Six hours of isolated carotid body hypoxia caused a progressive increase in ventilation above the acute hypoxic ventilatory response (Busch et al., 1985). Such time-dependent changes in ventilation did not occur with (a) 6 hours of carotid body hypercapnia (Bisgard et al., 1986a), (b) 4 hr of systemic hypocapnia (Bisgard et al., 1986b) or, (c) systemic arterial hypoxia (Weizhen et al., 1992). These results show the response is specific to carotid body hypoxia and not the result of increased carotid body stimulation or activity, or changes in P_{O_2} or pH at central chemoreceptors and respiratory centers in the brain. Plasticity induced by decreased P_{O_2} in the carotid body during the first hours of chronic hypoxia appears sufficient to explain ventilatory acclimatization for up to 6

hrs of hypoxia in goats. However, these results do not preclude other mechanisms of plasticity (e.g. in the CNS) occurring during longer hypoxic exposures, as discussed below.

4.4 Plasticity in the Central Nervous System (CNS)

The idea of chronic hypoxia inducing plasticity in the CNS to explain changes in the arterial chemoreflex actually precedes the idea of plasticity in the carotid body. Experiments in the 1970s were not finding support for the CSF pH theory of ventilatory acclimatization to hypoxia and altered responsiveness of medullary ventilatory control centers was postulated as an alternative explanation (Dempsey and Forster, 1982). Experimental evidence for this alternative included an increased ventilatory response to doxapram, a chemoreceptor stimulant, in subjects acclimatized to high altitude for 2 to 3 weeks (Forster et al., 1974). Assuming the effect of doxapram on carotid bodies is unchanged by chronic hypoxia, an increased ventilatory response to a similar dose of doxapram implies a greater ventilatory motor output for a comparable chemoreceptor afferent input to the CNS. Hence, such enhanced responsiveness of CNS respiratory centers can be described as an increase in the CNS gain of the HVR (Powell et al., 2000b).

More definitive evidence for an increased CNS gain of the HVR with chronic hypoxia was obtained from experiments measuring phrenic nerve activity in response to electrical stimulation of the carotid sinus nerve in anesthetized rats (Dwinell and Powell, 1999). Seven days of chronic hypoxia significantly increased the CNS gain of the HVR and there was a non-significant tendency for it to increase after only two days of hypoxia. In contrast, the physiological indicators of ventilatory acclimatization to hypoxia, i.e. metabolic rate, ventilation and arterial P_{CO_2}, do not change between 2 and 7 days of hypoxia in rats (Powell et al., 2000b). Therefore, experiments demonstrating a specific mechanism of plasticity acting at a specific time point in chronic hypoxia, may not apply with shorter or longer exposures. Hence, the demonstration of increased carotid body O_2-sensitivity with no change in CNS gain of the HVR in cats after 48 hours of hypoxia (Vizek et al., 1987), and similar ventilation and blood gases after 48 hours or longer exposures (Tatsumi et al., 1991), does not rule out increased CNS gain of the HVR at later times. Similar logic applies to the experiments showing ventilatory acclimatization in goats after 4 to 8 hours of hypoxia can be explained by increased carotid body O_2-sensitivity (Busch et al., 1985); experiments have not been performed that would disprove central plasticity with longer hypoxic exposures in other animals.

There are also chemosensitive areas in the brain that respond directly to O_2 (Neubauer and Sunderram, 2004) and CO_2 (Nattie, 2006). As mentioned above, central CO_2-chemoreceptors especially have been of great interest for changes in ventilatory control with chronic hypoxia. However, studies to date focused on changes in CSF pH as a stimulus and recent studies have not supported a mechanism involving central chemoreceptors for ventilatory acclimatization (Smith et al., 2001). Plasticity in either central O_2 or CO_2 chemoreceptors could play a role in control of breathing changes during chronic hypoxia but this has not been studied.

