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Time Domains of the Hypoxic Ventilatory Response and Their Molecular Basis

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Abstract

Ventilatory responses to hypoxia vary widely depending on the pattern and length of hypoxic exposure. Acute, prolonged, or intermittent hypoxic episodes can increase or decrease breathing for seconds to years, both during the hypoxic stimulus, and also after its removal. These myriad effects are the result of a complicated web of molecular interactions that underlie plasticity in the respiratory control reflex circuits and ultimately control the physiology of breathing in hypoxia. Since the time domains of the physiological hypoxic ventilatory response (HVR) were identified, considerable research effort has gone toward elucidating the underlying molecular mechanisms that mediate these varied responses. This research has begun to describe complicated and plastic interactions in the relay circuits between the peripheral chemoreceptors and the ventilatory control circuits within the central nervous system. Intriguingly, many of these molecular pathways seem to share key components between the different time domains, suggesting that varied physiological HVRs are the result of specific modifications to overlapping pathways. This review highlights what has been discovered regarding the cell and molecular level control of the time domains of the HVR, and highlights key areas where further research is required. Understanding the molecular control of ventilation in hypoxia has important implications for basic physiology and is emerging as an important component of several clinical fields.

Introduction

The hypoxic ventilatory response (HVR) is a complex interplay between several distinct mechanisms whose net effect varies depending on the pattern, duration, and intensity of hypoxic exposure. These interactions result in widely disparate physiological responses that induce either facilitation or depression of ventilation. Different hypoxic stimuli significantly alter the time-dependent mechanisms induced or the magnitude to which individual mechanisms are recruited, and thereby activate different time domains of the HVR. Furthermore, depending on the stimuli, multiple mechanisms may be recruited that have opposing, additive, occlusive, or synergistic (multiplicative) effects. Responses involve the activation and/or inhibition of several underlying pathways whose interactions and summation results in a final physiological ventilatory phenotype. As a result, small changes

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in hypoxic exposure times or intervals can drastically alter the physiological response to the stimulus. The physiological outcome may include short-term effects that temporarily alter synaptic activity or long-term effects that alter the strength of chemical synapses of respiratory circuits. Therefore, a given time domain of the HVR can affect future ventilatory responses, and thus constitutes a form of functional memory in the ventilatory control system.

Based on these observations, Powell et al. (1998) proposed to distinguish a given HVR by the following characteristic hallmarks: (i) the hypoxic stimulus paradigm (e.g., the pattern and intensity of hypoxic exposure); (ii) the time course of the response (e.g., seconds to years); (iii) the effects of this stimuli on the various physiological components of the HVR [e.g., alterations to tidal volume (V_T) versus respiratory frequency (f_R)]; (iv) whether these effects resulted in an increase or decrease of ventilation (e.g., potentiation or depression); and (v) the signaling molecules responsible for the manifestation of these responses (321). The stated goals of that brief review 18 years ago were to summarize the components of the HVR, to compare and contrast what was known about the various time domains of the HVR, and most importantly, to propose a common terminology to simplify scientific communication and thus facilitate research. Since that time, considerable progress has been made in the field, which has advanced our understanding of the body's response to hypoxia and shed light on the underlying mechanisms that mediate the various time domains of the HVR. In particular, significant advances have been made in the study of the molecular mechanisms of plasticity that underlie physiological responses to chronic sustained hypoxia and chronic or acute intermittent hypoxia. Also, spatially specific explorations have improved our understanding of how different neural circuits interact at various levels of physiology to effect integrated control responses in breathing. Through such studies, it is becoming increasingly clear that the physiological manifestations of the HVR (i.e., Hallmarks i-iv) are dependant upon how the underlying molecular, cellular, and neurotransmitter signaling pathways are modulated and interact in response to various patterns of hypoxia. Further, numerous recent studies have begun to demonstrate that not only can multiple molecular and signaling pathways induce similar physiological HVRs, but that the same molecular and signaling pathways can also induce different physiological HVRs depending on the duration for which these pathways are recruited. Finally, it is becoming apparent that these pathways can interact in complex ways at different levels of the respiratory control system to mediate different physiological responses due to small variations in the hypoxic stimulus.

There is one important idea in the 1998 description of the time domains of the HVR that has not changed, however. This is that plasticity in the ventilatory chemoreflex circuits (as defined later) is a major mechanism explaining time-dependent changes in ventilation and the HVR with different patterns of hypoxia, and that such changes in the HVR cannot be explained simply by time-dependent changes in the physiological stimuli for ventilation. This is discussed in depth in the sections on prolonged sustained hypoxia.

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Goals and scope

We propose that it is no longer appropriate to define a time domain of the HVR (with the HVR being the physiological response) according to its molecular mechanism and signaling pathway. Indeed, it is becoming increasingly clear that a given time domain of the HVR can be mediated by mechanisms of plasticity in numerous components of the respiratory control circuit that involve multiple, overlapping mechanisms at different time points during or following the hypoxic stimulus. Therefore, although the physiological manifestations of the HVR remain useful to characterize a specific time domain of the HVR for a given hypoxic stimulus, the underlying molecular and signaling mechanisms are not uniquely identified with a given HVR. Defining each unique molecular mechanism as a separate time domain of the HVR has become overly cumbersome and may be redundant. To resolve this dilemma, we now propose to define the time domains of the HVR as the physiological responses to different hypoxic stimuli (i.e., the elimination of Hallmark v discussed earlier), while emphasizing that the multiple potential underlying molecular time domains must be considered in experiments on mechanisms and in extrapolations about the physiological significance of a given time domain.

To address this new paradigm for time domains of the HVR, the primary goals of this review are to: (i) summarize the physiological responses of ventilation to the various patterns of hypoxia exposure described to date, that is, the time domains of the HVR, (ii) compare and contrast what has been learned about the underlying neurotransmitter, cellular, and molecular signals for each time domain of the HVR, and (iii) identify important unanswered questions and areas for future study. In addition, since any given physiological HVR is the result of the summation of hypoxic signals and responses from numerous levels in the respiratory control circuit, particular consideration will be given to the mechanisms in specific neural components of the ventilatory chemoreflex circuit. For example, how do responses from carotid body chemoreceptors, central chemoreceptors, central pattern generators, medullary integrative respiratory centers, cortical circuits that modulate breathing, and motor neuron pools contribute to an integrated ventilatory response? Finally, we will review emerging evidence that different signaling mechanisms result in a single time domain of the HVR and consider the physiological significance of this for different time domains of the HVR.

To be concise and to avoid redundancy we focus our review on studies examining responses to environmental hypoxia (also referred to elsewhere as hypoxic hypoxia) and other pulmonary causes of hypoxemia such as apnea, hypoventilation, or gas-exchange limitations in health and disease. Other factors limiting oxygen delivery in the body (e.g., heart failure, 39,181) or that increase oxygen demand or ventilatory drive (e.g., exercise, 11) will not be considered. We focus on mammals, including humans, the HVR of nonmammalian vertebrates having been recently reviewed elsewhere (252, 316). Other aspects of the HVR that have been covered elsewhere and will not be extensively considered here include the effects of development and aging (138, 359, 394), evolution (78), gender (26, 390), measurement techniques (394), and genetic differences in laboratory animals (17, 296).

In the ensuing sections, we will detail the physiological response of each HVR, as it relates to the definitive constraints of hypoxic time domains we outlined above (e.g., hypoxic

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stimulus paradigm; time course of the stimulus and response; physiological manifestation of the HVR, etc.) and also closely compare and contrast the underlying molecular and cellular signaling mechanisms identified to date in the study of each time domain. We will also discuss the animals and experimental preparations in which each HVR is studied, and conclude with an overview of the physiological and clinical significance of the various HVRs.

Synaptic plasticity and metaplasticity

As our knowledge of the underlying molecular mechanisms that mediate hypoxic ventilatory responses grows, it has become abundantly clear that synaptic plasticity in the relay circuits between chemoreceptors, ventilatory control circuits in the central nervous system (CNS), and respiratory motorneurons, is a common mechanism in most of the time domains of the HVR described below, and also in the control of ventilation and ventilatory rhythmogenesis in general (196, 255, 332). Therefore, it is useful to define synaptic plasticity for the purposes of this review.

Eccles, who received the 1963 Nobel Prize for his work on the synapse, wrote that plasticity in the CNS was the ability of individual synaptic junctions to respond to use and disuse by appropriate changes (82). In response to changes in stimuli or synaptic activation, a synapse may undergo potentiation (strengthening) or depression (weakening) of the connection that alters the postsynaptic response. Furthermore, these synaptic changes may be short-term or long-term in duration. Thus, four major forms of synaptic plasticity in vertebrates are short-term potentiation (STP) and depression, and long-term potentiation (LTP) and depression (3, 119). The form of plasticity that occurs at any given synapse depends on a number of neurotransmitter, neuromodulator, and protein changes. Typically, short-term plasticity results from changes in presynaptic neurotransmitter release or activity-dependent modification of proteins on the pre- or postsynaptic cell (e.g., phosphorylation) that alter postsynaptic responses to stimuli (440). Conversely, long-term plasticity typically involves the addition or removal of specific synaptic signaling proteins from the plasma membranes on cells (9, 108). The time required for recovery to baseline conditions following protein addition or subtraction is considerably longer than for changes in neurotransmitter release or phosphorylation, imbuing plasticity with long- or short-term characteristics, respectively (119).

In respiratory neural control circuits, plasticity has been defined as a persistent change in the control system (structural and/or functional) based on prior experience, which is in line with working definitions of plasticity set by the Society for Neuroscience in 2000 (255). Plasticity in respiratory control has been distinguished from modulation, which is defined as a neurochemically induced alteration in synaptic strength or cellular properties that is reversed when the neuromodulator is no longer present (255). This definition is consistent also with the concept of “memory” in the respiratory control system, used by Eldridge and Millhorn to describe some of the time domains of the HVR in a previous issue of the Handbook of Physiology (84). Additionally “metaplasticity” has also been defined in respiratory control as changes in the capacity to express plasticity depending on prior experience, or “plastic plasticity” (94, 255). The molecular and cellular basis for plasticity in respiratory control is

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presumably based on mechanisms similar to those understood to contribute to synaptic plasticity elsewhere in the CNS, as described in the aforementioned paragraph, and detailed in subsequent sections for different time domains of the HVR.

Physiological and Molecular Responses to Brief Hypoxic Exposures

The earliest time domains of the HVR occur during and following up to 5 min of a single hypoxic stimulation. Within this short time domain, three distinct ventilatory responses have been identified: the acute HVR, STP, and short-term depression (STD; Fig. 1). These HVRs are unique in their physiological etiology, but involve common molecular and neurotransmitter pathways, particularly, the acute HVR and STP. Occurring over seconds to just a few minutes, these time domains of the HVR are too brief for the underlying mechanisms to involve new protein synthesis; instead, they are more likely caused by interactions between cellular pathways that alter neurotransmitter release over the short-term. The primary mechanism of plasticity in these short-term time domains of the HVR appears to be enhanced synaptic transmission in the neural circuits between afferents from the peripheral and arterial chemoreceptors (carotid bodies), and respiratory motor neurons in the CNS. Such short-term plasticity appears to be activity dependent because it occurs with acute hypoxia or electrical simulation of the carotid sinus nerve in anesthetized, paralyzed animals. Therefore, short-term plasticity in the HVR begins with the acute carotid body response to hypoxia.

Acute hypoxic ventilatory response

The acute response to hypoxia is an immediate augmentation of ventilatory activity following the onset of a hypoxic stimulus, and typically includes increases in both f_R and V_T relative to baseline (321). To date, almost all animals studied exhibit such a hyperbolic increase in ventilation as a function of decreasing arterial oxygen saturation (Pa_{O2}) (30, but see also 300); however, the underlying changes in f_R versus V_T are highly variable between species (36, 256). The acute HVR persists throughout any given hypoxic stimulation and terminates immediately following the removal of hypoxia, that is, it begins and ends within one breath of the detection of a change in Pa_{O2} at the carotid body (321). Hence, as we define it, the acute HVR does not show plasticity. As an aside, most experimental attempts to measure the acute HVR likely include components of STP and/or hypoxic ventilatory decline (HVD) (see later). This is because these early time domains typically occur within a few minutes of the onset of hypoxia and it is often experimentally challenging to change O_2 tensions rapidly enough to permit accurate recordings of ventilatory responses in the first few minutes of exposure. For example, using standard plethysmography approaches, several minutes may be required to flush out normoxic gas mixtures with the hypoxic gas mixture in the animal chamber. As a result, researchers may refer to the first measurements of breathing in steady state as the acute response.

The acute HVR is a classic reflex response to sensory input from peripheral arterial chemoreceptors. The carotid bodies are the most important arterial chemoreceptor for the acute HVR in humans and most animals (95), and their adequate and physiological stimulus is Pa_{O_2} (198). Carotid body afferents travel in the carotid sinus nerve, which is a branch of

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the glossopharyngeal nerve, and their primary synapse in the CNS is in the nucleus tractus solitarii (nucleus of the solitary tract; NTS) (158), where glutamate is the primary excitatory neurotransmitter (discussed later). Afferent information from the carotid bodies is integrated with inputs from other sensory and higher CNS systems to evoke efferent responses in a variety of respiratory muscles, including the diaphragm, accessory inspiratory, expiratory, and upper airway muscle groups (84, 103, 208, 367).

The primary neurotransmitters that mediate carotid body chemoreceptor inputs to the NTS are L-glutamate, dopamine (DA), and substance P (SP) (125, 157, 169, 259, 325) (Fig. 2). The evidence for glutamatergic signaling in this pathway is particularly compelling. For example, during the onset of hypoxia, glutamate is released into the NTS within a timeframe that coincides with the acute HVR (throughout the first few minutes of a hypoxic bout); glutamate injection into the NTS mimics the acute HVR; and both the release of glutamate in response to a hypoxic stimulus and the acute HVR are abrogated by carotid body denervation (259, 339). Furthermore, NTS microinjection or systemic injection of glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) antagonists attenuates the acute HVR in a variety of species (8,56,142,259,290,375,388,389) and a cocktail of NMDAR and non-NMDAR blockers microinjected in the NTS completely blocks the ventilatory and cardiac response to carotid body chemoreceptor stimulation (403). Together, these data strongly support a central role for an enhancement of excitatory glutamatergic signaling between the carotid body and the NTS in the pathway that regulates the acute HVR.

In addition to the “through-put” excitatory responses mediated by glutamate, synaptic responses to acute hypoxia are “gated” such that the ventilatory response to changes in afferent input vary depending on the phase of the breathing cycle in which the stimulus occurs (84). Such gating is hypothesized to represent the activity of inhibitory neurotransmission mechanisms that oppose excitatory synaptic inputs mediated by glutamate release during a brief hypoxic episode. Indeed, several inhibitory neurotransmitters [e.g., DA or γ-amino butyric acid (GABA), 29,425] are released from carotid body afferents or other intermediary synaptic inputs and play a role in different time domains of the HVR [e.g., ventilatory acclimatization to hypoxia (VAH), see later]. However, the role of “gating” *per se* in various time domains of the HVR, except for STP, has not been investigated and there may be important differences in gating between species (84).

Other neurotransmitters that play a key role in the acute HVR include SP and DA. Both of these substances are found in the carotid body and CNS circuitry that mediate the acute HVR and can have differential effects at these two sites. SP is found in glomus cells and neuromodulates carotid body O_2 sensitivity by generally increasing carotid sinus nerve activity (197). The role of SP in the acute HVR beyond determining the level of afferent input to the reflex is less clear. Neurons from the petrosal ganglion can produce SP (170, 291) and SP mRNA is detected in petrosal ganglia but not the carotid body of rats (112,113). Neurokinin 1 receptors for SP are present on respiratory neurons in the NTS (212) and microiontophoretic application of SP excites respiratory neurons in the NTS (in cats, 148). Furthermore, intracerebroventricular injections of SP increase V_T and stimulate ventilation in anesthetized rats (147); however, SP mRNA expression does not increase in the carotid

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bodies or petrosal ganglia regions when rats are exposed to 1 h of hypoxia (112, 113), and a definitive role for SP in the CNS circuitry of the acute HVR remains to be demonstrated.

Similarly, DA has dual roles in the HVR involving both the carotid body and CNS circuitry of the reflex. DA is synthesized by tyrosine hydroxylase (TH) in glomus cells and is released from these cells when they are stimulated by hypoxia (125,129). Studies have shown that DA modulates the carotid body chemoreceptors at both pre- and postsynaptic type-2 DA receptors (D_2Rs) on glomus and afferent nerve endings, respectively (71,253). DA tonically inhibits carotid body neuronal output (25, 160, 393) and exogenous intravenous bolus administration of general DA or specific D_1R (but not D_2R) agonists depresses ventilatory activity (288, 410) via inhibition of carotid body neural activity (286). Conversely, antagonism of DA receptors in the carotid body stimulates neural activity and ventilation (139,305,402), and enhances the acute HVR (160, 171, 365).

In the CNS, DA plays an excitatory role in the acute HVR by acting on D_2Rs. Carotid body dopaminergic neurons terminate in the NTS and also in brainstem regions that regulate respiration (179,401). Antagonism of DA receptors in the CNS decreases the acute HVR independently of any effects on carotid body chemoreceptors (33,160,163,171,365). This finding has been confirmed in rats, mice, and cats, and supports the hypothesis that neuromodulation at DA receptors in the CNS potentiates the acute HVR (146, 160, 295). However, the net effect of DA acting simultaneously on D_2Rs at both peripheral and central sites appears to depress the HVR because ventilation, f_R, and V_T are all greater in mice with the D_2R gene deleted, compared to wild-type mice (164). As discussed in the section on VAH, the primary function of DA as a neuromodulator in both the carotid body and CNS may be to maintain optimal O_2 sensitivity in the acute HVR, for example, during chronic changes in O_2 during acclimatization to sustained hypoxia.

Finally, more recent studies have demonstrated an important role for ATP acting on purinergic (P2X) receptors in the NTS in mediating the acute HVR. In support of this, P2X receptors colocalize with carotid body afferent neurons in medullary regions involved in respiratory motor control, including in the NTS and the ventrolateral medulla (VLM) (132, 327, 341). *In vitro*ATP is released by carotid body afferent neurons during hypoxic episodes; the transmission of hypoxic signals in glomus cell cultures is abrogated by P2X antagonists (432). Similarly *in vivo*, the discharge rate of VLM second-order neurons increases with ATP application or hypoxia in a P2X-sensitive fashion (131,132). Further support for a role for ATP in the acute HVR comes from studies in mice with selective deletion of genes encoding for P2X receptors, in which the acute HVR and also the sensitivity of the carotid sinus nerve to decreases in Pa_{O2} are decreased in mice deficient in P2X receptor subunits (341).

Summarizing, experiments demonstrate that the acute HVR depends on (i) the net level of afferent traffic in the carotid sinus nerve, which involves multiple neurotransmitters and neuromodulators acting on the chemosensitive glomus cells and the glossopharyngeal nerve endings, (ii) excitatory glutamatergic neurotransmission in the primary synapse from the carotid sinus nerve in the NTS, which is subject to neuromodulation by at least DA, and likely also by ATP via P2X receptors, (iii) integration of information from other respiratory

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centers and inputs in the CNS, and (iv) activation of motor pathways to ventilatory and upper airway muscles. We propose that the molecular mechanisms of O_2 sensing and synaptic interactions between glomus cells and afferent nerve endings do not show physiologically significant plasticity during the acute HVR. The afferent response to changing Pa_{O_2} in carotid bodies *in vivo* typically has a latency of less than 300 ms and a peak response that occurs in less than 3 s from the onset of hypoxia and that is sustained throughout acute stimulation (198). This is expected if the effects of neuromodulators within the carotid body, which include vesicular-bound biogenic amines, purines, neuropeptides, gasotransmitters, and amino acids (198), do not change significantly with regard to the net afferent output in the carotid sinus nerve over short time domains. This is in contrast to plasticity in the molecular mechanisms of O_2 sensing and neuromodulation within the carotid body, which may contribute to plasticity in longer time domains of the HVR with chronic sustained or intermittent hypoxia [see VAH and long-term facilitation (LTF) later]. However, we propose as a working model that all time domains of the HVR depend to some degree on the neurochemical and molecular mechanisms that underlie the acute HVR, and particularly, the excitatory glutamatergic neurotransmission in the CNS. This concept is well illustrated by considering the mechanisms of STP.

Short-term potentiation

With hypoxic exposure of several seconds to a minute, there is a secondary increase in breathing that increases ventilation beyond changes mediated by the acute HVR. This secondary response is termed STP, or STP. Similarly, upon termination of a hypoxic stimulus, ventilation remains elevated for 1 to 2min before returning slowly to baseline levels. This period of ventilatory augmentation following the offset of the hypoxic stimulus is another manifestation of STP and these may represent “on and off” aspects of the same mechanism (Fig. 1) (84, 321). STP has been demonstrated in a wide variety of species and experimental states, following both direct hypoxic stimulation of intact animals and also direct electrical stimulation of carotid sinus nerves (247). To date, STP has been demonstrated in denervated cats, dogs, rabbits, and rats (43,84,144); in awake, sleeping, and exercising humans (15,102,118); and in awake ducks, goats, and mice (87, 190, 256). The physiological manifestations of STP (relative changes in f_R and V_T) vary between species, although there is a tendency for STP to be most apparent in increased V_T or phrenic nerve amplitude (84, 102). Furthermore, there is some variability in the time course of STP between and within species, depending on the experimental paradigm utilized [e.g., STP is progressively longer in awake relative to anesthetized preparations, and again so relative to decerebrate animals (34, 35); and is also prolonged with increasing severity of hypoxic stimuli (246)].

As with the acute HVR, STP can be induced by direct electrical stimulation of the carotid sinus nerve (247). This indicates that the underlying mechanism involves enhanced sensitivity in the reflex circuit between the carotid bodies and the CNS and not increased O_2 sensitivity of the carotid body *per se*. However, STP is not specific to the intersection of carotid sinus nerve afferents and the NTS, but may also occur at downstream bulbospinal synapses between NTS afferent neurons and individual motorpools. STP is evident in the output of the phrenic, inspiratory intercostal, and hypoglossal motorneurons (84, 103, 176)

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and can be induced or inhibited at discrete motor nerves individually or simultaneously (176).

As described above, the acute HVR is due to presynaptic glutamate release following hypoxic stimulation of the carotid body. STP occurs with persistent stimulation of the carotid sinus nerve for more than a few seconds of hypoxia, which results in maintained glutamate release from carotid body afferents. This sustained afferent input and release of glutamate prolongs stimulation of postsynaptic glutamate receptors, which has been shown to initiate synaptic LTP in other systems (e.g., the hippocampus). In this canonical form of LTP, sustained glutamate release causes receptor-mediated Ca^{2+} influx into postsynaptic neurons. Maintained Ca^{2+} influx through NMDARs leads to the dephosphorylation of neuronal nitric oxide synthase (nNOS) in postsynaptic neurons (10,79).When dephosphorylated, nNOS acts to increase NO formation (195,260), which diffuses in a retrograde fashion back across the synapse and into the presynaptic neuron. Here NO positively modulates guanalyl cyclase expression and activity (41, 195), which increases the activity of cyclic guanosine monophosphate (cGMP)-dependent protein kinase (261), and subsequently presynaptic glutamate release (263) to increase ventilation (192, 228, 322). Enhanced presynaptic glutamate release following the excitation of the presynaptic neuron then results in heightened sensitivity at the synapse by increasing the excitation of the post-synaptic neuron (i.e., this mechanisms increases the gain of the hypoxic signal from the carotid bodies to the CNS). We propose that this canonical pathway for synaptic LTP described elsewhere in the CNS operates at the synapse between carotid body afferents and neurons in the NTS to explain the STP time domain of the HVR (Fig. 3).

There is strong experimental evidence in support of a role for this glutamate/NO-mediated mechanism in the STP of the HVR. For example, NOS has been identified in the sub-nuclei of the NTS that receive input from the carotid body (40, 143). Additionally, NO donors injected directly into the NTS increase spontaneous discharge rates of neurons and also ventilation, while similar injection of nNOS antagonists inhibit ventilatory responses to hypoxia (287, 384). However, *in vivo* studies of the role of NO and nNOS in regulating STP are conflicting. For example, systemic nNOS inhibition or specific deletion of the nNOS gene attenuates STP of breathing following acute hypoxia in conscious mice in some studies (190), and cGMP accumulates in the brainstem of wild type, but not mutant nNOS knockout mice following acute hypoxic exposure (193). Similar results have been reported in both anesthetized rats and cats, in which administration of NOS inhibitors prior to a hypoxic exposure reduces the acute HVR and prevents hypoxia-mediated cGMP accumulation in the brainstem following acute hypoxia (143, 287). In conscious rats, systemic nNOS antagonism reduces hyperventilation during a 30-min hypoxic stimulus but not in the first minute of hypoxic exposure in one study (143). In contrast, NTS-specific NOS antagonism blunts the potassium cyanide-mediated increase in breathing at 5 and 10 min but not 30 min after treatment (134), and prevents the acute HVR after 1 minute (287). Conversely, others have reported that systemic or NTS-specific nNOS inhibition does not block STP in conscious mice (83), or rats (301). However, since this pathway is a common form of short-term synaptic plasticity that is conserved at glutamatergic synapses throughout the CNS, it is likely that enhanced synaptic sensitivity due to the activation of this pathway occurs not only at the synapse between the carotid body and the NTS, but also at the synapses between NTS

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secondary neurons and other respiratory centers in the CNS. Indeed, it is important to note that NO has opposing effects at the carotid body (inhibitory) versus the NTS (excitatory). Experiments examining regional-specific interactions would be useful to determine the spatial and temporal specificity of site-dependent synaptic memory in the overall STP respiratory response to hypoxia. Such studies would help resolve conflicting reports from similar study models.

Interestingly, STP was initially termed “after discharge” based on the concept of prolonged neural discharge following a brief stimulation (84). This is particularly prescient since the mechanisms that mediate the “off” form of STP following a hypoxic stimulus are likely essentially aftereffects of the mechanisms that mediate the onset of STP during the initial hypoxic stimulus. Indeed, the duration of the “off” phase of STP depends on stimulus intensity such that STP is prolonged with lower inspired oxygen in humans (246). As discussed earlier, during the early stages of STP this response is mediated by enhanced glutamatergic activity, which is usually due to increased presynaptic glutamate release. In the later stages of STP, large-scale Ca^{2+} influx through postsynaptic glutamatergic NMDARs, which underlies the early form of STP, leads to persistently elevated intracellular Ca^{2+}, the accumulation of which not only predisposes neurons toward excitability upon subsequent stimulation, but also contributes to the sustained phosphorylation of nNOS after the removal of the hypoxic stimulus (10, 41, 192, 195, 228, 260, 263, 359). Up to several minutes are required for neuronal Ca^{2+} to be returned to normal equilibrium through ion pump-mediated extrusion of the ion (183), which is a requirement for nNOS to return to its normal, inactivated state, to cease production of NO, and thereby return overall synaptic sensitivity to afferent stimulation to baseline levels.

STP during hypoxia itself thus likely represents the effect of accumulation of glutamate at the synapse, while STP during reoxygenation represents the slow rundown (return) to baseline of acute HVR-mediated glutamatergic potentiation that has occurred during the early form of STP (i.e., due to delays in clearing built-up Ca^{2+} in intermediary signaling neurons during hypoxia). Both of these events likely occur at all glutamatergic synapses in the HVR neural circuitry, including the synapses between carotid body afferents and NTS neurons, and synapses distal to the NTS in ventilatory pools of motor neurons. Since overall neuronal excitability is mediated by a balance of excitatory and inhibitory stimuli, secondary control mechanisms may induce inhibitory effects on certain motor neurons downstream of the NTS, overriding the enhanced excitability of STP mediated by elevated $[Ca^{2+}]$ in postsynaptic neurons. Such a mechanism would explain how STP might simultaneously be inhibited in some neurons but not in others (176), although this remains to be tested rigorously.

The physiological role of STP has been previously proposed to act as a smoothing influence in the control of breathing, which prevents reflex activation of the respiratory control system from proceeding too rapidly (84, 246). STP would, therefore, function as a stabilizing influence on the respiratory system (321). However, the glutamate receptor/NO-mediated pathway of STP is a common pathway of synaptic plasticity and it is more likely that these effects are unavoidable (albeit not undesirable) by products of the basic mechanisms of synaptic potentiation during prolonged stimulation, rather than representing an adaptation

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that was selected for to regulate the ventilatory response to hypoxia *per se* in a stable manner. Taken altogether, these data suggest that if mechanisms exist to stabilize the respiratory response to changing levels of oxygen availability, then they will be more likely mediated by inhibitory inputs to various levels of the HVR neural circuit that balance excitatory effects of glutamatergic activation.

Summarizing, the evidence discussed above supports our working model for the excitatory release of glutamate underlying the acute HVR, as well as both the onset and decay phases of STP. With prolonged activation of this pathway during sustained hypoxia, the synaptic mediators of this second, short-term time domain of the HVR begin to involve cellular and molecular pathways and presumably plasticity in the neural circuit of the HVR synapses between carotid body afferent and the NTS, and potentially elsewhere in the ventilatory control network. We hypothesize that the underlying mechanisms of plasticity at these additional synapses involve the same LTP mechanism described earlier for the primary synapse of the HVR in the CNS.

Short-term depression

STD of the HVR manifests as a decrease in f_R following a transient overshoot in f_R that occurs during short-term hypoxia and lasts from seconds to minutes; it is also seen as a return to normal levels of f_R after a transient undershoot in f_R immediately following a hypoxic episode (Fig. 1) (321). This second “off”-phase of STD is also termed posthypoxic frequency decline (PHFD, 52) and is thought to be mediated in part by an increase in expiration time (T_E). Whereas the acute HVR and STP represent the physiological manifestations of basic conserved principles of enhanced synaptic transmission and early synaptic plasticity that are consistent across multiple synapses in neural circuits between the carotid body and ventilatory motor neurons, STD appears to be a time domain of the HVR that is mediated by a highly specific molecular signaling pathway downstream of both the carotid body and NTS. Furthermore, while the acute HVR and STP have been observed in all species and experimental preparations studied to date, the occurrence of STD varies between experimental preparations, species, and even strains within a given species.

STD was first identified as a decrease of f_R (of phrenic nerve activity) during or following hypoxia or carotid sinus nerve stimulation in anesthetized rats (144). Since this initial study, much of the research efforts to characterize the underlying mechanisms of STD have focused upon examination of the “off” response following the cessation of hypoxia (i.e., PHFD). Although it is generally assumed that the “on” and “off” mechanisms of STD are the same, research into the “on” mechanism is too sparse to draw meaningful conclusions. Nonetheless, research examining the “off” mechanism has demonstrated that STD is most likely effected in the CNS circuitry for the HVR, since it persists in awake carotid body-denervated rats (55). Specifically, the ventrolateral pons region is critical for STD because lesions here abolish STD in anesthetized rats (52), while agonism of excitatory synaptic inputs (i.e., glutamatergic) to this region mimic STD and increase T_E specifically (177).

The ventrolateral pons includes portions of the noradrenergic A_5 cell group and experiments demonstrate a role for catecholamines in STD. Lesions of the A_5 cell group in the ventrolateral pons blocks STD in conscious rats (353) and systemic injection of α_2-

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adrenoreceptor antagonists abolishes STD in anesthetized rats (13, 14). However, intracerebroventricular antagonism of α_2-adrenoreceptors does not prevent STD (53), suggesting that α_2-adrenoreceptors are not required but rather modulate the response (13). Serotonin (5-HT), also appears to be involved in STD since antagonism of 5-$HT_{2A/C}$ receptors augments STD in rats (188), and antagonism of 5-HT_{1A} receptors is required to reveal the occurrence of STD in mice (267) (Fig. 4). It is interesting to note that 5-HT and α_2-adrenoreceptors also interact to mediate respiratory responses to intermittent hypoxia (see LTF section below), and it is tempting to speculate that mechanisms that regulate LTF also play a role in the mediation of STD. Further research is required to delineate the specific mechanisms that underlie this unique time domain of the HVR. Nonetheless, together these results demonstrate that unlike the acute HVR and STP, STD is mediated by the balance of a complex interplay of signaling intermediates in respiratory brainstem neurons.

Initially it was believed that STD occurred only in anesthetized rats (52, 144, 321) because it is not detected in anesthetized cats or goats (84, 399), or in sleeping humans (140). However, researchers have since identified this mechanism in conscious rats and mice (55, 267) and also in awake humans (377). Additionally, this time domain of the HVR is sensitive to genetic differences, as indicated by the observation that the magnitude of STD can differ between strains of rats and also within substrains (144, 215, 379). It is also notable that this form of respiratory plasticity appears to occur independently of the carotid body and NTS, highlighting the key fact that plasticity in the respiratory response to hypoxia occurs at multiple levels of ventilatory control circuits.

Interestingly, the magnitude of STD depends on the history of hypoxic exposure or stimulations of the carotid sinus nerve such that previously stimulated preparations respond less robustly (i.e., have reduced STD) to subsequent stimulations than naïve preparations, even when phrenic nerve activity has returned to baseline (13,14,215). Therefore, STD exhibits metaplasticity, wherein the history of stimulation (hypoxia at carotid bodies or electrical stimulation of the carotid sinus nerve) modulates the future responses to subsequent stimuli. For this reason, further research may eventually demonstrate that STD is mechanistically more closely related to HVRs that occur in response to intermittent hypoxic protocols (see later).

Summarizing, the paucity of information regarding the physiological impact of STD and its underlying molecular mechanisms make it among the least well-understood time domains of the HVR. Studies to date indicate that this time domain may play an important role in “smoothing” out the augmentation of ventilation in the first few minutes of breathing but more work is required to confirm this hypothesis, to explore its role in additional species, and to explain discrepancies between experimental results from awake versus anesthetized preparations. Many of the characteristics of STD are unique and particularly interesting as, unlike other time domains, STD (i) seems to be entirely regulated within a single specific region of the CNS (i.e., the ventrolateral pons), (ii) affects a single component of ventilation (f_R), and (iii) is the first occurrence of metaplasticity in the HVR. Numerous questions remain to be answered in the study of STD. For example, the “on” stage of this time domain is particularly poorly understood, as are the underlying cellular and molecular mechanisms.

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Finally, potential similarities and overlap between what is known about the molecular components of STD and those of LTF deserve greater research attention.

Hypoxic ventilatory decline

HVD (Fig. 1) is a decrease in ventilation from the level of the acute HVR and/or STP when hypoxemia is sustained for 5 to 30 min in adult animals (321). In the literature, HVD has also been referred to as ventilatory “roll-off” of the HVR or hypoxic ventilatory “depression,” although we explicitly recommend against this latter terminology for reasons explained below. HVD occurs when hypoxemia is sustained for at least 3 to 5 min in adult animals (321) and can persist for as long as 8 weeks in humans during sustained hypoxia at high altitude (167, 350, 351). Thus, this time domain bridges the temporal gap between short-term time domains of the HVR (e.g., STP) and long-term time domains [e.g., VAH and hypoxic desensitization (HD), see later].

HVD is difficult to study because it is not observed in all animals and experimental preparations. HVD varies depending on sex, age, arousal state, anesthesia, and hypoxic stimuli (394). Initially, it was believed that the heterogeneity of this response was due primarily to the state of arousal within any given species since in some animals HVD is only observed in the anesthetized state; however, in many species HVD is observed in both anesthetized/asleep and also in awake animals, calling this definition into question. For example, HVD has been observed in awake humans (155,167,350,351), awake and anesthetized cats (405), awake rats (229,382), anesthetized rats, as a decline in phrenic nerve activity (156,375), and in anesthetized rabbits (136), but is not observed in awake dogs (44, 60). Perhaps more confounding, in awake goats some groups detect HVD (120,121,229,382), while others do not (2,76,285). Studies on awake rats that have not reported an occurrence of HVD (2) may be explained by the short time course of HVD (109), such that ventilation may have already stabilized at a lower level by the time steady state HVR measurements were made.

HVD is different from the biphasic HVR observed in neonates and most small rodents, wherein metabolic rates decrease in response to hypoxia as part of a thermoregulatory response to low oxygen that induces body temperature decreases (270). Also, HVD occurs independent of the poikilocapnia that accompanies the acute HVR. HVD is observed in isocapnic hypoxia (278), but it is estimated that 10% to 20% of HVD in awake humans may be caused by increases in cerebral blood flow with hypoxia that lowers P_{CO2} at chemosensitive sites in the CNS (394). The time-course of the offset of HVD is generally longer than the onset. For example, in humans, HVD occurs within 25 min of hypoxia but persists for 60 min after the removal of the initial hypoxic stimulus (62, 81). Interestingly, the recovery time following HVD can be shortened by breathing oxygen-enriched gas mixtures (81, 278), and to date, this is the only time domain of the HVR identified that can be directly modified by this approach. However, HVD is not changed during 2 days to 8 weeks of hypoxia in humans at high altitude (167, 350, 351) (see also VAH section).

To our knowledge, HVD has only been observed in phrenic nerve activity and ventilation, but has not been examined in other respiratory motor outputs. In most experiments, HVD modifies V_T with minimal effects on f_R, distinguishing it from STD, which is characterized

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by increased f_R with no observed change in V_T (see earlier).However, HVD decreases f_R in some preparations (230), which illustrates variability in HVD with sex, age, arousal state, anesthesia state, and hypoxic stimuli (394).

Initially, it was thought that HVD was caused by depression of the ventilatory chemoreflex response, somewhere distal in the reflex arc to the arterial chemoreceptors. In support of HVD being a mechanism in the CNS, carotid sinus nerve activity does not decline during sustained hypoxia in animal studies, although this has never been measured in humans or conscious preparations (reviewed by 394). In support of a decrease in O_2 sensitivity during HVD, the acute HVR between normoxia and a fixed level of hypoxia decreases in humans after 25 min of hypoxia (81). This suggests decreased O_2 sensitivity of the acute HVR; however, subsequent studies showed ventilation is not as depressed in normoxia after hypoxia as it was depressed during sustained hypoxia. In fact, the slope of the HVR between two levels of isocapnic hypoxia (e.g., Sa_{O2} = 90% and 80%, respectively) does not decrease in sustained hypoxia (351) (Fig. 5). These results led to several experiments testing if HVD was independent of changes in O_2 sensitivity of the ventilatory chemoreflex. As illustrated in Figure 5, human studies generally supported the idea that HVD is a decline in ventilation without significant decreases in ventilatory O_2 sensitivity during 14 to 120 min of sustained hypoxia (110,351). However, there is also evidence in humans for a decrease in ventilatory O_2 sensitivity: (i) measured in the first few minutes of hypoxic exposure (22,110) (Fig. 5) or (ii) estimated from sophisticated models of ventilatory responses to central versus peripheral chemoreflexes (211).

Teppema and Dahan (2010) noted several observations that support a role for the arterial ventilatory chemoreflex in HVD even if a change in O_2 sensitivity is not the main cause of this time domain. First, the magnitude of HVD is proportional to the magnitude of the acute HVR, as summarized in Tables 1 and 2 of Teppema and Dahan (2010) that show the effects of various pharmacological interventions on the acute HVR and HVD in humans and animals, respectively. Second, bilateral carotid body denervation eliminates the acute HVR and HVD in awake cats and humans (Fig. 5 in 394). These authors discuss how anesthesia may uncouple HVD from arterial chemoreceptor activity and explain an independent effect of CNS hypoxia on HVD in anesthetized cats and humans. The mechanism for HVD that is independent of arterial chemoreceptors is not clear, but the effect of hypoxia to increase cerebral blood flow and decrease P_{CO2} at central chemosensitive sites (see earlier) may contribute. Systemic adenosine receptor antagonists block HVD in carotid body denervated cats (175, 250, 426) and dogs (175, 250, 426). In addition, naloxone blocks opioid receptors and blunts ventilatory depression from carboxyhemoglobinemia in carotid body denervated cats (279). Both adenosine and opioid receptors are coupled intracellularly to inhibitory G-proteins that may modulate cellular processes in neurons in the NTS or elsewhere in the CNS respiratory control circuits to induce the physiological HVD. Finally, it is known that higher brain centers can depress ventilation independently of arterial chemoreceptors as demonstrated by brain transections at or below the intercollicular level increasing ventilation in hypoxic, carotid body denervated, awake rats (229).

The neurochemical mechanism of HVD that does depend on arterial chemoreceptor input is hypothesized to be inhibitory neurotransmission between the carotid body afferents and

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medullary neurons, or within other CNS respiratory circuits. Regulation via the activity of adenosine receptors is a potential mechanism, and adenosine release increases with hypoxia and inhibits ventilation centrally (80). However, the most compelling evidence implicates GABA as the primary effecter of HVD (Fig. 6). For example, intracerebroventricular perfusion of GABA or GABA receptor agonists decreases ventilation during normoxia and hypoxia in awake rats (382), while systemic administration of $GABA_A$ receptor antagonists abolishes the HVD in awake rats and in anesthetized cats (161, 245). This later effect has since been localized to the NTS, where the GABA concentration increases during HVD in awake rats (382). GABA antagonists injected directly into the NTS markedly reduce HVD in intact animals but this effect is not observed in carotid body-denervated animals (382). Together, these data indicate that HVD is mediated by GABAergic inhibition in central components of the respiratory control circuit, but that chemoreceptor stimulation to induce this HVR is critical.

Although HVD does not appear to be due to direct modulation via the NO/glutamate pathway that underlies the acute HVR and STP as described earlier, there is evidence that HVD (and to some extent STP) are mediated by *S*-nitrosoglutathione (SNOG), which is formed by nNOS (217). SNOG is degraded by SNOG reductase, which decreases tissue *S*-nitrosylation status and increases the activity of various proteins in the CNS (154), including NMDARs (385). Through these interactions, SNOG increases ventilation in conscious mice when injected intracerebroventricularly (217). Recent studies in conscious SNOG reductase heterozygous and homozygous knockout mice showed that HVD was reduced or abolished when SNOG reductase was genetically ablated (297). Further studies are required to determine which synaptic proteins are modulated by this pathway in the regulation of HVD.

In addition to a potential role for NOS in mediating HVD via modulation of protein *S*-nitrosylation, it is also possible that the canonical NO/glutamate pathway contributes indirectly to the accumulation of GABA during HVD. A primary means of removing excessive glutamate from the synapse following its release from afferent neurons during sustained hypoxia is the direct conversion of glutamate to GABA via the activity of the glutamic acid decarboxylase (GAD), whose activity has been shown to increase in hypoxic mammalian brain (278,343). HVD may thus represent the long-term physiological outcome of enhanced glutamate release following the acute HVR and STP; that is, the accumulation of glutamate may induce activation of GAD, which would convert excess glutamate to GABA, thus leading to the accumulation of GABA in the NTS. This action would oppose the excitatory effects of glutamate and may induce HVD (156, 184). Examinations of the role of GAD in glutamate/GABA cycling in the NTS during hypoxia would be a fascinating addition to the literature. Glutamate also may directly excite GABAergic interneurons in the NTS, which would result in the release of inhibitory GABA even as glutamate is exciting postsynaptic glutamatergic neurons in the NTS or at other downstream sites in the CNS(382). The modulation of GABAergic interneurons in the NTS during hypoxia also begs further investigation; and together, these two mechanisms may connect NO/glutamate receptor signaling to this inhibitory stage of the physiological responses to hypoxia.

Indirect support for this hypothesis comes from studies in which DA administered to awake humans or anesthetized rabbits prevents the acute HVR and STP, which in turn prevents the

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occurrence of HVD (62, 136). This suggests that upregulating inhibitory dopaminergic input during hypoxia opposes the excitation of the NTS mediated by excitatory inputs from the carotid body, which would in turn limit the release of glutamate at the NTS. Without glutamate to induce GABA release and/or to serve as a substrate for conversion to GABA, the accumulation of GABA would thus be prevented and HVD during reoxygenation would not occur. In support of this, peripheral DA administration abolishes the HVD in humans (62). Alternatively (or perhaps additionally), dopaminergic inhibition at the CNS may selectively inhibit GABAergic interneurons in the CNS, and ablate the synaptic release of GABA at the NTS. This second hypothesis is supported in particular by the observation that intracerebroventricular injection of DA directly into the CNS of rabbits inhibits the HVD (136). Further examination of putative causative links between the acute HVR/STP and HVD, and the role that DA plays in mediating the eventual accumulation of glutamate and potentially GABA is warranted to test these questions. For example, it is known that DA can mediate ventilatory responses during the hypoxic phase that then set the tone for HVD following the removal of the hypoxic stimulus (potentially by abrogating excitatory glutamate release and thus inhibitory GABA accumulation, or by directly inhibiting GABA release from inhibitory interneurons). Blocking dopaminergic signaling during the transition to normoxia following hypoxic stimuli in carotid body-intact versus denervated animals on a species by species basis would help resolve this pathway.

DA is hypothesized to play a direct role in HVD also, independent of its inhibitory effects at the carotid body, but the evidence for this is not as clear as for GABA. DA is a key inhibitory neuromodulator in the carotid bodies but it facilitates the acute HVR by actions in the CNS (163). The independent effects of DA at peripheral and central sites have been studied with DA receptor blockers that do or do not cross the blood brain barrier or local administration of drugs to the CNS. Smatresk et al. (1983) first demonstrated the potential for this approach by simultaneously measuring ventilation and carotid body responses to hypoxia in anesthetized cats with haloperidol (a DA antagonist that crosses the blood brain barrier) (365). These authors showed that DA inhibits the carotid body but facilitates the CNS gain of the HVR, as haloperidol decreased carotid body activity but increased ventilation for given changes in Pa_{O2} (Fig. 7).

Systemic inhibition of dopaminergic receptors with haloperidol abolishes HVD in awake or anesthetized cats, but peripheral DA receptor blockade with domperidone (which does not cross the blood brain barrier) increases the acute HVR (consistent with inhibitory effect of DA on carotid bodies) and does not change HVD (392). Intracerebroventricular DA administration induces respiratory depression in anesthetized rabbits with intact or denervated carotid bodies (136), consistent with central DA causing HVD, although it is not clear that ventilatory depression in hypoxic, carotid body denervated animals is the same thing as HVD. These animal results indicate DA contributes to HVD by a CNS mechanism. In contrast in humans, peripheral but not central dopaminergic inhibition abolishes HVD (62, 306). Thus, the role of DA in HVD varies between species and may be due to inhibition of the carotid body chemoreceptors, which would decrease the HVR and HVD, and/or inhibition in the CNS that decreases the HVR.