It is also possible that chronic hypoxia could affect interactions between central and arterial chemoreflexes. There is recent evidence for functional and anatomical links between the carotid body ventilatory chemoreflex and CO_2-sensitive areas in the retrotrapezoid nucleus (Takakura et al., 2006). Also, caudal regions of the nucleus of the solitary tract is the site of both the primary synapse from carotid sinus nerve afferents (Gonzalez et al., 1994) and CO_2 chemosensitivity that stimulates ventilation (Dean et al., 1990;Coates et al., 1993). However, most evidence indicates the interaction between central and peripheral chemoreception is additive (Cunningham et al., 1986). Hence, changes in central chemoreceptive drive would not be predicted to increase the slope of the HVR, which is observed in chronic hypoxia. There is some evidence for a multiplicative interaction between central and peripheral ventilatory chemoreflexes in humans but it is not large (Robbins, 1988). However, increasing central

chemoreceptive drive actually decreased the slope of the isocapnic HVR in the normoxic control rats while chronic hypoxia increased the slope (Powell et al., 2000a).

5. Physiological significance of carotid body plasticity in hypoxia

The increased HVR observed during acclimatization to hypoxia is physiologically significant by increasing ventilation, which reduces the impact of environmental hypoxia on oxygen levels in the body. However, longer exposures to hypoxia at high altitude for years to generations "blunts" the HVR (Weil et al., 1971). The blunted HVR is a very general response observed in birds and mammals that are native to high altitudes (Black and Tenney, 1980;Weil, 1986), although not in all human populations (Beall et al., 1997; Leon-Velarde and Richalet, 2006). This suggests a blunted HVR may result from natural selection and be an advantage in hypoxia at altitude. It can be speculated that a blunted HVR is advantageous by reducing the oxygen cost of breathing *only if* there are other adaptations. For example, Andean high altitude natives have more efficient metabolic processes in hypoxia compared to lowlanders (Rupert and Hochachka, 2001). Further research is necessary to determine if a blunted HVR is advantageous during very long-term hypoxia.

The significance of carotid body plasticity for chronic hypoxemia with disease is not clear either. Although much of the basic research done on this topic uses the rationale that it will help us understand heart and lung disease and devise better treatments, we still do not know if plasticity occurs in the carotid bodies and chemoreflexes of chronically hypoxemic patients. This is because the problem is extremely difficult to study in patients with chronic lung disease for several reasons. First, the ventilatory chemoreflexes are extremely variable between individuals but the genetic basis for this is unknown. Therefore, it is impossible to estimate the healthy normal ventilatory response in an individual before he or she had the disease. Also, there are no longitudinal studies of the problem in populations susceptible to chronic lung disease to overcome this limitation to date. Second, individual patients with lung disease may have different degrees of abnormality in gas exchange and lung mechanics. Such changes in the efferent arm of ventilatory chemoreflexes can have very powerful effects on the physiological control of arterial blood gases and this is difficult to distinguish from problems with the sensory or integrative components of the reflex. For example, Younes (1995) pointed out that ventilation can increase enough to overcome a six-fold increase in respiratory resistance if ventilation is stimulated by an increase in Pa_{CO_2} that is too small to reliably measure. Finally, it is difficult to determine if specific mechanisms described for experimental animals (e.g. plasticity in the afferent vs. CNS integrating components of the HVR) occur in patients because of limitations in interpreting the results from reflex studies in humans (Powell et al., 2000a). Hence, the significance of plasticity in the HVR for lung disease remains to be determined also.

6. Conclusions

Chronic hypoxia causes plasticity in O_2-sensitive chemoreceptors and the hypoxic ventilatory response reflex. Ventilatory acclimatization to hours to months of hypoxia is a robust and universal response that reduces the impact of chronic hypoxia on oxygen levels in the body. Yet acclimatization results from a wide variety of species-specific molecular and neurochemical mechanisms that operate over very different time domains. Some of these mechanisms may be redundant. Hence, carotid body plasticity with chronic hypoxia is consistent with the "chemosome" hypothesis that proposes multiple O_2-sensors to explain the wide range of O_2 sensitivity in the carotid body (Prabhakar, 2006). Similarly, multiple levels of plasticity in the arterial chemoreflex (i.e. both sensory and central integrating components) appear to have evolved in concert so ventilatory sensitivity to hypoxia is optimized for the prevailing level of environmental oxygen.

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