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It is also worth noting that the HVD was initially proposed to be an energy-conserving mechanism during hypoxia (278), and decreased neural activity and mechanical activity (e.g., breathing) are known to preserve energy in hibernating or hypoxia-tolerant species during long-term hypoxic or hypothermic episodes (78,252). However, HVD lasts into the normoxic recovery period when energy conservation is not critical, so it seems more likely that HVD is simply the physiological side-effect of synaptic clearing mechanisms that act to return synaptic tone to baseline levels following excessive glutamate release due to prolonged hypoxic stimulation. It is more likely that synaptic glutamate accumulation leads to synaptic GABA accumulation at the NTS, which in turn triggers cell-based responses in the CNS that induce systemic reductions in ventilation for a short period of time. The observation that oxygen breathing shortens HVD (Easton et al., 1988) is consistent with this model as such a treatment would abolish carotid body afferent signaling and minimize glutamate release. This would allow for more rapid removal of accumulated synaptic glutamate by conversion to GABA, and thus shorten the HVD.

Summarizing, HVD is an important medium-term time domain of the HVR that occurs with several minutes of hypoxia exposure and persists after the offset of hypoxia. HVD thus bridges the gap between short-term responses to acute hypoxia (i.e., the acute HVR and STP) and long-term responses to chronic hypoxia (e.g., VAH, see next section). HVD has proven difficult to study; experimental results are highly variable between species, gender, genetic strain, and experimental preparation; which greatly complicates a meta-analysis of the molecular mechanisms that underlie this time domain. Nonetheless, studies to date consistently implicate plasticity in the reflex arc between the carotid bodies and the NTS as the primary location of HVD. However, debate remains regarding the respective roles of upregulating inhibitory (i.e., dopaminergic, GABAergic) and downregulating excitatory (i.e., glutamatergic) signaling in mediating this plasticity and the final physiological response.

Physiological and Molecular Responses to Prolonged Hypoxic Exposures

When hypoxia is prolonged for more than a few minutes, additional hypoxic ventilatory responses occur. Specifically, when hypoxia is prolonged beyond the STP stimulus window by several days or weeks, mechanisms termed VAH, and ventilatory deacclimatization to hypoxia (VDH) occur; whereas in response to life-long hypoxia a mechanisms termed HD has been identified (321).

Ventilatory acclimatization to hypoxia

When hypoxemia is prolonged for hours to months, additional time-dependent increases in ventilation occur that further improve Pa_{O2} (Fig. 1) (413). (Note that we are considering the persistent hyperventilation in normoxia after chronic hypoxia as a different mechanism: VDH, see later.) VAH has been described in a wide variety of animals, including humans (reviewed by 394), goats (97,366), ponies (98), dogs (37), cats (21, 404), rats (2, 293), and mice (163, 164, 194, 295). VAH occurs in response to prolonged exposure to environmental hypoxia (e.g., at high altitude) and there are no significant differences in the manifestation of VAH in either hypobaric or normobaric hypoxia reported. Therefore, it is assumed that VAH

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occurs with any prolonged hypoxemia, although its role in chronic lung disease remains to be established (see later).

The magnitude of VAH increases with the magnitude of the hypoxic stimulus (75, 331), although a proper dose-response curve has not been measured under strictly comparable conditions to date. The time course of VAH varies considerably between species and may take hours to days to become completely manifest. For example, VAH is fully induced within 4 to 6 h in goats, but requires about two days in rats (30, 293, 320), and days to weeks in humans, perhaps depending on the level of experimental hypoxia used (reviewed by 394). Of the experimental models studied to date, the time course of VAH in rats is most similar to humans, with most of the change occurring over the first few days of hypoxia and relatively small or nonsignificant changes over 1 to 2 weeks (293, 350). In awake humans and in rats, VAH manifests primarily as an increase in V_T (2, 100); however, the contributions from each component of ventilation vary between species. For example, in goats, cats and mice, VAH involves increases in both V_T and f_R (85, 163, 390).

In the 1960s, it was hypothesized that changes in central CO_2-sensitive chemoreceptor stimuli during chronic hypoxia explained VAH (358). However, this “unified control theory” was subsequently disproved by additional measurements of pH in cerebrospinal fluid (CSF) showing alkalosis persists during VAH and, similar to arterial pH, compensation seems to lag rather than lead time-dependent increases in ventilation during chronic hypoxia (68, 413). Other potential roles for central CO_2-sensitive chemoreceptors in VAH, as well as in normoxia after chronic hypoxia or VDH, are considered below. However, experiments establishing that VAH could not be explained by time-dependent changes in chemoreceptor stimuli changed the paradigm for acclimatization. Stimulus-response relationships are *not* fixed in the ventilatory control system and must be plastic or adapting to chronic changes in environmental stimuli.

Subsequent studies have shown that plasticity occurs at two levels within the HVR reflex arc, as discussed in the following sections. Chronic sustained hypoxia increases (i) carotid body chemoreceptor O_2 sensitivity and (ii) the “CNS gain of the HVR,” defined as the change in ventilatory motor output for a given change in afferent input from arterial chemoreceptors (320). Increased CNS gain of the HVR was actually postulated by Forster and Dempsey (1981) before the discovery of plasticity in carotid body O_2 sensitivity, to explain VAH when the unified control theory involving pH of CSF broke down. Changes in static and dynamic lung mechanics in people sojourning to high altitude also cannot explain time-dependent increases in ventilation, implying changes in ventilatory drive (114), and static lung mechanics are not changed by chronic hypoxia in rats (320). However, we are not aware of any studies on neuromuscular transmission in chronic hypoxia, or of phrenic motor neurons that play a critical role with HVR plasticity in intermittent hypoxia (see later).

Arterial chemoreceptor plasticity in ventilatory acclimatization to hypoxia— Considerable attention has been devoted to the role of the carotid body in explaining VAH and it is clear that VAH does not occur when the carotid body is denervated (20, 38, 96, 285, 294, 406). Chronic hypoxia changes the anatomy, ultrastructure, chemosensory mechanisms, and neurotransmitters in the carotid body such that glomus cell sensitivity to hypoxia is

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increased. Structurally, the carotid body roughly doubles in size with glomus cell hypertrophy, hyperplasia, and angiogenesis (70, 238), although the functional significance of such changes are unknown. As a result, P_{O2} at putative chemosensory sites on glomus cells should increase, if they change at all, based on decreased diffusion distances from arterial blood in chronically hypoxic carotid bodies (238). The covering of glomus cells with sustentacular cells decreases with chronic hypoxia (200), and this increases the potential surface area for gap junctions, which could potentially increase sensitivity to oxygen changes (90).

The effect of chronic hypoxia on carotid sinus nerve discharge has been quantified in several animal preparations exposed to hypoxia ($Pa_{O2} \approx 40$ Torr) for hours to days. Anesthetized goats increase discharge 3.6-fold over 4 h (calculated from Table 5 in Ref. 276); *in vitro* rat preparations increase 1.9-fold over 7 to 16 days (Fig. 5 in Ref. 45); anesthetized cats increase 1.6-fold (calculated from Table 1 in Ref. 406). Differences between studies presumably reflect different durations of hypoxic exposures as well as species differences (e.g., the rapid appearance of HD in cats, which involves other mechanisms; see below). However, all of the studies are consistent in supporting that changes in O_2 sensitivity are a function of P_{O2} and not CO_2 or pH.

Chemosensory mechanisms in the carotid body affected by chronic hypoxia that could increase O_2 sensitivity include NADPH oxidases [NOX; which produce reactive oxygen species (ROS) that modulate glomus cell activity] and O_2- sensitive K^+ channels (198, 317, 394). Voltage-gated Ca^{2+} channels and Na^+ currents also increase with chronic hypoxia, which increases glomus cell excitability (198, 394). There is also evidence for O_2-sensitive Na^+ channels on afferent nerve endings of the carotid sinus nerve in the carotid body. Faustino and Donnelly (2006) described persistent Na^+ currents in carotid sinus nerve afferents that play a critical role in carotid body O_2 sensitivity (92), and these may explain results such as the recovery of chemosensory activity in the carotid sinus nerve after removal of the carotid body (258), or species differences of individual neurotransmitters or neuromodulators on chemoreception between species (198,317). However, it is difficult to study these fine nerve endings and their potential role in plasticity with chronic hypoxia remains to be investigated.

At the neurotransmitter level, there is evidence in support of roles for several neuromodulators of the carotid body in contributing to VAH. These have been expertly reviewed elsewhere (198, 317), and will not be extensively discussed here. Also, studies since 1995 are summarized nicely in Table 3 of Teppema and Dahan (2010) and earlier work is reviewed by Bisgard and Forster (28). The experimental evidence is strongest for contributions of DA, endothelin, and cytokines to increased carotid body O_2 sensitivity or carotid sinus nerve activity with chronic hypoxia. Other neurochemicals in the carotid body that change with chronic hypoxia, but that do not necessarily contribute to increased chemoreceptor activity and VAH, include several other neuropeptides (e.g., SP, neuropeptide y, vasoactive intestinal peptide, and calcitonin generelated peptide), angiotensin II, melatonin, and ATP. Acetylcholine and norepinepherine (NE) have excitatory effects on the carotid body but chronic hypoxia does not clearly increase these effects (198).

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The effects of DA on D_2Rs in the carotid body are interesting because they are inhibitory and tend to balance excitatory effects of D_2Rs in the CNS (see later). Chronic hypoxia causes dramatic increases in carotid body DA levels and would be predicted to increase the inhibitory effects of DA on carotid bodies (199). However, the effects of DA at the carotid body change during chronic sustained hypoxia (166). Blocking peripheral D_2Rs with domperidone (a D_2R antagonist that does not cross the blood brain barrier) increases ventilation in goats exposed to 4 to 6 h of hypoxia (173, 174) and in humans exposed to 8 h of isocapnic hypoxia (305); this is consistent with observations of increased DA release during early sustained hypoxia. In contrast, blocking peripheral D_2Rs with domperidone after 1 to 2 days of sustained hypoxia has no effect on ventilation in rats (25, 163) or cats (393). This is consistent with decreased D_2R inhibition at the carotid bodies explaining VAH, and involves, at least, decreased D_2R expression (165) and depletion of DA in the carotid body (25). However, the inhibitory effects of D_2Rs in the carotid body return after 7 to 8 days of hypoxia in rats as D_2R expression and carotid body DA levels increase above normoxic control levels (25, 165). It is hypothesized that restoring carotid body D_2R inhibition under ambient conditions (i.e., chronic hypoxia) restores maximum chemosensitivity so an organism has the ability to respond to changes in O_2 around the new chronic level (163). This is consistent with the general principle of homeoplasticity, in which sensory systems reestablish control levels of sensitivity after exposure to chronic perturbations (400). This remains to be tested by exploring the role of D_2Rs in chronically hypoxic subjects over the full range of carotid body stimulation, including deeper levels of hypoxia than those studied in normoxic control animals.

Endothelin 1 (ET-1) is a potent vasoconstrictor but there is evidence that it plays a role in VAH by increasing the O_2 sensitivity of carotid bodies by neural versus vascular effects. ET-1 is a vasoactive peptide that increases with chronic hypoxia under the influence of hypoxia-inducible factor-1α (HIF-1α) (319) in O_2-sensitive tissues such as the carotid body (45), and enhances the carotid body response to hypoxia mediated by endothelin A receptors on glomus cells. For example, chronic hypoxia increases the expression of ET-1 and endothelin A receptors in glomus cells, whereas blocking endothelin A receptors reverses the increased hypoxic sensitivity of carotid bodies during chronic sustained hypoxia (45). It is not known if ET-1 in the CNS has a role in VAH (see later), but it does have specific neuromodulatory effects on glutamatergic neurotransmission in the CNS (431).

Recently, inflammatory cytokines have been shown to be important for the increased O_2 sensitivity of carotid bodies with chronic sustained hypoxia (220). Expression of mRNA for monocyte chemoattractant protein-1, interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNFα) all increase in the carotid bodies of rats exposed to chronic sustained hypoxia. Increased cytokine mRNA is associated with increases in immune cells (ED1+ macrophages) in the carotid body, but there is also evidence for increased expression in the glomus and sustentacular (chief) cells in the carotid body, as also reported by other laboratories (206). Treating rats with anti-inflammatory drugs during exposure to chronic hypoxia blocks or blunts the increases in cytokine expression in the carotid body, and importantly, blocks the increase in carotid body neural activity and O_2 sensitivity measured in *in vitro* preparations (220). Liu et al. (2011) proposed that chronic hypoxia increases carotid body O_2 sensitivity by the mechanisms responsible for hyperalgesia (i.e., increased

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pain sensation to a noxious stimulus) (219). In support of this, they point out that hyperalgesia depends on cytokines released from activated immune cells and glia, including IL-1β, IL-6, and TNF- α, which increase sensory nerve excitability by affecting several possible ion channels. Moreover, anti-inflammatory drugs block both increases in cytokines and hyperalgesia. The physiological significance of inflammatory signaling in the carotid bodies was demonstrated in rats treated with ibuprofen during acclimatization to chronic hypoxia, which completely blocked VAH (315). As discussed later, this also involves inflammatory signaling in the CNS. The mechanisms by which chronic hypoxia increase proinflammatory cytokines in the carotid body remain to be determined, but it may involve HIF-1α (315). The role of HIF-1α in both carotid body and the CNS plasticity are considered together in a separate section later.

Summarizing, contributions of the carotid body to VAH include plasticity in molecular mechanisms of chemoreception and neurotransmitters or neuromodulators. It is interesting that all of the neurochemical mechanisms in the carotid body have demonstrated potential parallel mechanisms in the CNS and are under the control of HIF-1α, as discussed in the next two sections.

Central nervous system plasticity mechanisms of ventilatory acclimatization to hypoxia—As described earlier, studies in animal models report a 1.6- to 3.6-fold increase in the frequency of action potentials from carotid body afferents with chronic sustained hypoxia. The next question is if this is sufficient to explain VAH or if additional mechanisms, such as an increased CNS gain of the HVR, are necessary to explain VAH. Answering this requires measuring both changes in ventilation and carotid body activity in normoxic control and chronically hypoxia subjects, which is experimentally challenging. There are two studies using this approach that provide strong experimental evidence *against* plasticity in the CNS contributing to VAH, and both involve relatively short exposures to sustained hypoxia.

First, Weil's group measured the acute HVR and carotid body O_2 sensitivity in cats exposed to 2 days of hypoxia and calculated a "translation index" for carotid sinus nerve activity into ventilation (406). They found no significant differences in the translation index after 2 days of sustained hypoxia, but it is worth noting that this index can change, as it significantly decreased after 3 to 4 weeks of sustained hypoxia in cats that showed HD (391, and see later). Second, Bisgard and his colleagues performed several ventilatory studies in goats and showed ventilation increased almost twofold after 4 h at Pa_{O2} = 40 Torr (Fig. 5 in Ref. 30). This can be compared to the 3.6-fold increase in carotid body activity under similar conditions, so ventilatory responsiveness to arterial chemoreceptor afferent input certainly is not increasing. Also, the ventilatory response to carotid body stimulation with sodium cyanide in goats was not changed by 4 h of sustained hypoxia, indicating no change in reflex responsiveness to arterial chemoreceptor afferent input (86). Hence, changes in carotid body O_2 sensitivity adequately explain VAH over 4 to 48 h in goats and cats, respectively.

In contrast, more experiments find strong evidence supporting increases in the CNS gain of the HVR with longer sustained hypoxia. The Wisconsin group found significant increases in ventilatory response to sodium cyanide and doxapram in ponies exposed to 3 to 4 days of

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hypoxia, and to doxapram in humans exposed to 2 to 3 weeks of hypoxia (98, 100). Interpreting these results as increased CNS gain of the HVR assumes that the drugs have the same effect on arterial chemoreceptors before and after chronic hypoxia, which is probably reasonable (320). More recent experiments actually measured the effects of doxapram on carotid body activity in rats and showed it did not change after 2 days of sustained hypoxia, while the ventilatory response to doxapram increased significantly as predicted for an increased CNS gain of the HVR (417). Even more definitive evidence for an increased CNS gain of the HVR was obtained from experiments quantifying the relationship between arterial chemoreceptor input and phrenic nerve output in normoxic control and chronically hypoxic anesthetized rats (77). Assuming no changes in the efferent arm of the HVR or lung mechanics, as discussed above, this experiment isolates the effects of chronic hypoxia on ventilatory motor responses to the CNS. Figure 8 shows that 7 days of sustained hypoxia significantly increased the effect of a given level of carotid sinus nerve stimulation on phrenic burst frequency and minute activity, analogous to f_R and inspired ventilation, respectively (reproduced from Fig. 3 of 320). Similar trends were observed after 2 days of sustained hypoxia although they were not quite significant. Hence, in all species studied except cats, which have some unusual time domains of the HVR with, for example, HD occurring in only 28 days, the CNS gain of the HVR appears to increase between 2 days and 4 weeks of sustained hypoxia.

The neural substrate, or possible sites, for plasticity in the CNS gain of the HVR includes the respiratory nuclei and pathways in the HVR reflex. Summarizing from several excellent reviews (72,180,273), afferent input from the carotid and aortic chemoreceptors travel in the glossopharyngeal and vagus nerves, respectively, to the caudal NTS. The NTS contains bulbospinal neurons that project directly to phrenic motor nuclei (PMN) and propriobulbar (premotor) neurons projecting to the ventral respiratory group (VRG). The rostral VRG (rVRG) contains bulbospinal neurons that form the primary projection to PMN. The rVRG also receives projections from most respiratory nuclei, including the pre-Bötzinger complex (PBC), which contains neurons generating the respiratory rhythm (368). Nuclei involved in central CO_2 chemosensivity can also contribute to the HVR. The NTS receives the primary input from carotid body afferents and has intrinsic CO_2 chemosensitivity (51). The PBC also has neurons that are directly affected by hypoxia; so chronic hypoxia could have direct effects on rhythm generation. Nuclei involved in central CO_2 chemosensivity can also contribute to the HVR. The retrotrapezoid nucleus (RTN), which is the classic ventral medullary site for central CO_2-sensitive chemoreception, receives input from the carotid bodies (via the NTS) and projects to various respiratory nuclei (137).

Recent results show a hyperadditive (multiplicative) increase in ventilatory sensitivity of central chemoreceptors to CO_2 with increased carotid body stimulation in awake dogs (31). These authors hypothesized that increased carotid body activity with chronic hypoxia could contribute to VAH and VDH, but it is difficult to explain the increased phrenic nerve response in chronically hypoxic rats with a fixed level of carotid sinus nerve stimulation from these results (77). One possibility for modulating the CNS gain of the HVR is direct O_2 sensitivity in respiratory control circuits. O_2-sensitive sites in the CNS that affect breathing include the PBC, which is located in the rostral ventrolateral medulla (RVLM) near C1 sympatho-excitatory neurons that are also excited by hypoxia (374, 380). The breathing

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response to hypoxic stimulation of the PBC is augmented breaths and sighs (333, 374, 381). The putative molecular mechanism for central O_2 sensitivity is heme-oxygenase 2 (HO-2), which is only expressed constitutively in RVLM neurons that depolarize in response to hypoxia or cyanide (61).

HO-2 can affect neural activity by direct effects on ion channels, indirect effects of reaction products (i.e., CO or biliverdin, or less likely iron), and/or downstream targets of these products, such as ROS (280). Ionotropic glutamate receptors can modify the effects of hypoxia on the PBC, but they are not necessary for the response (373). Similarly, the NTS receives the primary input from carotid body afferents and has intrinsic CO_2 chemosensitivity (51, 57). The HVR is blunted in transgenic mice with the HO-2 gene deleted (5) but there is no strong evidence that central O_2 sensitivity contributes to VAH (318). Chronic hypoxia decreases HO-2 levels in the RVLM, and this is compensated by an increase in HO-1, which is an inducible form of heme oxygenase (280). The increase in HO-1 contributes to the recovery from an initial decrease in the frequency of sighs and sympathetic activity with chronic hypoxia but it does not affect the overall ventilatory response during chronic hypoxia (381). Hence, heme oxygenases modulate hypoxic sensitivity of the neural networks generating sighs and sympathetic activity, but there is no evidence for their direct effects on the CNS gain of the HVR with chronic hypoxia. Finally, when discussing central O_2 sensitivity, there are important precedents in the literature on central CO_2 sensitivity that should be considered (318). For example, Guyenet and co-workers have pointed out a distinction between "CO_2 chemosensitivity" of CNS neurons versus central "CO_2 chemoreceptors." This distinction may be important in considerations of central O_2 sensitivity also (e.g., see Ref. 74). Similarly, the significance of multiple sites of central O_2 sensitivity (281) is no more obvious than it is for central CO_2 sensitivity (137), and requires further study.

Physiological mechanisms in these CNS pathways and nuclei that could mediate plasticity with chronic hypoxia include, at least, neurotransmitters and modulators, growth factors, inflammatory signals, and O_2-sensitive gene expression. We will discuss catecholamines first because of the dual role for DA at D_2Rs in both the carotid bodies (see earlier) and the CNS. This is illustrated in Figure 7, which plots ventilation versus carotid sinus nerve activity measured simultaneously in anesthetized cats (365). Blocking D_2Rs in the carotid bodies and CNS with haloperidol increases carotid body activity for a given Pa_{O2}, but decreases ventilation for a given carotid body activity. Assuming no change in the efferent arm of the ventilatory chemoreflex, this implies that D_2R activation in the CNS decreases the threshold for a ventilatory response to carotid body activity and increases the CNS gain of the HVR. The cogency of this assumption has been discussed and validated (320).

The excitatory effect of D_2Rs in the CNS on the HVR were first described by Hedner et al. (1982) and opposed the known inhibitory effects of D_2Rs in the carotid bodies (146). Experiments in rats and mice support the idea that D_2R activation contributes to the increased CNS gain of the HVR with chronic hypoxia, primarily through an effect on f_R, but it takes a week for this effect to develop (163, 166, 320). After only 2 days of sustained hypoxia, the excitatory effect of D_2R in the CNS on f_R actually decreases, but after 8 days, it returns and becomes more prominent during hypoxia, which is the new "normal" or chronic

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O_2 level for the animals (163, 166). Expression of D_2R mRNA changes in the caudal NTS during this time have been examined but the changes do not parallel the effects of D_2R on the HVR; other mechanisms must be involved, such as mRNA stability, protein levels, membrane insertion, and/or signal transduction (165,320). Hence, D_2Rs in the CNS contribute to the increased CNS gain of the HVR after one week of sustained hypoxia. However, the primary function of D_2Rs in VAH appears to be maintaining chemoreceptor and ventilatory sensitivity in an optimum range in different chronic O_2 environments, through the principle of homeoplasticity (400), as discussed for carotid body plasticity earlier.

In addition to DA, glutamate is another likely candidate to mediate the CNS component of VAH, as it is the major excitatory neurotransmitter for the HVR in the NTS. Of particular interest are glutamatergic synapses between the carotid sinus nerve and the NTS. As discussed earlier, carotid body afferents terminate within the NTS (216), and recently, evidence has begun to emerge suggesting glutamatergic NMDARs located within the NTS play a key role in the CNS component of VAH. Figure 9A illustrates a unique and essential role for NMDARs in VAH; the acute HVR is abolished by blocking NMDARs in the NTS of chronically hypoxic, awake rats (299). This is consistent with the effect predicted from systemic NMDAR blockade (336), but also surprising. One would predict an increased HVR simply from increased carotid body activity with chronic hypoxia (see earlier). Further experiments will be necessary to directly test the role of NMDARs on the CNS gain of the HVR (e.g., by comparing responses to fixed levels of carotid sinus nerve activity in control and chronically hypoxic subjects).

In contrast, Figure 9B shows that non-NMDA glutamatergic receptors (i.e., α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors, AMPARs) contribute to the ventilatory response to the ventilatory chemoreflex under all conditions (i.e., acute or chronic hypoxia and hypercapnia not shown) and they do not play a unique role in VAH (299), but see also (302). This is consistent with the effect of glutamate at AMPARs on respiratory premotor neurons, which is an “additive effect,” in contrast to a “multiplicative” effect of GABA receptors (236). Hence, the overall increase in ventilatory drive with chronic hypoxia, which is observed in acute hypoxia, normoxia, or hypercapnia, could result from increased AMPAR activity with tonic increases in afferent activity from carotid bodies that occurs with chronic hypoxia (317). The normal effect of AMPARs is to activate and desensitize rapidly, thereby mediating fast excitatory synaptic transmission, this contrasts with the role of NMDARs receptors, which is to generally control neuronal excitability by modulating repetitive firing behavior and contributing to nonlinear behaviors and use-dependent synaptic plasticity (337).

It is interesting to note that with regard to glutamatergic synaptic transmission, VAH shows similarities to canonical LTP (i.e., a long-lasting enhancement of signal transmission between two neurons), since it enhances respiration for hours to weeks after removal of the hypoxic stimulus and because increased respiration occurs via elevated excitatory signaling in respiratory circuits (i.e., potentiation). As described earlier, canonical LTP in the hippocampus is mediated by enhanced glutamatergic activity, which can be due to either increased presynaptic glutamate release (induced by retrograde NO signaling from the

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postsynaptic neuron) and/or increased postsynaptic sensitivity to glutamate (due to increased glutamatergic AMPAR or NMDAR expression or sensitivity) (228). NMDARs are central to both forms of LTP as their activation is preceded by AMPAR activation, and also because postsynaptic NMDAR-mediated Ca^{2+} influx leads to the phosphorylation of nNOS to increase NO formation and subsequently pre-synaptic glutamate release (10, 41, 192, 195, 228, 260, 263, 359).

Specific to the HVR, elegant experiments in awake rats provided the first *in vivo* evidence that NO acts as retrograde messenger by exerting positive feedback on glutamate release in the NTS to augment the HVR (287). There is also some evidence supporting a potential role for NO in VAH. For example, nNOS mRNA, protein expression, and enzymatic activity increase following 24 h of hypoxia in rat CNS (324), and following 2 weeks of chronic sustained hypoxia in murine medulla (83); however, these authors did not attempt to manipulate NMDARs or examine the underlying mechanism of LTP directly. Controversially, these authors also reported that systemic injection of a nNOS antagonist reduces murine VAH, while others have shown that normoxic respiration increases and the HVR is augmented in nNOS null mice (193). Furthermore, systemic or NTS-specific inhibition of nNOS in chronically hypoxic rats has no effect on VAH (301). Confoundingly, NO has opposing effects at the carotid bodies (inhibitory) versus the NTS (excitatory), which makes these *in vivo* results difficult to interpret (49, 192, 287).

Notably, glutamate levels in the NTS return to baseline within minutes of hypoxic onset and phrenic nerve activity decreases (259, 339), indicating that new protein synthesis or receptor modification is required to sustain or enhance the HVR during longer term hypoxia (i.e., VAH), which occurs during chronic sustained hypoxia and potentially in chronic lung disorders. Indeed, in other areas of the CNS, it has been demonstrated that with prolonged activation of the canonical glutamate/NO LTP pathway, the expression of involved proteins, such as NMDARs and nNOSs increase to further enhance the sensitivity of the system (10). Similarly, in mice and rats exposed to chronic hypoxia, the expression of phosphorylated AMPAR and NMDAR subunits and also nNOS increase in the NTS (83, 299, 301, 335, 434). Overall though, while there is clear evidence that glutamatergic neurotransmission is central to the CNS component of VAH, the role of NO and nNOS in mediating this effect remains controversial, and results to date point at potential species differences.

Beyond ionotropic glutamatergic transmission, the effects of metabotropic glutamate receptors (mGluRs) remain to be investigated. Activation of mGluRs in neurons causes a rise in intracellular Ca^{2+} mediated by phospholipase C (354), and this mechanism has been linked to synaptic plasticity (239). Both metabotropic and ionotropic glutamate receptors in the NTS are known to be involved in cardiovascular reflexes, which share many common properties with ventilatory reflexes (123), as are ionotropic glutamate receptors in the NTS (436).

Moving from excitatory to inhibitory synaptic transmission, GABA is also a strong candidate for increasing the CNS gain of the HVR considering its multiplicative effect on respiratory premotor neuron activity, which is in contrast to the additive effect of AMPARs (236). $GABA_A$ and $GABA_B$ receptors are both present in the caudal NTS and chronic

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hypoxia reduces the sensitivity of $GABA_A$ receptors in neurons isolated from the caudal NTS of rats (397). Moving to whole animal models, Chung and colleagues (2006) studied the effect of blocking $GABA_A$ receptors in the caudal NTS of awake rats and found no effect on ventilation on the HVR in awake, normoxic control rats or on ventilation in chronically hypoxic rats (50). However, $GABA_A$ receptor blockade in chronically hypoxic rats decreases ventilation during normoxia, that is, it reduces VDH, the persistent hyperventilation after chronic hypoxia. The effects of blocking $GABA_A$ and $GABA_B$ receptors simultaneously are similar but the reduction in VDH is primarily through an effect on $V_T$in contrast to an effect on f_R with $GABA_A$ receptor blockade alone. Hence, GABA does not appear to contribute to the increased CNS gain of the HVR with chronic hypoxia in rats *in vivo* (although the study size is small), and the difference between GABA and NMDA receptor effects provides evidence that VAH and VDH are independent mechanisms, as discussed later.

Recent electrophysiological studies of NTS second-order neurons in *ex vivo* brainstem slices provide evidence of roles for additional membrane proteins in mediating changes in neuronal excitability in chronic sustained hypoxia. For example, in neurons from nonacclimatized rats, hypoxia activates an inhibitory ATP-sensitive K^+ (K_{ATP}) channel-mediated conductance that opposes neuronal excitation (433, 434). In neurons from chronic sustained hypoxia-conditioned rats (7 days in 10% O_2), this inhibitory current is markedly reduced, likely due to a significant reduction of K_{ATP} channel subunit expression in the NTS, and electrically evoked postsynaptic currents (eEPSC) are larger. In addition, the astrocytic inhibition of K^+ currents is reduced in chronic hypoxic (24 h in 10% O_2) rats relative to normoxic controls (4). These mechanisms would render NTS second-order neurons considerably more excitable to stimulation following acclimatization to chronic sustained hypoxia. Electrical stimulation via nonglutamatergic pathways may thus be enhanced by this post-synaptic mechanism and underlie a portion of the GluR antagonist-insensitive ventilatory response. Signaling pathways for such mechanisms of plasticity are not known; however, chronic sustained hypoxia has been shown to modify a wide variety of signaling molecules, and potential candidates will be discussed next.

NE is an important neurotransmitter in A2 neurons of the NTS, which receives input from arterial chemoreceptors; chronic hypoxia increases NE turnover and TH expression (i.e., the rate-limiting enzyme for NE synthesis) in caudal A2 regions (376). Such changes are not observed in acute hypoxia or in chronic hypoxia after carotid body denervation. Unfortunately, the effect of NE on the CNS gain of the HVR has not been measured *in vivo*; however, *in vitro* electrophysiological recordings from rat NTS second-order neurons demonstrate that: (i) eEPSC are larger in neurons from CSH-acclimatized rats, (ii) NE inhibits these eEPSCs in a dose-dependent fashion, (iii) NE-dependent inhibition of eEPSCs is greater in neurons from CSH-acclimatized rats, and (iv) this inhibition occurs via activation of presynaptic α2-adrenoreceptors (434). Further experiments are required to evaluate the role of NE and TH in modulating ventilatory responses to hypoxia *in vivo*.

Inflammatory signaling is also involved in the increased CNS gain of the HVR, similar to the effects described above for inflammatory signals in increased O_2 sensitivity of carotid bodies. Popa and colleagues (2011) treated rats with ibuprofen during acclimatization to hypoxia and this completely blocked VAH without affecting the persistent hyperventilation

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in normoxia (i.e., VDH). Ventilation is predicted to be elevated after chronic hypoxia from an increased CNS gain of the HVR even if carotid body activity was not increased, so the results indicate that ibuprofen also blocks the increased CNS gain of the HVR. Chronic hypoxia increases mRNA for inflammatory cytokines (IL-1β and IL-6, but not TNF-α) in biopsies of the NTS, and this is blocked by ibuprofen, similar to results for the carotid body (219). Hence, similar mechanisms may be involved in plasticity in both peripheral and central nervous systems. The exact mechanism by which chronic hypoxia induces inflammatory signals is not known but may involve HIF-1, which regulates innate immunity and is hypothesized to have evolved to allow phagocytic cells to operate efficiently in the hypoxic microenvironment of infected tissues (439). Hypoxia indirectly activates NF-κB, which promotes transcription of TNF-α and other cytokines (340), but NF-κB also promotes expression of HIF-1α, which makes the interaction complex. The important point is that ibuprofen has anti-inflammatory actions independent of its classical effect to inhibit cyclooxygenases 1 and 2, which control the production of prostanoids that can sensitize nociceptors (219). However, potential effects of prostaglandins on the CNS gain of the HVR (or carotid bodies) have not been ruled out.

Growth factors, which play critical roles in plasticity of the HVR with intermittent hypoxia (see later), contribute to VAH also. Activating platelet-derived growth factor-β (PDGF-β) receptors in the NTS of rats attenuates the acute HVR and eliminates HVD (133). However, the inhibitory effect of PDGF-β receptor activation on the HVR wanes during sustained hypoxia as PDGF-β receptor protein decreases (7). This suggests that disinhibition of the PDGF-β effect contributes to VAH in rats. However, further work is necessary because HVD is not as prominent in all rat experiments as it was in the PDGF-β study and HVD persists during chronic hypoxia in humans and it does contribute to VAH (351).

Lastly, erythropoietin (Epo) mediates important changes in the HVR through nonerythropoietic effects in the CNS in rats and mice (330). Effects of Epo on ventilatory control have been demonstrated in transgenic mice that overexpress human Epo in the brain (TG21 mice) (372). TG21 mice have a greater acute HVR and VAH is greater than in wild type mice. Injecting recombinant human Epo intravenously alters the breathing pattern without changing ventilation in wild type mice, presumably by effects on the carotid body. However, the importance of Epo in the CNS for the HVR was demonstrated in carotid body denervated mice; severe hypoxia (6% O_2) evokes a reduced but significant acute HVR in denervated Tg21 mice, in contrast to ventilatory depression in denervated wild-type mice. Subsequent studies showed that chronic hypoxia decreases the level of soluble Epo receptor, which is an endogenous antagonist of Epo. This increases circulating Epo levels and stimulatory effects on the HVR. The importance of this change for VAH, and a putative CNS site of the action, was demonstrated by intracerebroventricular infusions of soluble Epo receptor in acclimatized mice, which reverses VAH and lowers brain Epo levels. Moreover, rats raised at high altitude have increased Epo receptor expression in the brainstem, but not the forebrain (355). The exact mechanisms by which Epo induces CNS plasticity to mediate VAH is not certain but are likely related to neuroprotective mechanisms of Epo in stroke pathology (371) and may involve extracellular signal-related kinase (ERK) and protein kinase B (Akt) signaling, which also contribute to plasticity in intermittent hypoxia (see later). Effects of Epo on VAH may involve HIF-1 also. VAH is blunted in transgenic female

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mice over expressing Epo in the brain and periphery, and estradiol is known to decrease expression of HIF-1 (111). This effect is reversed by ovarectomy but also by carotid body denervation so CNS effects remain to be demonstrated.

O_2-sensitive gene expression and plasticity in ventilatory acclimatization to hypoxia—The time course of VAH is suggestive of changes in gene expression and, indeed, O_2-sensitive gene expression appears to be involved. HIF-1 is the most widely studied transcription factor and it appears to coordinate several aspects of oxygen transport in response to hypoxia. As covered in recent reviews (326,356), HIF-1 was discovered as the transcription factor for Epo. It binds to an eight base pair segment of hypoxia response elements in O_2-sensitive genes to activate their transcription. HIF-1 activity depends on phosphorylation and its binding to DNA is redox-sensitive. HIF-1 is a heterodimer composed of HIF-1α and HIF-1β, which is also known as the aryl hydrocarbon receptor nuclear translocator. Both HIF-1 subunits are expressed constitutively, but HIF-1α is subject to degradation under normoxic conditions. Prolyl hydroxylases (PHD) act on two specific prolyl residues in the oxygen-dependent-degradation domains (ODD) of HIF-1α subunits in the presence of oxygen, Fe^{2+} and 2-oxogluterate. In normoxia, the ODD binds to the Von Hippel-Lindau protein, which provides recognition for the E3 ubiquitin ligase complex and leads to proteasomal degradation of HIF-1α. Factor inhibiting HIF (FIH) acts similarly by hydroxylating asparagine residues in the carboxy-terminal-trans-activation domain of HIF-1α to block association of HIF-1 with CBP/p300 transcriptional coactivators.

HIF-1α is not necessary for the normal HVR in mice but it does contribute to O_2 sensing in the carotid body and VAH (319). Kline et al. (2002) studied transgenic mice with only one copy of the HIF-1α gene (the homozygous “knock out” is embryonic lethal) and found no difference in the acute HVR or carotid body morphology compared to wild-type mice (191). However, carotid body neural responses to hypoxia were decreased in the HIF-1α heterozygous mice by unknown mechanisms so other ventilatory drives not dependent on HIF- 1α must have compensated.

HIF-1α increases in both the carotid body and CNS respiratory centers after only 1 h of hypoxia (304) and effects of HIF-1α at both sites play a role in VAH. The f_R response to hypoxia decreases with chronic hypoxia inHIF-1$\alpha^{+/-}$ mice, in contrast to an increase in wild-type mice (191). The depressed O_2 sensitivity of the carotid bodies in HIF-1$\alpha^{+/-}$ mice probably contributes to this depressed VAH. Deleting one copy of HIF-1α can also affect many of the mediators of plasticity in carotid bodies with chronic hypoxia, including DA (via TH), ET-1, and Epo. Carotid body neural activity would need to be measured in chronically hypoxic HIF-1$\alpha^{+/-}$ mice to distinguish arterial chemoreceptors versus CNS plasticity in explaining differences in VAH. However, preliminary results with homozygous HIF-1α deletion restricted to the caudal NTS showHIF-1α contributes to an increased CNS gain of the HVR (124), similar to the deletion of one copy of HIF-1α gene in the carotid body. This is not surprising considering many of the mediators of CNS plasticity in chronic hypoxia are affected by HIF-1α, including Epo and inflammatory signals.

There is also evidence for a role of HIF-1α in human VAH in studies of patients with Chuvash polycythemia (122, 369). This is a rare autosomal recessive genetic disease that

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impairs, but does not ablate, the regulation of HIF by interfering with the normal function of von Hippel-Lindau tumor suppressor protein to degrade HIF. Individuals with Chuvash polycythemia exhibit signs of VAH (hypocapnia and increased HVR) without any prior exposure to hypoxia (369).

The roles of other O_2-sensitive transcription factors in VAH have not been investigated as extensively as HIF-1α. HIF-2α is a likely candidate considering the role of Epo in VAH (see earlier) and that HIF-2α is the major physiological regulator of Epo (326). Also, HIF-2α and HIF-3α increase in the carotid body with both chronic sustained and intermittent hypoxia, in contrast to HIF-1α,which only increases with sustained hypoxia (206). The specific roles of the different HIF-1 subunits in the HVR and VAH are unknown. FIH affects ventilation and the HVR as evidenced by an increased ventilation/metabolism ratio, hypocapnia, and a compensatory respiratory alkalosis in normoxic transgenic mice with the FIH gene deleted. In terms of ventilation, normoxic FIH null mice appear to be acclimatized to chronic hypoxia, but they have normal hematocrits and reduced Epo responses to acute hypoxia. Hence, the effects of FIH on ventilatory control may not be mediated uniquely through HIF and further study is needed to test their role in VAH. Finally, activator protein-1 is involved in carotid body plasticity with chronic intermittent hypoxia (CIH) (323), but it has not been studied in chronic sustained hypoxia. Hypoxia-inducible gene expression, mediated by factors other than HIF-1 or HIF-2 are reviewed elsewhere (326).

Summarizing, VAH is one of the better-understood time domains in terms of mechanisms. VAH involves cellular remodeling at the level of the carotid body and neurochemical changes that increase chemosensitivity to changes in Pa_{O2}. It also involves synaptic remodeling within the NTS and other synaptic interfaces in the respiratory control circuit. Both of these steps are under the control of O_2-sensitive gene expression that ultimately results in time-dependent increases in ventilation during days to weeks of hypoxia. Some cellular mechanisms of this time domain are known, but we know essentially nothing about the signaling pathways (in comparison to LTF with intermittent hypoxia, see later) and the picture continues to develop as more and more modulators are identified that act either at the carotid bodies or within the CNS to mediate VAH.

Ventilatory deacclimatization to hypoxia

A key aspect of VAH is that following the removal of a hypoxic stimulus the increased systemic sensitivity to oxygen persists. As a result, individuals and animals will typically hyperventilate in normal oxygen environments for hours to weeks following the removal of hypoxic stimuli. Previously, this response has been defined as VDH (30, 321). VDH and VAH show similar but not identical time courses when sustained hypoxia is stopped and started. In fact, most of the experimental evidence addressing the relationship between physiological mechanisms for VDH and VAH are based on the time course for changes in ventilatory drive (and the arterial P_{CO2} set point) and O_2 sensitivity (i.e., the acute HVR). For example, the time course for a decrease in Pa_{CO2} in humans during the first 24 h of 3 to 5 days at high altitude (4300 m) is similar to the time course for increased Pa_{CO2} in the first 24 h after normoxia is restored (69). Similar results are reported for ponies, and coupled with measurements showing a negative correlation between ventilation and CSF $[H^+]$, it has

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been argued that changes in pH of the CSF, brain interstitial fluid, or arterial blood do not cause VAH or VDH (68). However, while these experiments exclude a potential mechanism for VDH, they are not strong evidence for VDH simply being the symmetrical elimination of VAH mechanisms. As discussed earlier for the role of DA in the carotid body and CNS in VAH, multiple mechanisms can explain a given level of ventilatory drive or HVR at different time points during sustained hypoxia.

Other experiments comparing the time course of VDH and VAH suggest different mechanisms. For example, Sato et al. found that upon returning to sea level after 14 days at 3800 m, the acute HVR returned to control levels while endtidal P_{CO2} remained less than control, that is, VDH persisted longer than VAH (350). In contrast, during VAH, end-tidal P_{CO2} stabilized after 2 to 4 days, while the HVR was still increasing at 14 days. Forster et al. (1971) found the opposite in humans acclimatized to 3100 m for 45 days; end-tidal P_{CO2} returned to normoxic baseline levels within 7 days of return to sea level, while the isocapnic HVR remained elevated for up to 45 days (99). Acid-base status and ventilatory responses to other stimuli (e.g., exercise, CO_2) were back to the normoxic baseline at this time, which supported the idea that the effects are unique to hypoxia. These results indicate that further study is necessary and experiments should focus on potential differences between plasticity of the acute HVR and O_2 sensitivity versus normoxic ventilatory drive, which causes persistent hypocapnia, during VDH.

Although known stimuli for H^+ and CO_2-sensitive chemoreceptors cannot explain VAH or VDH, some experiments suggest hypocapnia may be involved in VDH. For example, VDH does not occur after VAH in goats exposed to isocapnic hypoxia for 4 to 6 h, whereas it does occur when hypoxia is accompanied by hypocapnia (30). In contrast, human studies find no difference in VDH after 8 to 48 h of isocapnic versus poikilocapnic hypoxia (91, 387). The nature of VDH was different in these studies compared to other human studies of high-altitude acclimatization, however. Robbins laboratory found an increase in the slope hypercapnic ventilatory response curve (ventilation vs. end-tidal P_{CO2}) with no change in intercept (159), while longer periods of acclimatization usually increase the intercept of the hypercapnic ventilatory response (413). There is one other human study with longer exposure to hypoxia (100 h), but isocapnia was not maintained as precisely as in the studies from Robbins' laboratory (58). The intercept of the hypercapnic ventilatory response increased and VDH was observed after 75 h of poikilocapnic hypoxia, but not isocapnic hypoxia. Further experiments are necessary to explain species differences and the effects of hypoxic and hypocapnic exposure durations on VDH.

Interactions between arterial chemoreceptors and central chemosensitive areas may be involved in VDH as discussed earlier for VAH. In addition to network affects, plasticity in CO_2 sensitivity of central "chemoreceptors", that is independent of synaptic effects, could play a role in VDH. This has been studied with simultaneous measurements of intracellular pH and action potential responses to hypercapnia in neurons in the solitary complex (including the NTS and dorsal motor nucleus of the vagus) of perfused medullary slices under synaptic blockade (284). The percentage of CO_2- sensitive neurons inhibited by hypercapnia was significantly greater in chronically hypoxic versus normoxic rats (28% vs. 8%) while the percentage of neurons activated by hypercapnia and chemosensitivity (i.e., the

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change in action potential frequency relative to a change in intracellular pH) were not altered by chronic hypoxia. These results are opposite to those expected for the increased CO_2 sensitivity reported in VDH (413), but the CO_2-inhibited neurons could be "activated" by hypoxic hypocapnia. However, it is difficult to correlate cellular chemosensitive responses to hypercapnia of neurons from one brainstem region with whole animal ventilatory responses because there are multiple chemosensitive regions and the role of each in ventilatory control is not known (272). The RTN is argued to be the most important area of chemosensitivity in the brainstem (137), and this area should be tonically activated by carotid body chemoreceptors in chronic hypoxia with inputs via the caudal NTS (386). Determining how chronic hypoxia affects the cellular CO_2 sensitivity of RTN neurons in comparison to other regions of the brainstem could help us understand their potential role in VDH.

The quantitative role of increased O_2 sensitivity in carotid bodies with chronic hypoxia is not clear, although this must contribute to VAH (317). Similar to the increases in carotid body discharge during sustained hypoxia discussed earlier (see the section on VAH), carotid body discharge increases 1.9-fold when normoxia is restored after 4 h of hypoxia (Pa_{O2} = 40 Torr) in anesthetized goats (285) or 4.9-fold in *in vitro* preparations from rats exposed to hypoxia for 7 to 16 days (45).

Finally, it is possible that VAH and VDH do in fact involve the "on" and "off" components of the same mechanisms, but that these two phases simply have different time courses. For example, if protein synthesis takes longer than degradation, an element of plasticity involving this protein may wane more quickly in VDH than it waxes in VAH. Since peripheral and central VAH are primarily due to morphological changes and new protein synthesis at the carotid body and NTS during chronic sustained hypoxia, it stands to reason that following the removal of such a hypoxic stimulus, the return to normal of the protein and morphological phenotype of these tissues could take a different period of time then their initial remodeling takes during hypoxia. The persistent hyperventilation and enhanced sensitivity to oxygen that is characteristic of VDH would then be due to the slow deprogramming of these tissues and removal of proteins that enhance synaptic signaling from the neuronal plasma membranes. Based on such a model, the fact that VDH is not coupled to VAH in goats following just 4 to 6 h would be expected, as this timeframe would not be sufficient for significant new protein synthesis to occur. It is possible, therefore, that in goats, although VAH is fully manifest within 4 to 6 h of treatment, this early response does not involve new protein synthesis. Conversely, with long-term hypoxia, secondary mechanisms involving new protein synthesis likely occur in goat (as in other models) and that VDH would occur coupled to VAH in this model as well.

Summarizing, VDH is a one of the least-understood time domains in terms of mechanisms; the cellular and molecular underpinnings of VDH have received almost no research attention. VDH may be mediated in part by changes related to hypercapnia or chemosensitivity but is most likely simply the "off" phase of VAH. As we begin to understand more about the mechanisms of VAH, this hypothesis has become simple to test and further experiments are warranted to answer this question.

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Hypoxic desensitization

When humans are exposed to prolonged chronic hypoxia (i.e., for years or a lifetime) their acute HVR is blunted and both ventilation and ventilatory sensitivity to changes in Pa_{O2} are decreased relative to normal subjects who are acclimatized to hypoxia for a shorter period of time (24, 46, 201, 357). This phenomenon is termed HD and is illustrated in Figure 10, which summarizes one of the early experimental studies documenting a “blunted” HVR in high-altitude people (414). Although HD was probably the first time domain of the HVR to be recognized, it remains among the poorest understood time domains of the HVR. In particular, the underlying cellular and molecular mechanisms that mediate HD are entirely unknown, although recent evidence shows differences between high altitude native populations that appear to have a genetic basis.

Previously, it was hypothesized that HD is a mechanism selected for energy conservation that reduces the energy spent on breathing once nonventilatory modes of acclimatization [such as increased metabolic efficiency or increased oxygen carrying capacity in the blood (78)] have had time to be upregulated (413), although experimental evidence for this is lacking. In this context, it is interesting to note that in addition to HD, another commonly observed hallmark exhibited by high-altitude sojourners is erythropoiesis, which increases red blood cell mass and blood viscosity (419, 420). However, despite such long-term enhancements to oxygen delivery systems during prolonged hypoxic exposure, HD persists, and physical and mental performance for most lowland peoples is impaired during prolonged exposures to hypoxia at altitude (418, 422, 424). Furthermore, individuals from low-altitude populations who migrate to high altitude suffer from a number of hypoxia-related diseases and struggle to reproduce at these elevations (178, 209, 266, 421). Conversely, individuals from populations that have lived at high altitude for numerous generations reproduce and have normal levels of physical activity and mental acuity at high altitude, suggesting fundamentally different physiological responses to chronic hypoxia. These differences also suggest that the hypoxia of altitude has exerted substantial evolutionary selective pressure on populations that have lived at elevation for generations, leading to the emergence of beneficial genetic or epigenetic modifications that better support life in chronic hypoxia by altering cellular or systemic function beyond breathing alone. Therefore, this time domain of the HVR may involve interplay between physiological and genetic adaptations instead of solely physiological acclimatization mechanisms mediated by molecular plasticity in the ventilatory reflex circuits, at least in human populations that have dwelled at altitude the longest.

In general, high-altitude populations are defined as peoples dwelling at elevations more than 2500 m, and who thus live in chronically hypoxic environments due to the low ambient oxygen pressure at such altitudes (i.e., hypobaric hypoxia). These human populations are mainly found in the high plateaus of Central Asia (e.g., Tibet), Africa (e.g., Ethiopia), and the South American Andes. Of these regions, the Tibetan plateaus has been colonized for approximately 25,000 years, making Tibetans among the longest dwelling groups of humans at high altitude presently identified, and also live in one of the highest populated regions on earth at approximately 4000 m (6, 264). Conversely, the Andean plateau was colonized more recently (~8000-11,000 years ago) (6). (Note: most studies of physiological responses to

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hypoxia in humans have focused on these two populations, and so we will primarily focus on these two groups for the purposes of this review.) Interestingly, these high altitude populations exhibit different susceptibilities to their hypoxic environments and divergent physiological responses that generally correlate with the length of time the population has been at altitude (see later). It is likely that different high-altitude populations have evolved adaptive mechanisms to tolerate hypoxic stress, with different combinations of mechanisms designed to increase the delivery of oxygen to tissues, and/or to reduce physiological oxygen requirements at the cellular and systemic levels (205,209). For example, Tibetan highlanders, who have lived at the highest altitude for the longest period of time, do not exhibit increased hemoglobin concentration with increased elevation (i.e., due to increased hypobaric hypoxia) (244, 307, 360), indicating that such adaptations to increase the oxygen carrying capacity of the blood may not be ideal for prolonged exposure to hypoxia.

With this in mind, it is informative to note that significant differences have been reported between the ventilatory responses to hypoxia exhibited by the various populations of highland natives that have lived at elevation for many generations, and also relative to lowland natives, including those that have lived at altitude for their entire life-time and high-altitude sojourners, who have recently migrated to altitude (e.g., Europeans, Sherpas, or Han Chinese). [Note that Sherpas migrated to altitude in the mid-1500s, and are thus a very recent addition to high-altitude populations (378).] For example, lowland-born humans exhibit increasing HD that correlates with the length of time that they have dwelled at high altitude (115, 116, 438). Conversely, relative to fully acclimated individuals who migrated from lowland populations (i.e., that exhibit fully manifested HD), high-altitude native Tibetans do not exhibit hypoventilation or a decrease in their HVR (115, 116, 438). Instead, Tibetans (162), but not Sherpas (201, 349), have HVRs that are comparable to sea-level residents at sea level (24), that is, they do not exhibit reduced f_R at altitude and their HVR is not blunted. In between these two extremes, Andean natives, whose ancestors have dwelled at altitude for a far shorter period of time than Tibetans, typically have a lower fR and Pa_{O2} in hypoxia than lowland dwelling individuals or Tibetan highlanders (i.e., they exhibit HD) (46, 201). High-altitude residents in the Rocky Mountains of North America show similar results (414). Furthermore, the sensitivity of the hypoxic ventilatory responses is markedly blunted in Andeans (204, 248, 357), and this reduction is thought to be mediated in part by reduced peripheral chemoreceptor sensitivity (414), which develops in adolescence (202, 203).

The lack of hypoventilation in Tibetans breathing hypoxia was compared comprehensively to Andeans in a study examining 320 Tibetans and 542 Bolivian Aymara between the ages of 9 to 94 years and was found to persist among men, women, children and the elderly (24). Figure 11 shows that Tibetans had a twofold greater acute HVR than Andeans; note the greater variation within the Tibetan population (24). Interestingly, recent results from low altitude native subjects indicate that a low acute HVR is the most common finding, such that a frequency distribution of the acute HVR is positively skewed (Gaio and Powell, unpublished). Additional experiments are necessary because previous publications report a normal (unskewed) distribution for the acute HVR in healthy lowlanders, in contrast to a positively skewed distribution for the acute HVR in relatives of patients with hypoventilation syndrome or endurance athletes (412). Together, these results suggest that the Tibetans may

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have more genetic variation allowing for selection of a higher HVR than lowlanders or Andean highlanders.

Nonetheless, the observation that North and South American high-altitude populations exhibit reduced f_R and a blunted HVR remains unexplained (24). Furthermore, this respiratory phenotype may not be a positive adaptation, as it is has been suggested that a low HVR is associated with increased susceptibilities to altitude-related illnesses, such as chronic mountain sickness (CMS) or acute mountain sickness (AMS) (265). In support of this, Tibetans typically exhibit extremely low rates of such illnesses relative to migrated Han Chinese, Andeans, or Rocky Mountain natives (307). Careful analysis between Andean and Tibetan populations have largely ruled out differences in gross physiology, size, hormone levels, age, development, gender, health, disease-state, or fitness as factors to explain these differing ventilatory response to hypoxia between altitude adapted populations (24), suggesting that genetic differences may underlie these disparate, and often maladaptive, physiological responses. Indeed, recent careful studies comparing the physiological adaptations to chronic hypoxia between different populations of life-long highlanders and acclimatizing lowlanders have begun to reveal key differences in their ventilatory responses and sensitivity to both O_2 and CO_2suggesting fundamentally divergent mechanisms dependent upon very long timescale (i.e., ancestral) historical exposure to chronic hypoxia (363, 364).

These different strategies exhibited by populations whose exposure to chronic hypoxia vary on the scale of several years to several thousand years clearly support the hypothesis that developmental differences due to growing up under chronic hypoxia, or acclimatization effects due to long-term exposure to hypoxia, do not exert the same effects on the control of breathing as genetic modifications due to multigenerational exposure to a hypoxic environment. Indeed, these findings strongly support a genetic basis for alternative physiological responses that mitigate the need for HD in populations that have dwelled at altitude for 10,000s of years. For example, genetic or epigenetic changes that permanently alter whole-animal oxygen demand in the longest dwelling high altitude human populations to better match environmental oxygen availability would effectively reset the oxygen needs for these humans to suit their hypoxic environment. As a result, physiological responses to hypoxia in such populations resemble those of lowland humans breathing normoxia because hypoxia at altitude is now the subjective normoxia for this population. In other words, Tibetans are now adapted to experience hypoxia at altitude as a “normal” oxygen level, in the same way that lowlanders experience sea-level oxygen-availability as normal (normoxia). As a result, they do not exhibit HD because they are not in subjective hypoxia at altitude. Conversely, high-altitude Andean populations have presumably not dwelled at altitude for a sufficiently long period of time to drive genetic mutations and resulting population-wide cellular or molecular adaptations, and instead rely still on physiological acclimatization, as evidenced by: HD, environment-induced changes to carotid body sensitivity, increased erythropoiesis, and resulting susceptibility to altitude-related illnesses.

This hypothetical framework is supported by recent genome-based analysis of hypoxia-adapted populations searching for gene changes linked to persistent hypoxia due to life at high altitude. Results from these studies have demonstrated that low oxygen availability in

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high-altitude regions does indeed exert a substantial evolutionary selective pressure on human populations. Two groups initially reported results from genome-wide screens examining the genetic basis of altitude adaptation in Tibetan highlanders whose ancestors have resided at altitude for 10,000s of years, relative to previously lowland-dwelling populations that have more recently migrated to high altitudes (Europeans or Han Chinese). Both of these studies reported divergence of the endothelial Per-Arnt-Sim domain protein 1 (*EPAS1*) gene, which is also known as HIF-2α (23, 427), and which regulates the induction of erythropoiesis by hypoxia (334). Since these initial studies, numerous other groups have reported similar findings (27, 308, 408, 423). In addition, these and other studies have reported divergence in the expression of *EGLN1*, which encodes for PHD2, a key regulatory enzyme in the HIF pathway that mediates the degradation of HIF during hypoxia (23,222,361,427). Such genetic divergence may contribute to the hypoxic adaptation of Tibetan natives that have dwelled at altitude for generations. Furthermore, it is particularly notable that this divergence represents the strongest instance of natural selection discovered in a human population to date, highlighting the ability of hypoxic environments to drive genetic adaptation on a population-wide level (361, 427).

Recent studies of human populations provide some physiological support for divergent HIF signaling as mediators of some of the systemic differences between Tibetan and Andean or lowland populations. For example, Robbins' group recently contrasted ventilatory, hematological, and cellular differences between Han and Tibetan populations living at sea level (313). These authors found that relative to lowland Han Chinese, Tibetans had lower hemoglobin concentrations, higher metabolism-corrected ventilation, and blunted ventilatory responses to both acute and chronic (8 h) hypoxia. Furthermore, analysis of peripheral blood lymphocytes revealed that both basal and also hypoxia-induced recruitment of HIF-2α and HIF-target genes was reduced in Tibetans, consistent with previous genome-wide studies. Importantly, this study also suggests that the vascular adaptations of Tibetans are not reversed by a lifetime at sea level, further supporting the hypothesis that these changes occur at the genome-level and are not easily reversed. Conversely, Andeans, who have lived at altitude for considerably less time, undergo some reversal of HD after two or more years at sea level (135). In addition, other researchers have recently reported a splice variant in the Tibetan *EGLN1* gene that encodes for a PHD2 isoform with a lower K_m value for oxygen (222), which would promote increased HIF degradation under hypoxic conditions and reduced erythropoiesis. It remains unclear, however, whether these HIF-related adaptations are solely responsible for limiting excessive erythropoiesis in Tibetans or if they also underlie pulmonary, vascular, metabolic or other adaptations. Further studies are required to sort out these questions.

Using a similar genome-wide approach, multiple groups have recently sequenced the genome of animals native to high altitude. For example, domestic yaks (*Bos grunniens*), which are adapted to life at high altitude on the Qinghai-Tibetan Plateau (329). Yaks exhibit numerous physiological adaptations to hypoxia, including enlarged lungs and heart, absence of hypoxic pulmonary vasoconstriction, and higher metabolism. By genetic comparison with closely related low-altitude cattle, a number of genes were identified that have undergone positive selection driven by generational chronic hypoxia in yak. A significant number of these genes were grouped in the hypoxia functional category. Specifically, these authors

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identified two regulators (*Adam17 and Arg2*) and a target gene of Hif-1a (*Mmp-3*), which is involved in angiogenesis, vasodilatation, and energy metabolism. Notably, *Adam17* has also been shown to be divergent between Tibetans and lowland dwellers (361). Several genes related to nutrition/ metabolism pathways also exhibited positive selection in yak (329). Beyond yaks, recent studies have also described mutations to the *EPAS* and HIF pathways that correlate with long-term life in hypoxia and increasing altitude in Tibetan dogs (130, 409) Tibetan wolves (435), and Tibetan pigs (73).

Such genome-based tools are an excellent first step in studying the underlying mechanisms of adaptations to long-term hypoxia; however, they do not address the physiological mechanisms of HD specifically, and the dearth of suitable animal models or human tissue in which to explore molecular and synaptic mechanisms that may underlie potential adaptations represents a considerable hindrance to further study. Nonetheless, the results of genome screens provide tantalizing clues that indicate the occurrence of systemic metabolic depression at the cellular level, which agrees with early hypotheses that with life-long exposure to hyperbaric hypoxia, breathing work may be reduced in the face of decreased cellular oxygen demand for metabolic needs. Indeed, a key obstacle in the study of the physiological and molecular mechanisms of HD is that, until very recently, reasonable animal models of this condition had not been identified. Furthermore, information available from animal-based studies that purport to mimic HD is conflicting. For example, cats exhibit HD following just 2 weeks of hypobaric hypoxia (simulating an altitude of 5500 m) and one study reported decreased carotid body sensitivity and CNS gain of the HVR under similar conditions; however, HD in cats reverses far more rapidly upon return to “sea-level” than in humans and another study reported that carotid body sensitivity increased under the same conditions in cats (20,391,413). Together, these results indicate that animal models acclimatized to chronic hypoxia for such short periods of time do not reasonably mimic the physiological effects of HD due to prolonged (years to a lifetime) exposure to chronic hypoxia.

Recently, researchers have turned to novel rodent models of life-long hypobaric hypoxia adaptation, including plateau pika (*Ochotona curzoniae*) and plateau zokor (*Myospalax refuescens*). Although research into the hypoxic adaptations of these rodents is at an early stage, some work regarding ventilatory control has been undertaken in pika in particular. Pika have lived at high altitude (~5000 m) for more than 30 million years and are thought to be completely acclimated to life in chronic hypoxia (314). Relative to rats, pika have lower hemoglobin concentrations, a blunted hypoxic vasoconstriction response to hypoxia, and thin-walled pulmonary arterioles to facilitate rapid gas exchange (117). Interestingly, HIF-1α protein expression is modified in pika relative to rats (210) and also in a number of other animal models of hypobaric or normobaric hypoxia, such as naked mole rats (186), which also exhibit a blunted HVR in acute hypoxia (300). When taken together with the common regulation of HIF family genes in the adaptation to life-long hypoxia in high altitude human populations and also in high altitude animal populations such as Tibetan dogs, pigs, wolves, and yaks, a common pattern becomes clearly apparent. Among the potential targets of HIF family genes in respiratory control are Epo (which increases the oxygen carrying capacity of blood), vascular endothelial growth factor (which regulates angiogenesis, lung morphology, and brain function), and several signaling intermediates

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known to interact with hypoxic plasticity in the respiratory control circuits, including heme oxygenase, TH, and NO (319). In relation to the HVR, and as discussed earlier, HIF-1 is known to target several key neuromodulators that have been linked to control of various time domains of the HVR, including ET-1, nNOSs, and TH (319). Taken together, these interactions are promising starting points from which to explore the molecular and neurological control of HD in animal models of this time domain of the HVR.

Summarizing, our understanding of the physiological effects of life-long and multigenerational hypoxic exposure is in its infancy relative to most other time domains of the HVR. It now seems that HD is actually a spectrum of mechanisms that range from physiological plasticity due to lifelong hypoxia, to population-wide genetic adaptations that are the result of natural selection for hypoxia during life at altitude dating back over millennia. Specifically, physiological responses in humans that have migrated to altitude in their lifetime or populations that have migrated to altitude in the last several thousand years tend to promote changes in the control of breathing and in the oxygen delivery systems in the body; however, these adaptations also appear to contribute to maladaptive diseases and pathologies at altitude. Conversely, the longest dwelling human populations at altitude do not exhibit a typical HD response, but instead appear to rely on adaptations outside of the control of breathing, and that likely alter cellular or systemic oxygen demand (78). It remains to be determined if the time for adaptation to altitude or the ancestral genetic variability explains the apparently different strategies exhibited by Tibetans and other high altitude human populations. Going forward, combined physiological and genomic studies of adaptations in high-altitude animal models and human populations should help answer this question.

Physiological and Molecular Responses to Episodic Hypoxic Exposures

Two distinct HVR time domains have been described that occur in response to repeated episodes of hypoxia. These are termed PA and LTF (321). PA refers to an increase in the HVR observed in the hypoxic component of respiration during successive episodes of identical hypoxic stimuli (Fig. 1). Conversely, LTF is defined as the progressive increase in respiratory motor output during normoxia following intermittent hypoxic episodes, which tends to increase over 15 to 60 min and persists for minutes to hours after the stimulus (Fig. 1). LTF in particular is an intriguing and unique time domain of the HVR as it occurs during the normoxic recovery period following the hypoxic stimulus but has no component that is manifested during the hypoxic stimulus. Occurring over minutes to a few hours, the underlying mechanisms of these two time domains involve both altered neurotransmitter and neuromodulator activity and also new protein synthesis. The primary mechanism of plasticity in these time domains appears to be alterations of neuromodulators that occur both at the carotid body and also at synaptic connections between afferents from the CNS components of the respiratory control circuit and respiratory motor neurons that innervate respiratory muscles. Whether or not these two mechanisms represent the hypoxic trigger and posthypoxic response components of the same or connected plasticity pathways remain to be determined.

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Progressive augmentation

PA refers to an increase in the HVR observed in the hypoxic component of respiration during successive episodes of identical hypoxic stimuli (Fig. 1). PA was first identified in the inspiratory intercostal nerve activity of anesthetized cats during successive 2 min periods of carotid sinus nerve stimulation at 5 min intervals (103). In this paradigm, respiratory nerve responses induced during successive hypoxic episodes increase relative to the previous episode, from approximately 65% to more than 90% of maximal activity. Since this initial study, PA has been described in a wide variety of arousal states, animals, and regions of the respiratory control circuits. PA has been described in the carotid sinus, hypoglossal, and phrenic nerves of anesthetized rats (59, 104), and the genioglossus muscle of humans (141). Furthermore, ventilatory PA has been described in awake ducks (256), goats (399), rabbits (370), and awake and sleeping humans (141,207,407). In contrast, several studies reported an absence of PA in response to intermittent hypoxic protocols in rats (240, 292) and humans (185,232,237). However, this absence was likely due to uncontrolled CO_2which was decreased in all of the aforementioned studies. In support of this, PA has been observed in most studies in which isocapnia was maintained (141, 214, 232, 345, 346, 348, 398).

PA typically involves increases in f_R and V_T and can last for at least 1 to 2 h after hypoxic stimuli in rats and humans (104,232). PA has been defined as a distinct mechanism from STP because it occurs during stimulus episodes separated by 5 min, which exceeds the time constant of STP (103,321). PA was also defined as distinct from LTF, since it is not inhibited by blocking methysergide-sensitive 5-HT receptors, which until recently, were believed to block all mechanisms of LTF (14, 19, 103). However, as we postulate in the introduction, molecular mechanisms no longer provide reliable criteria for defining a time domain of the HVR when multiple mechanisms result in similar responses, and indeed, a recently described novel pathway of LTF is also 5-HT insensitive (65,153,283) (see later), calling into question this characteristic as a qualification of LTF and as a distinction between LTF and other time domains of the HVR. Indeed, there is some evidence that PA may be mediated in part by 5-HT, since intermittent application of 5-HT to isolated rat carotid bodies induces PA of peripheral chemoreceptor activity (311). Taken together, these studies indicate that dependence on serotonergic mechanisms should not be a distinguishing characteristic between PA and LTF.

Conversely, there are many similarities between these intermittent HVRs: (i) both PA and LTF refer to an increase in ventilation during and after successive episodes of identical hypoxic stimuli; (ii) both mostly occur simultaneously and in response to the same stimulus patterns (103,207); (iii) neither PA nor LTF is now considered to be explicitly 5-HT dependant or independent (65, 153, 207); and (iv) both PA and LTF are inhibited by antioxidants and specifically NOX inhibitors (207,225,226,309,311) (see later). Taken together, these data support a mechanistic connection between the control of PA and LTF. However, it remains to be determined whether or not PA is required to induce LTF and if these two time domains of intermittent hypoxia rely upon the same molecular pathways of initiation.

Mechanistically, little is known about the underlying molecular pathways that mediate ventilatory PA, and reports to date have sometimes been conflicting. For example, some

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researchers report a role for 5-HT signaling in PA (311), while others argue that 5-HT does not play a role in this time domain (103). There is, however, general agreement that the induction of PA involves ROS production from NOX, since ROS scavengers or NOX inhibitors abolishes PA in isolated carotid bodies and also in awake rats and humans (Fig. 12) (207, 309–311). Conversely, considerably more is known about the induction and underlying mechanisms of LTF and the potential overlap between these two mechanisms means that these may also be the mechanisms of PA. Further experiments are required to accurately determine if PA and LTF are the hypoxic and posthypoxic components of the same molecular pathway; however, regardless of whether or not they share molecular pathways, they are clearly distinct time domains of the HVR, as we posit that the physiological HVR must be defined independently of its molecular underpinnings.

Long-term facilitation

Intermittent hypoxia causes LTF of respiratory motor nerve activity, which manifests as a persistent increase over the normoxic baseline for an hour or more after the acute HVR (298, 303). In general, ventilatory LTF primarily involves increases in V_T and lasts for up to 90 min after the cessation of the final hypoxic stimulus episode. LTF has been observed in a wide variety of animals, both as increased ventilation or enhanced phrenic nerve activity in awake or anesthetized animals, respectively (105, 256, 269, 321, 395). Ventilatory LTF (mediated by LTF of the phrenic nerve) has been observed in anesthetized cats (103,251) and rabbits (370), conscious dogs (44), ducks (256), and goats (399), in awake or in anesthetized mice and rats (14, 144, 292, 312, 395), and in awake or sleeping humans (12, 269, 407). In addition to LTF of the phrenic nerve, LTF has been reported in the vagal nerve in rats (383), in the hypoglossal nerve in cats (231), rats (104, 106, 243), and humans (48,141), and in the upper airway muscles in rats (289).

There is considerable heterogeneity in the magnitude and duration of LTF between species depending upon the exact hypoxic induction protocol utilized; the effects of various protocols between species have recently been expertly reviewed (234, 257, 274). Ventilatory LTF is difficult to study experimentally and appears to depend on sleep-wakefulness state, species, and the hypoxic induction protocol; this topic has also been reviewed recently (234). Most of the experimental work defining molecular mechanisms of LTF has been done in anesthetized animal preparations (primarily in rat) and focuses on phrenic LTF. However, other studies show that ventilatory LTF may be mediated by the sum of synaptic changes in genioglossal, hypoglossal, and intercostal motor responses, in addition to phrenic responses (103, 234, 243, 321). The hypoxic stimulus for LTF must be intermittent hypoxia as LTF is not induced by continuous hypoxia of the same duration as a bout of intermittent hypoxic episodes (e.g., 25 or 50 min) (76, 416).

Mechanistically, of the physiological time domains of the HVR, the underlying molecular pathways that mediate ventilatory LTF of the phrenic nerve following either episodic stimulation of the carotid sinus nerve or episodic hypoxia are the most completely understood. Until recently, 5-HT type 2 receptor (5-HT_2R) activation during, but not after, intermittent hypoxia was known as the primary molecular mechanism for LTF (16,19,107). Experimental evidence for this includes the observations that LTF induced by intermittent

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hypoxia or carotid sinus nerve stimulation is prevented by 5-HT_2R blockade with the general 5-HTR antagonist methysergide (14), or ketanserin, a specific 5-HT_2R antagonist (214, 242, 437).

The working model for the 5-HT_2 mechanism of LTF is as follows (Fig. 13). First, episodic hypoxia activates serotonergic Raphe neurons in the medulla, which results in the release of the neuromodulator 5-HT near phrenic motor neurons; such 5-HT release has been measured when the carotid sinus nerve is electrically stimulated (42), and there is strong evidence for it occurring with hypoxia as well (18, 88, 89, 187, 268, 339). Episodic activation of 5-HT_2Rs leads to synthesis of brain-derived neurotrophic factor (BDNF) in the spinal cord near the phrenic motorneurons. Evidence supporting this includes observations that intrathecal BDNF administration induces LTF without a hypoxic stimulus, and that blocking BDNF translation and protein synthesis with RNAi approaches abolishes hypoxia-induced LTF (18). BDNF subsequently activates high-affinity receptor tyrosine kinases (TrkB), which in turn activate ERK1&2 (18, 189, 415). ERK1&2 regulate glutamatergic receptor sensitivity at the postsynaptic membrane in other systems (342) and putatively this results in LTF (47, 93, 214, 242). Presumably, this involves inserting glutamate receptors into the postsynaptic membrane and/or phosphorylating them to enhance sensitivity to pre-synaptic inputs, as described for other glutamatergic systems (218, 221, 347).

Further study, however, challenged the idea that serotonergic inputs are necessary to induce LTF. For example, activation of α1-adrenergic receptors can induce phrenic nerve LTF independently of 5-HT receptors via a pathway that is mediated by Akt instead of ERKs (153,282), and LTF persists in mice genetically depleted of 5-HT in their CNS (150). A more recent study demonstrated that α1-adrenergic receptor activation is sufficient but not necessary to induce phrenic nerve LTF (168). Interestingly, both α1-adrenergic receptors and 5-HT receptors are coupled to G_q-proteins, a class of g-protein that is linked to the activity of phospholipase C (32), suggesting that these two types of receptors may converge on a common pathway and serve as common activators of LTF. Thus α1-adrenergic receptor activation may be one of many redundant trigger mechanisms that converge on G-proteins to induce LTF. It has been proposed that these mechanisms form a pathway termed the “Q” Pathway (Fig. 14) (65).

Recently, another molecular pathway capable of inducing LTF has been reported and it is mediated by spinal cord adenosine type 2A receptors (A_{2A}Rs) (127, 151, 283) and 5-HT_7Rs (127, 151, 152, 283). A_{2A}Rs signal through adeny-late cyclase-coupled G_s proteins and accordingly, this pathway has been termed the “S” pathway (Fig. 14) (65). Support for a more general role for G_s signaling in LTF is the observation that 5-HT_7Rs, which also utilize G_s, can induce long lasting phrenic motor facilitation (152). It is possible that 5-HT_7Rs play a role in the enhanced LTF observed with CIH (see later). Interestingly, the S Pathway involves activation of immature TrkB independently of BDNF synthesis and this pathway proceeds through the activation of PI3K/Akt but does not involve ERKs (127). The S and Q pathways are simultaneously initiated by intermittent hypoxia, but they tend to limit each other, since blocking only one pathway increases LTF (65, 151). This interaction is typical of G_s and G_q proteins, which interfere with each other via a well-described crosstalk mechanism in other systems (344), and may involve ROS (151), which are involved in LTF

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(207, 223–226). The physiological significance of these dual mechanisms for the HVR may involve the recent discovery that the severity of the intermittent hypoxic stimulus determines which pathway induces LTF, such that more severe hypoxic episodes (25–30 mmHg Pa_{O2}) preferably induce the S Pathway, whereas during moderate hypoxia (45–5 mmHg Pa_{O2}), the balance is shifted to favor regulation by the Q Pathway (283).

In addition to these pathways, molecular mechanisms mediated by vascular endothelial growth factor (66), and Epo (64), have also recently been described, and both pathways interact with ERK and Akt signaling (63–66, 352). It remains to be seen whether these mechanisms are components of the Q or S Pathways, or if they represent entirely new molecular mechanisms that mediate LTF.

It is interesting to note that although intermittent but not chronic hypoxic exposures are required to induce LTF, a single bolus injection of BDNF is sufficient to activate LTF. This raises important questions about the activation of this hypoxic response and how the system differentiates between hypoxic exposures. Presumably, since BDNF application alone is sufficient to induce LTF, BDNF is either not upregulated by a single chronic hypoxic exposure, or other molecular pathways interfere with the activation of BDNF to prevent the occurrence of LTF following a nonintermittent bout of hypoxia. It will be important to determine how the system is able to differentiate between a single hypoxic episode and a train of intermittent episodes with regard to the activation of BDNF synthesis and the initiation of LTF.

CIH, studied by exposing animals to several hours of intermittent hypoxia per day for between 4 days to 5 weeks, increases the magnitude of LTF (289). Increased LTF with CIH involves both elevated carotid body chemoreceptor responses to a given hypoxic stimulus [sensory LTF (310)] and increased CNS gain of the HVR, which is demonstrated by a potentiated phrenic nerve response to electrical stimulation of the carotid sinus nerve (214). Enhanced LTF due to CIH has been observed to date in cats (338), dogs (182), and rats (241, 242, 289, 309). This effect has been reported in animals treated with hypoxic episodes using hypoxic durations of between 15 s (plus 68–85 s of graded hypoxia during the cycle phase between hypoxia and normoxia) and 5 min followed by 5 min of normoxia for several days, but does not occur in response to chronic sustained hypoxia (310), although the mechanism underlying this change was manifest as increased chemoreceptor sensitivity to oxygen (309). CIH induces new synthesis of the proteins that mediate the LTF pathway (411) and increases the magnitude of phrenic nerve LTF (214, 310). Interestingly, LTF after CIH still depends on 5-HT_2Rs, but the increment in LTF with chronic versus acute IH involves central (vs. carotid body) effects of a different subtype of 5-HT receptor, which is sensitive to methysergide (214). This finding provided early evidence that LTF could be regulated by multiple mechanisms (see earlier).

As with PA, a key role for ROS has been described in the mediation of LTF in response to either acute intermittent hypoxia (AIH) or CIH, most likely in relation to the activity of 5-HT or NOX (Fig. 15). This relationship was first demonstrated in rat and mouse carotid bodies *ex vivo* in which episodic application of 5-HT induces carotid body LTF, the activity and phosphorylation of NOX, and ROS production (311, 312). In these experiments, 5-

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HT_2R or NOX antagonists or general ROS scavengers prevented LTF, and LTF did not occur in NOX knockout mutant mice (311, 312). Interestingly, this molecular mechanism has been linked to the activity of HIF-1: CIH-induced LTF does not occur in heterozygous HIF-1 knockout mice, and CIH-mediated increases in ROS production also do not occur (312). Further support for this pathway comes from measurements of rat phrenic nerve activity since superoxide scavengers or inhibitors of NOX can inhibit LTF of the phrenic nerve (223–226).

There is also some evidence of a role for NO/ glutamatergic LTP in the LTF of phrenic nerves during CIH. For example, episodic hypoxia-induced LTF of the phrenic nerve is attenuated in nNOS null mutant mice (190); and NMDAR antagonists substantially reduce ventilatory LTF in CIH rats (213); however, increases in NMDAR subunit expression are localized to the carotid body, not the NTS (220), and whole-cell NMDAR currents are reduced in the NTS (67).

An important caveat to this research is that the majority of studies have been undertaken in young male animals (primarily Sprague-Dawley rats). Sex hormones regulate plasticity in the CNS, including ventilatory plasticity responses to intermittent hypoxia (26,390). Research has demonstrated that the magnitude of phrenic nerve LTF is markedly reduced in aged male Sprague-Dawley rats (13months) relative to young male rats (3–4 months), while LTF of the hypoglossal nerve is abolished in the older population (428). This effect has been linked to the expression of sex hormones, and LTF of the phrenic and hypoglossal nerves is similarly abrogated in gonadectomized or aged male Fischer 344 rats relative to young intact animals, such that (i) the decrease in hypoglossal LTF correlates with decreased expression of the sex hormones testosterone, progesterone, and oestradiol (429), and (ii) testosterone supplementation reverses the effects of gonadectomy (430), or aging (277). Furthermore, the expression of LTF has also been shown to vary between various strains of rats, such that AIH-induced changes in 5-HT signaling and LTF of the phrenic and hypoglossal nerves are not observed in Brown Norway rats, but are more pronounced in Lewis rats relative to Fischer 344 rats (17, 128). These findings suggest that genetic and epigenetic differences may also contribute to the extent to which LTF is induced by intermittent hypoxia.

Summarizing, PA and LTF are distinct time domains of the HVR that occur during and following intermittent hypoxic episodes, respectively. It is currently unclear if they depend upon the same underlying molecular mechanisms but evidence to date suggests this may be the case. What is known regarding the role of ROS and NOX in the molecular underpinnings of PA is consistent with similar studies of their role in LTF, but PA remains understudied and further research is required. The idea that different molecular mechanisms explain LTF versus VAH (which also increases normoxic ventilatory drive after the stimulus is removed, see earlier) has also been called into question because a critical argument for this idea was that VAH does not involve 5-HT (321). Notably, LTF is the first time domain of the HVR in which multiple distinct underlying molecular mechanisms have been identified. The physiological significance of separate mechanisms for LTF remains to be determined but new evidence indicates they may be important with different degrees of hypoxia. In particular, differences in sensitivity of the Q versus S pathway to various patterns of intermittent hypoxia remain to be tested. Although LTF is perhaps the best-understood time

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domain of the HVR in animal models, in humans, the mechanisms of LTF have received considerably less attention than other time domains of the HVR. Mechanistically, similar effects of ROS scavengers as inhibitors of ventilatory LTF have been demonstrated in humans as have been reported in animals (207); but all other components of the various LTF pathways described in animal models remain untested in humans. Further research is required to understand the mechanisms of LTF in humans, and also its clinical significance.

Summary and Significance

Terminology

One of the primary objections of the 1998 hypoxic time domains review was to propose and define a common set of terms for each time domain of the HVR, to "…simplify communication among researchers and to promote the integration of results from different experimental preparations and laboratories" (321). In this aspect, the previous review was largely successful and most of the terminology proposed has been adopted and propagated throughout the field of respiratory biology. Nonetheless, with rapidly increasing advances in our knowledge of, in particular, the molecular mediators of the HVRs, the previously established definition of each time domain now require updating. For example, the acute response did not "catch on" widely with the field.

For the reasons discussed earlier, we now propose that the time domains of the HVR be defined as physiological responses that are independent of their underlying molecular pathways. This would also simplify the nomenclature being developed for the different mechanism causing LTF (e.g., phrenic vs. ventilatory vs. sensory LTF, etc.). This in no way should detract from the importance of the various molecular mediators of each HVR described to date; however, we believe that this approach will simplify communication between researchers; and moreover, is in keeping with the approach taken in the umbrella field of neuroscience within which most of these mechanisms fall. For example, synaptic plasticity mechanisms are generally defined as mediating short- or long-term potentiation or depression. There are numerous pathways that have been described that contribute to these basic overarching synaptic outcomes, but they are not each defined as unique forms of synaptic plasticity; instead they are defined as different molecular mechanisms of specific physiological responses. For example, STP (of synaptic plasticity, not respiratory drive) includes synaptic augmentation, synaptic facilitation, and posttetanic facilitation, but is referred to generally as STP. Similarly, the occurrences of synaptic plasticity at different regions of the CNS are not each given unique names. For example, LTP of cerebellar or cortical synapses are both termed LTP. The same approach should be carried over to the study of respiratory biology. Thus phrenic LTF and motorneuron LTF are not unique time domains, but instead should be described as LTF of the phrenic or motor neurons, respectively; that is, the same physiological mechanism occurring in different regions of the relay circuits that control respiration.

Clinical significance

At sea level, hypoxia is a relatively rare occurrence for healthy individuals; however, it is a common component of a wide variety of clinical disorders, and several of the time domains

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of the HVR are representative of the hypoxic stress induced by a variety of pathologies and diseases. For example, chronic obstructive pulmonary disease (COPD) has been diagnosed in more than 13 million Americans and is the third leading cause of death in the United States, costing an estimated $42.6 billion in annual health care expenses and lost productivity (1). COPD is expected to become the fourth leading global cause of death by 2030 due to increased prevalence of smoking (235). Chronic sustained hypoxemia is one of the main complications of COPD and the most common treatment of COPD is long-term oxygen therapy, which has been clearly demonstrated to be beneficial for COPD patients (249). Unfortunately, oxygen therapy is costly and impinges upon quality of life. Enhancing the endogenous VAH may produce a larger, more rapid physiological response to hypoxemia and alleviate systemic hypoxemia in lung disease, thereby reducing or eliminating the need for oxygen therapy in COPD patients (362). Conversely, in those patients where increased ventilation leads to dyspnea (shortness of breath), blocking the VAH in combination with oxygen therapy may more effectively manage COPD. However, enhancing and/or blocking VAH requires knowledge of the specific synaptic pathways that mediate VAH, and therefore elucidating these pathways is physiologically and clinically essential.

Similarly, individuals who live their lives or visit at high-altitude experience persistent hypoxia that is associated with a wide variety of high-altitude illnesses, such as AMS and CMS. In particular, CMS is associated with increased mortality due to moderate to severe pulmonary hypertension and increased susceptibility to cardiac pathology and neurological stroke (209, 262). Common symptoms of CMS include breathlessness, sleep disturbance, cyanosis, venous dilation in the hands and feet, headache, club finger, and tinnitus. These, along with dizziness, fatigue, and depressed or forgetful mental states contribute to significant morbidity, and CMS patients often struggle to work or lead productive lives (266). CMS has been classified as a global public health concern that potentially threatens more than 140 million people living at high altitude in Bolivia, Chile, China, India, Peru, Tibet, and the United States, among others. Nonetheless, Tibetan populations of high-altitude natives rarely develop altitude-related illnesses and have profoundly different ventilatory responses to hypoxia. Understanding how these individuals adapt is of significant clinical interest, as it may provide insight into the treatment of altitude-related illnesses via the modulation of HD in high-altitude natives or sojourners.

Finally, LTF is thought to be involved in sleep-disordered breathing such as obstructive sleep apnea (OSA) or sudden infant death syndrome (227, 233). The activity rate of the Raphe nucleus (within which 5-HT release that is critical to LTF of the phrenic nerve occurs) is generally depressed during sleep, whereas the activity of this nucleus is generally saturated in awake animals (149, 172). Therefore, effects of episodic hypoxic stimulation on Raphe nucleus activity are likely to be considerably more profound during periods of deep sleep. Indeed, LTF is more readily induced during deep non-REM sleep than during light sleep or wakefulness (271). However, whether LTF is the cause or the solution to sleep disordered breathing remains unknown, and studies have indicated that LTF is enhanced in individuals who have previously experienced intermittent hypoxia (101). For instance, in OSA patients experimentally exposed to intermittent hypoxia during sleep, the induction of ventilatory LTF was greater than in healthy control individuals (207). Nonetheless, whether or not

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intermittent hypoxia even mimics the impact of intermittent breathing-related hypoxia on physiology remains unknown.

More recently, LTF has been linked to improving control of breathing following acute spinal cord injury in rodents and humans. A key challenge after spinal cord injury is respiratory failure due to the disruption of brainstem-spinal cord projections that mediate ventilation via the activity of spinal cord respiratory neurons. It has been hypothesized that increasing plasticity in these neurons may improve outcomes for spinal cord injury patients and AIH has been shown to induce plasticity in these neurons (227, 254). Recent studies in rodent models of spinal cord injury and also clinical trials in human patients indicate that AIH does indeed increase the plasticity in these neurons and improve control of ventilation following acute spinal injury (126,145,275,328,396). Therefore overall, understanding the molecular and physiological mechanisms of the various time domains of the HVR may have profound impact on the treatment of a wide variety of pathologies and offer a unique and important strategy into many clinically relevant questions.

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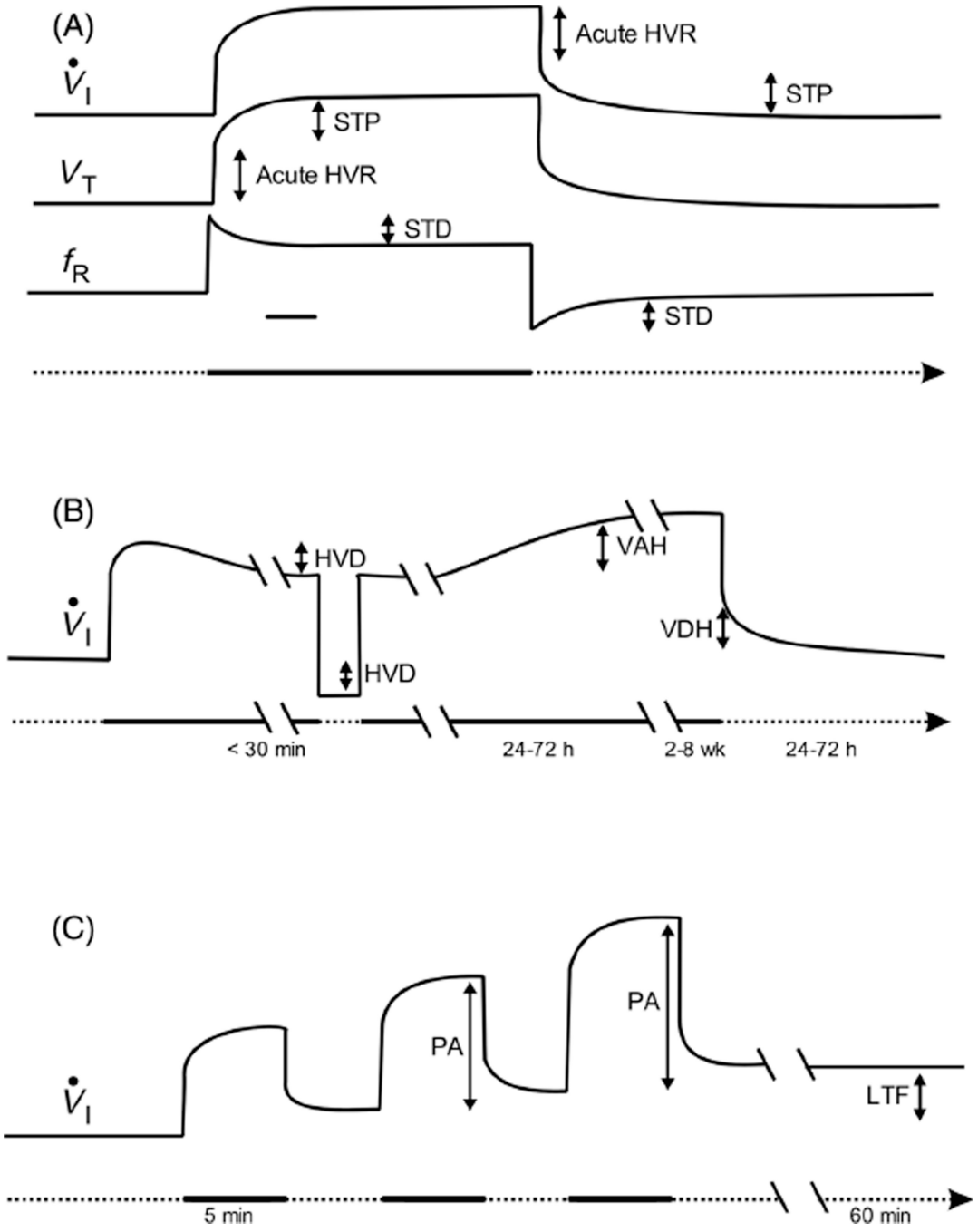

Figure 1.

Time domains of the HVR. Ventilation ($\dot{V}_I$), tidal volume (V_T), and respiratory frequency (f_R) as a function of time during normoxia (dotted baseline) and different durations and patterns of hypoxia (solid baseline). (A) The acute HVR is followed by STP and STD during brief (seconds to minutes) hypoxic exposures. (B) Ventilatory responses during and after sustained (minutes to days) hypoxic exposures include HVD, VAH, and VDH described in later sections; HD is not shown.) (C) Ventilatory responses during and after intermittent hypoxic exposures include PA and LTF (described in later sections).

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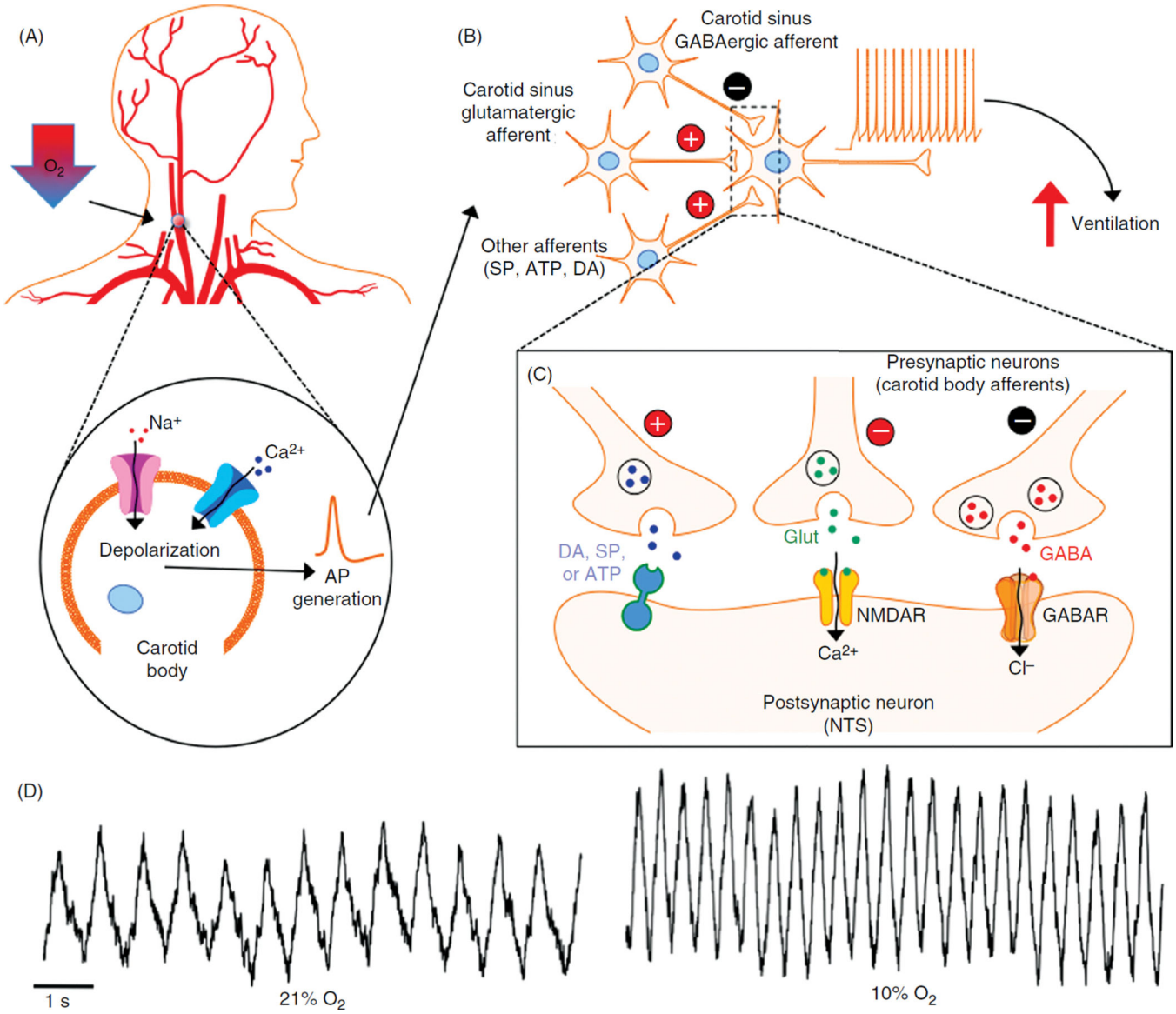

Figure 2.

Molecular mechanisms of the acute HVR. Acute hypoxia of seconds to minutes causes increased ventilation during the hypoxic stimulus. (A) During short-term hypoxia, decreased Pa_{O_2} is sensed by the peripheral chemoreceptors (carotid bodies). Hypoxia-mediated excitatory ion (Na^+ and Ca^{2+}) influx induces depolarization of the carotid bodies, which leads to the generation of action potentials that then propagate along the carotid sinus nerve. (B) Excitatory glutamatergic carotid body afferent neurons along with SP and inhibitory GABAergic neurons synapse at the NTS. If the summation of these opposing signals is net-excitatory, action potentials are generated in the NTS neurons that communicate to the respiratory motorneurons and increase ventilation via excitation of the phrenic nerve. (C) At the synapse between carotid sinus nerve afferent neurons and the NTS, pre-synaptic release of SP, glutamate, and GABA induce opposing effects on the excitability of the NTS postsynaptic second-order neurons and induce excitatory signal propagation that increases

downstream respiratory drive (i.e., the acute HVR). (D) This increased drive manifests as an increase in ventilation (raw data is from rats acutely breathing normoxic or hypoxic gas mixtures (Pamenter and Powell, unpublished).

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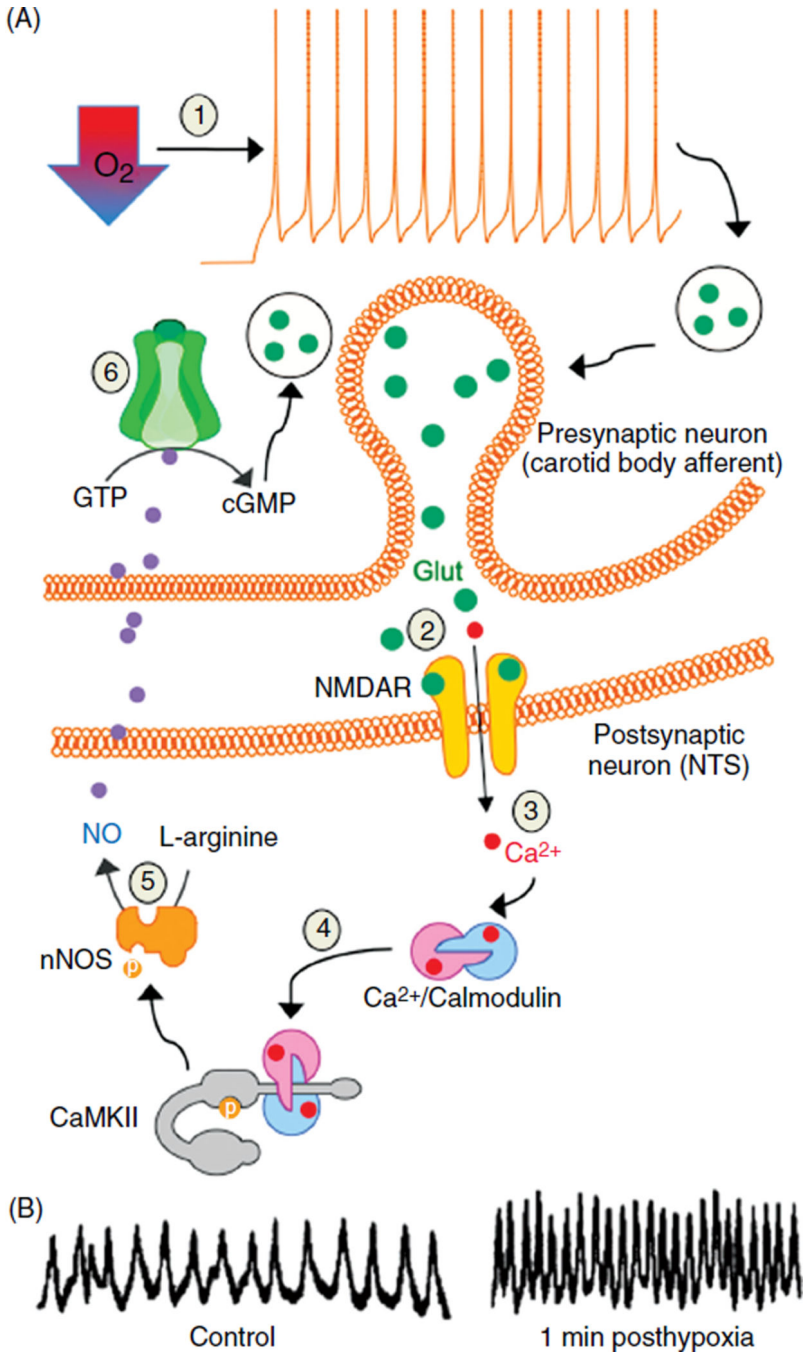

Figure 3.

Molecular mechanisms of STP. Acute hypoxia of several minutes increases ventilatory drive and normoxic (baseline) ventilation remains elevated for several minutes poststimulus. (A) During short-term hypoxia: (i) carotid sinus nerve activity is elevated, which leads to (ii) sustained glutamate (glut) release from carotid body afferent neurons that stimulates NMDARs in postsynaptic NTS second-order neurons. Maintained activation of postsynaptic glutamate receptors causes (iii) intracellular Ca^{2+} accumulation, which (iv) binds with calmodulin and activates CaMKII. CaMKII then (v) modifies membrane-dissociated nNOSs,

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stimulating production of NO, which rapidly defuses back across the synaptic cleft and (vi) stimulates guanalyl cyclase-mediated production of cGMP. cGMP then enhances presynaptic glut release and thus enhances the excitatory signal propagation that increases downstream respiratory drive (i.e., STP). (B) Physiologically, STP manifests as an increase in breathing frequency; reprinted with permission (190).

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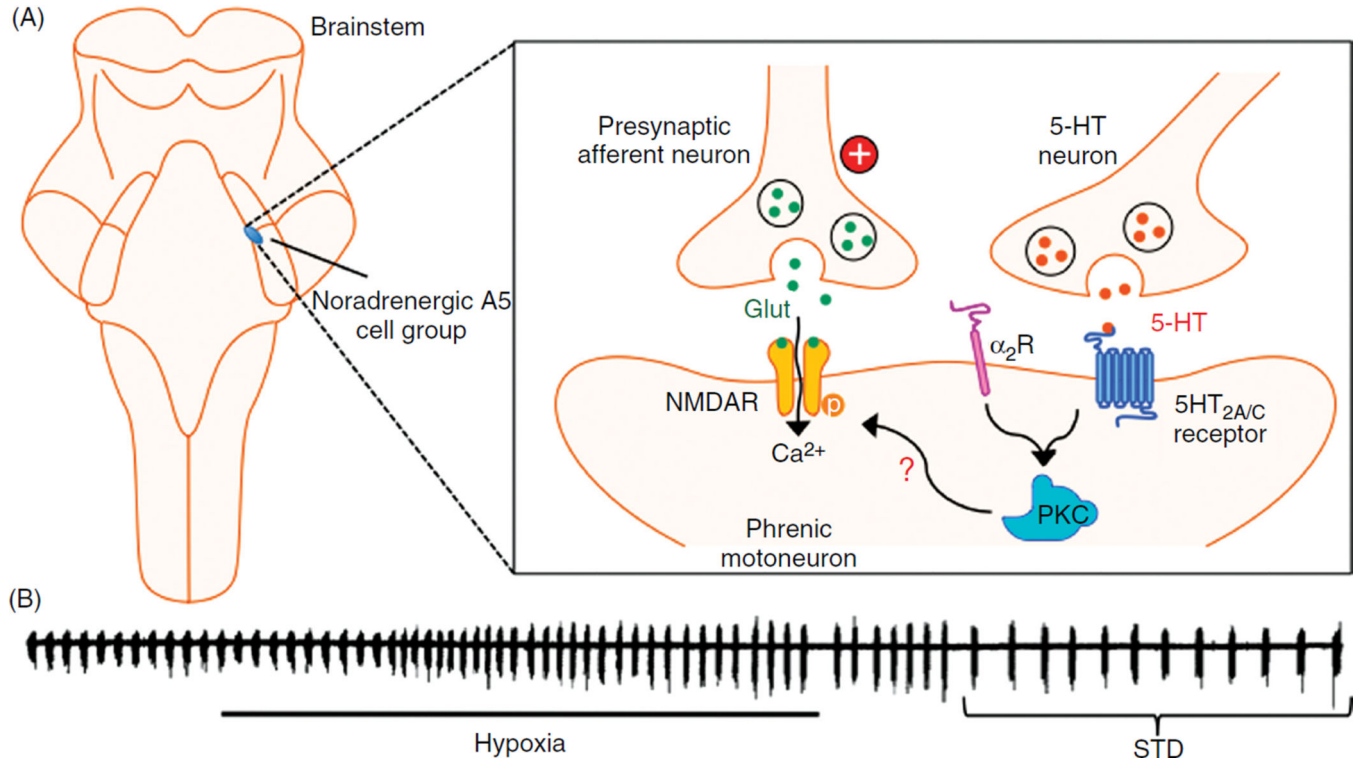

Figure 4.

Molecular mechanisms of STD. Acute hypoxia of seconds to minutes can lead to a short-term decrease in breathing frequency via an incompletely understood pathway. (A) Acute hypoxia induces activation of serotonin (5-HT) type 2A and 2C receptors and/or α_2-adrenoreceptors within the noradrenergic A5 cell group. Activation of $5\text{-HT}_{2A/2C}$ or α_2-adrenoreceptors modulates glutamatergic NMDARs via an unknown pathway, and presumably decreases NMDAR activation. (B) Physiologically, activation of this pathway leads to decreased phrenic nerve activity and decreased ventilation (due to reduced breathing frequency); reprinted with permission (54).

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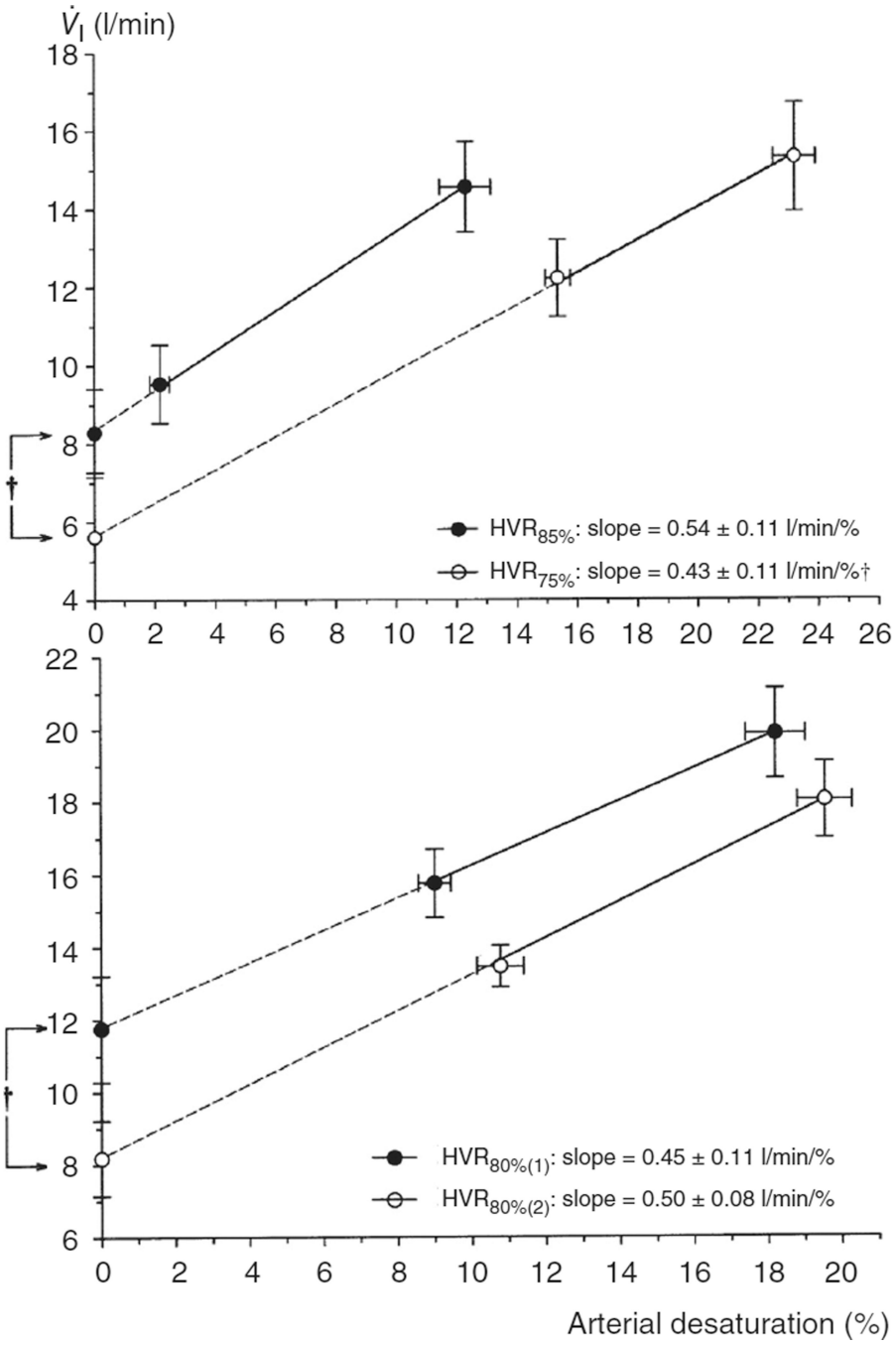

Figure 5.

HVD in humans involves a rapid decrease in O_2 sensitivity followed by larger decreases in ventilation without changes in O_2 sensitivity. (Upper panel) The slope of the HVR between baseline in mild hyperoxia and Sa_{O2} = 85% for 3 min is significantly greater than the slope between Sa_{O2} = 75% and 85% after 25 min of hypoxia (Sa_{O2} = 85%). (Lower panel) However, most of the decrease in ventilation during sustained hypoxia is explained by a significant decrease in ventilation without a change in O_2 sensitivity; the slope of the HVR between Sa_{O2} = 90% and 80% is not significantly different after 8 versus 14 min of hypoxia

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(Sa_{O2} = 90%), but there is significant HVD (illustrated as a decrease in the Y intercept predicting ventilation at Sa_{O2} = 100%). We propose a more accurate way to quantify HVD is the decrease in ventilation predicted within the range of sustained hypoxia studied, for example, the change in ventilation predicted at Sa_{O2} = 85% between the two HVR measurements in the lower panel; reprinted with permission (109).

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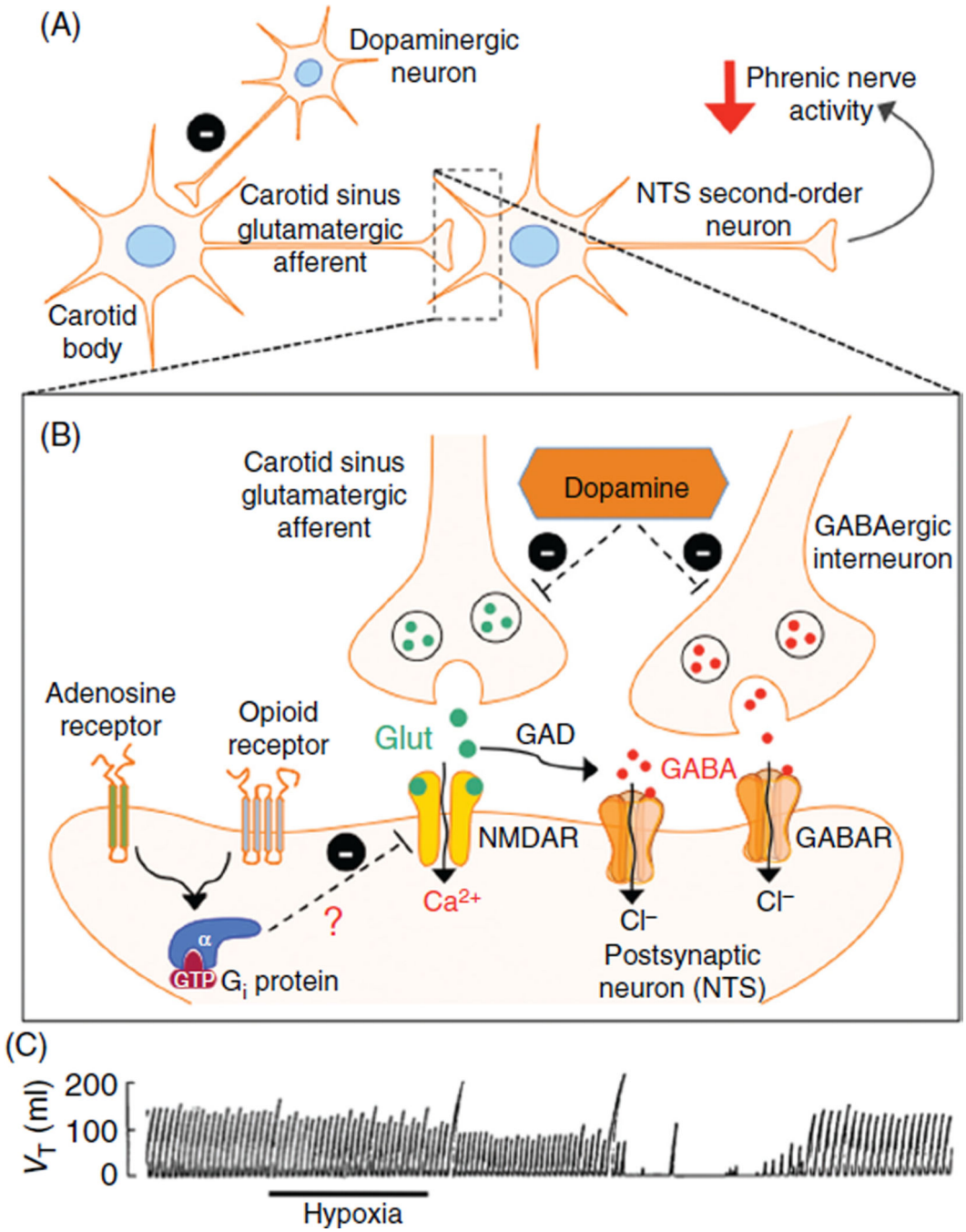

Figure 6.

Molecular mechanisms of the HVD. Acute hypoxia of 5 to 30 min leads to a decrease in ventilatory drive, or a hypoxic apnea. The underlying mechanism of this HVR is poorly understood but evidence indicates mediation at both the peripheral chemoreceptors (A) and within the CNS (B). (A) At the peripheral chemoreceptors, DA release may inhibit hypoxia-mediated carotid body depolarization and reduce carotid sinus nerve activity and thus downstream phrenic nerve activity. (B) At the synapse between the carotid sinus nerve and NTS second-order neurons, GABA accumulation due to chronic activation of GABAergic

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interneurons or conversion of excessive synaptic glutamate to GABA by GAD inhibits the electrical excitability and downstream phrenic nerve activation. DA may act on either the carotid sinus nerve afferent or GABAergic interneurons to decrease GABA accumulation via these putative pathways. Activation of adenosine and opioid receptors has also been implicated in this pathway, potentially via inhibition of glutamatergic NMDAR activity mediated by inhibitory g protein signaling. (C) Physiologically, STP manifests as decreased ventilation (data shown are from hypoxic chemodenervated dogs; adapted from (426) with permission.

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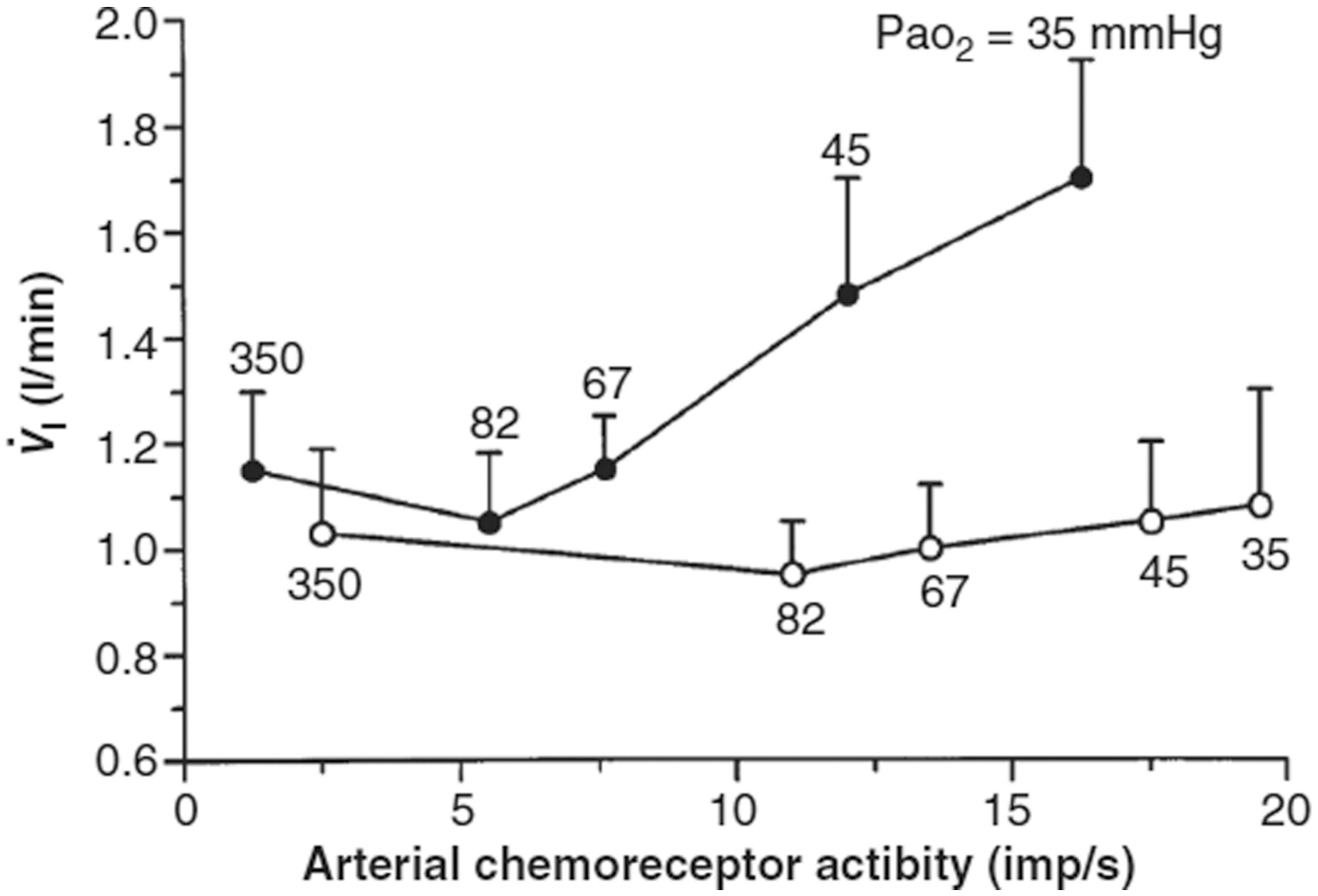

Figure 7.

Opposing effects of DA on carotid body chemoreceptor sensitivity and ventilation. Ventilation ($\dot{V}_I$) and arterial chemoreceptor activity measured simultaneously in anesthetized cats with haloperidol (open symbols) versus control (filled symbols). Haloperidol blocks D_2Rs (i) in carotid bodies, which increases chemoreceptor activity for a given P_{O_2}, and (ii) in the CNS, which decreases the CNS gain of the HVR so $\dot{V}_I$ is less for a given chemoreceptor activity. Reprinted with permission (320); adapted from (365) with permission.

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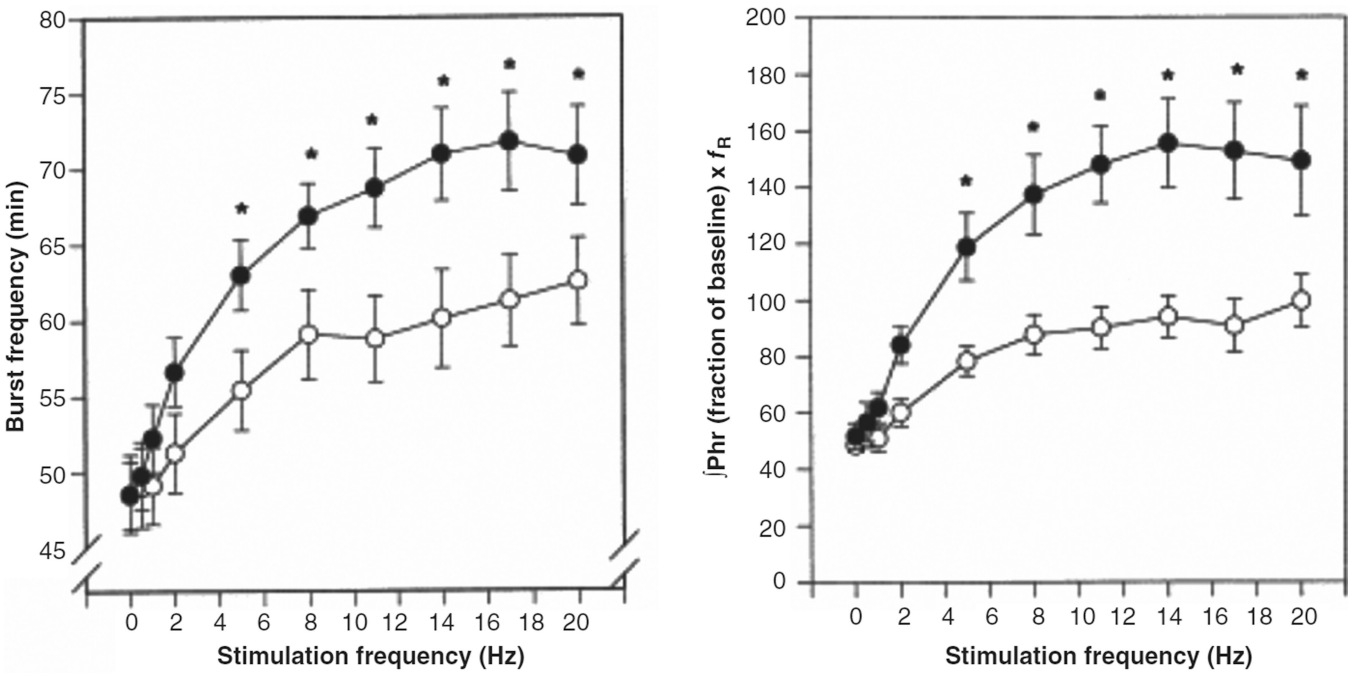

Figure 8.

VAH involves increases in the CNS gain of the HVR. Neural output to respiratory muscles (left, phrenic burst frequency; right, phrenic burst frequency X integrated phrenic amplitude; analogous to f_R and $\dot{V}_I$respectively) increases in chronically hypoxic (filled symbols) compared to normoxic control rats (open symbols) for any given level of electrical stimulation of the carotid sinus nerve; reprinted with permission (320).

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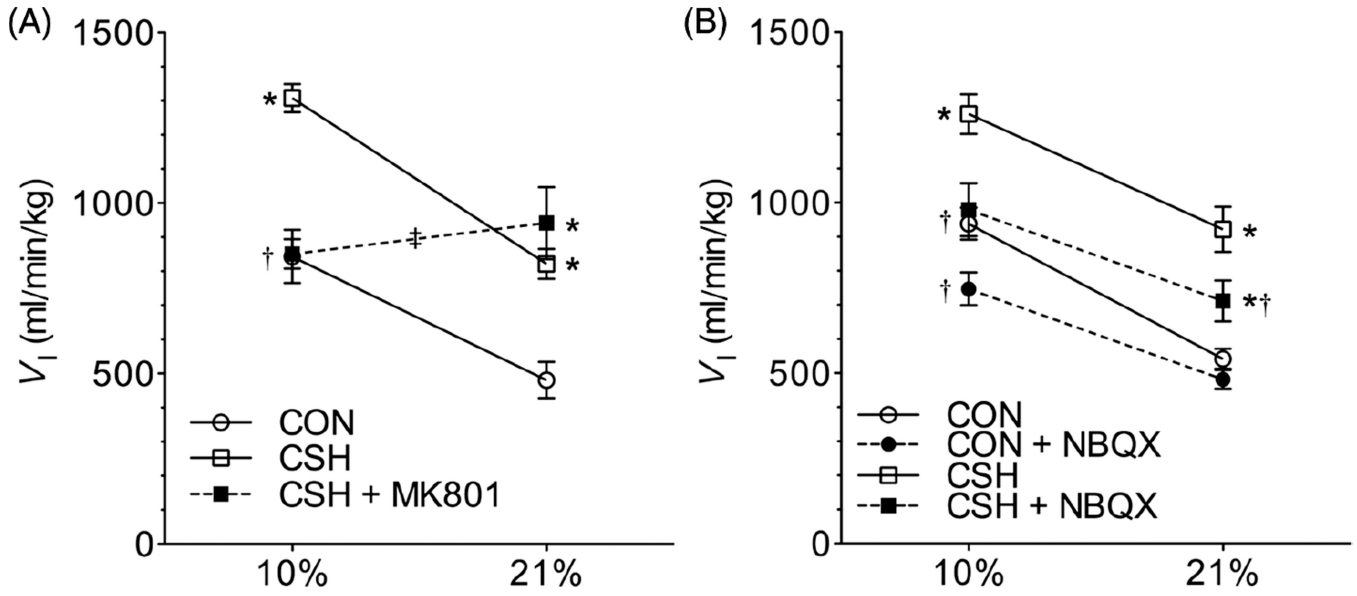

Figure 9.

VAH is mediated by glutamatergic NMDAR, but not AMPAR receptors. (A) The acute HVR is abolished by blocking NMDARs withMK801 in the NTS of chronically hypoxic, awake rats (CSH) and has no effect on chronically normoxic (CON) rats. (B) Non-NMDA glutamatergic receptors (blocked with NBQX) contribute to the ventilatory chemoreflex response all conditions (i.e., acute or chronic hypoxia (and also hypercapnia, not shown) and do not play a unique role in VAH; adapted from (299) with permission.

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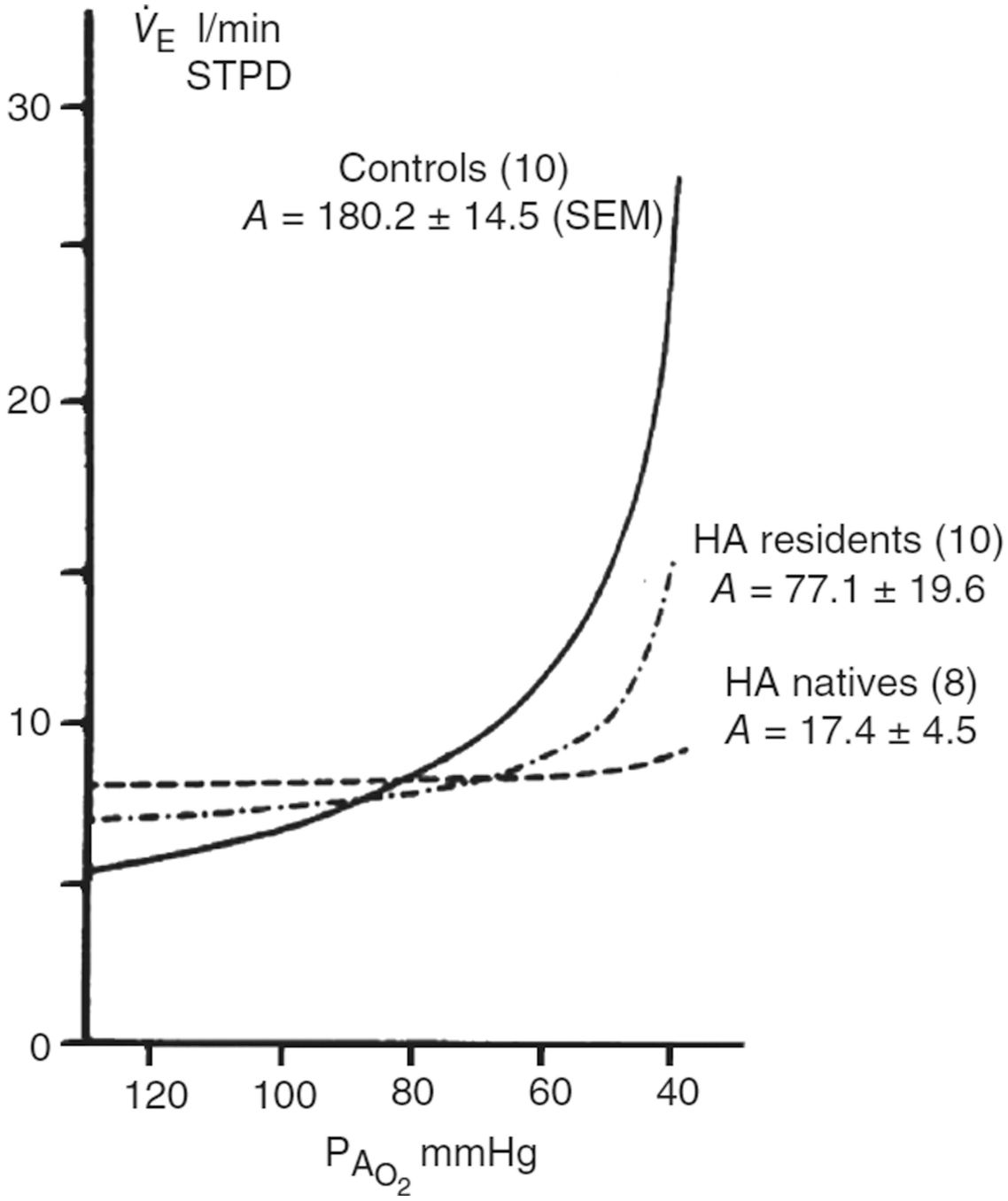

Figure 10.

Hypoxic ventilatory drive is reduced in high altitude natives. Relationship between ventilation and arterial oxygen tension in low altitude control human subjects, high altitude (HA) residents (3–39 years at altitude) and HA natives (born and live at altitude); reprinted with permission (413).

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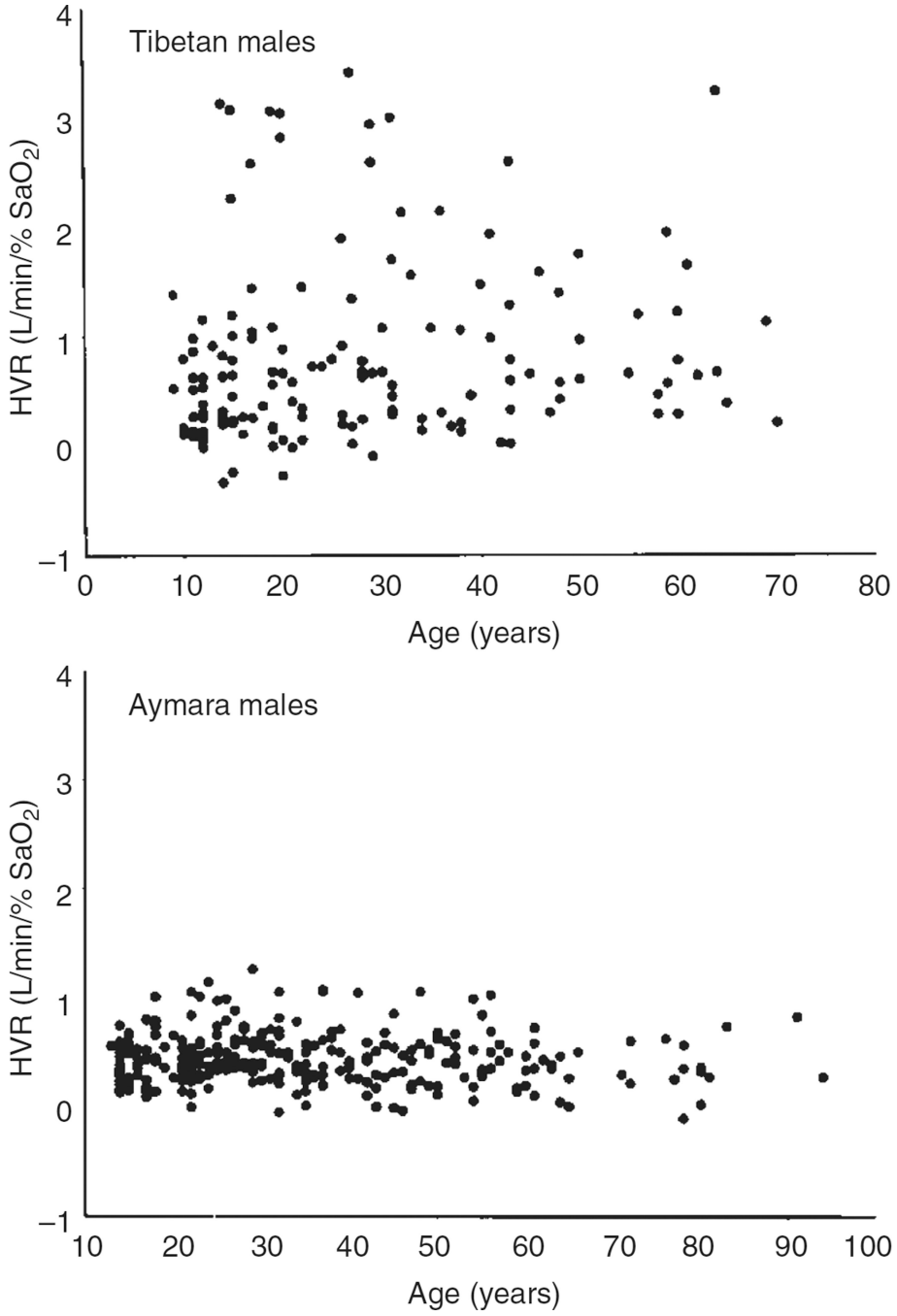

Figure 11.
High-altitude natives from Tibet (upper panel) have greater and varied HVRs than high altitude native Andeans (Aymara; lower panel). Adapted from (24) with permission.

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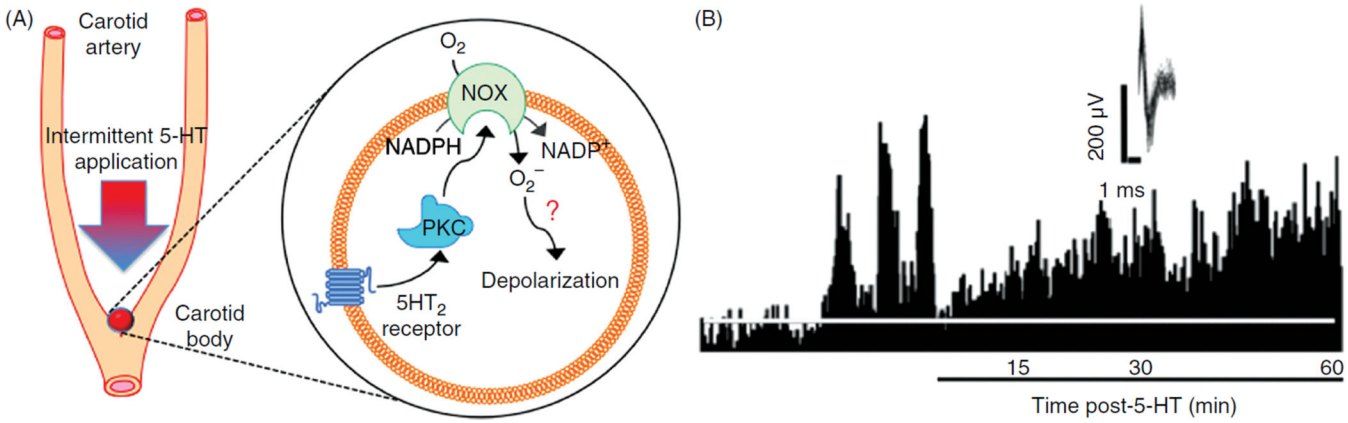

Figure 12.

Putative molecular mechanism of PA at the carotid body. (A) AIH or repeated acute application of serotonin (5-HT) to carotid bodies induces PKC-mediated phosphorylation of carotid body NADPH oxidase (NOX) subunits and increased production or superoxide (O_2^-). Superoxide production leads to enhanced carotid body membrane potential depolarization and increased afferent neuron firing. (B) This effect is manifested in progressive augmentation of phrenic nerve activity during the hypoxic episodes (or during 5-HT application, arrows), followed by LTF of phrenic nerve activity in the normoxic period following the intermittent stimulus; adapted from (311) with permission.

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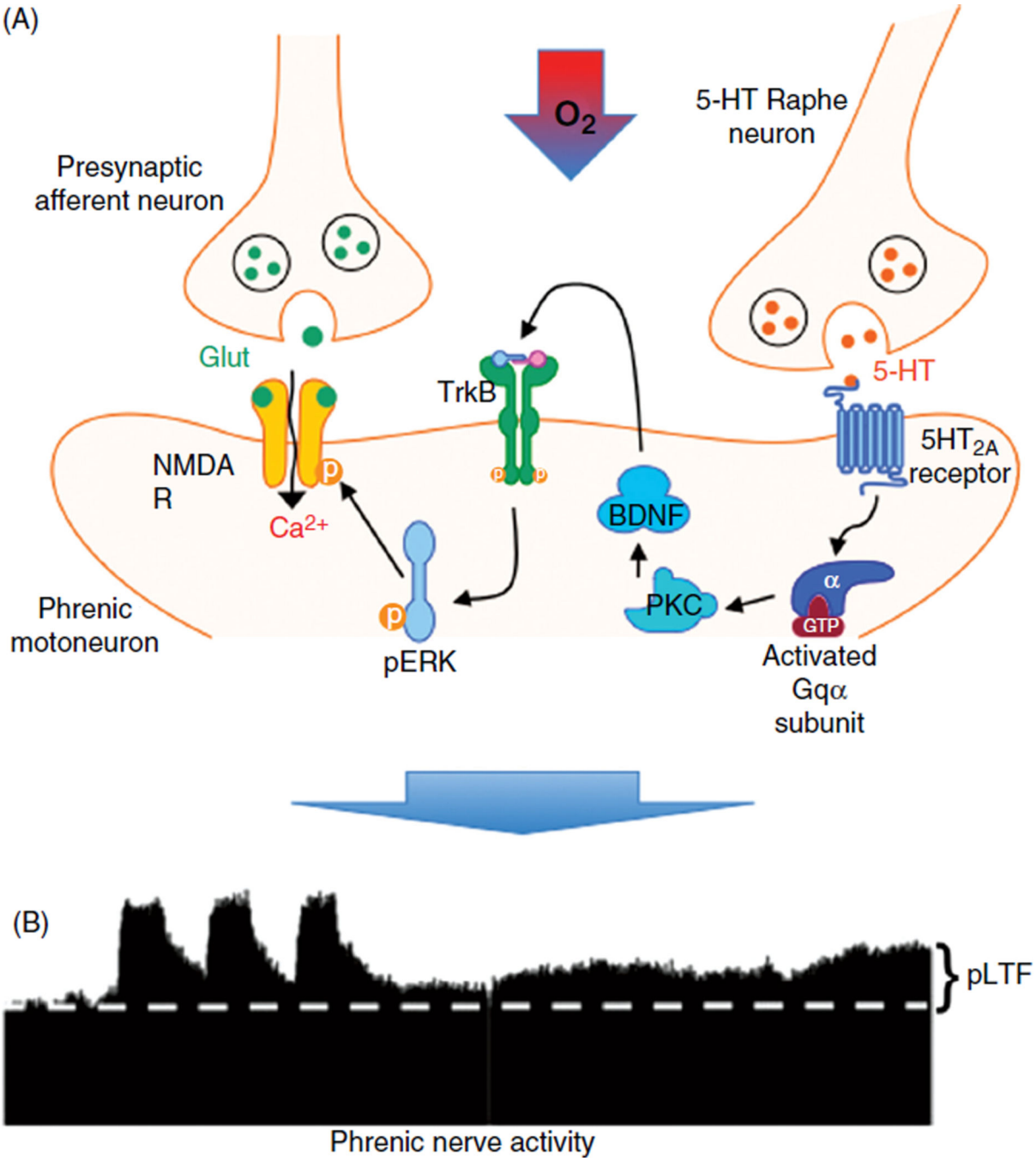

Figure 13.

Classic model of the molecular basis of phrenic LTF. Intermittent hypoxia (IH) increases ventilatory drive during acute hypoxia and normoxic (baseline) ventilation remains elevated for over an hour after IH. (A) Carotid body stimulation by IH releases 5-HT from neuromodulatory Raphe neurons, which binds to 5-HT type 1A and 2A receptors on phrenic motorneurons. 5-HT activates G_q protein signaling cascades to activate protein kinase C (PKC) and induce the synthesis of BDNF. BDNF binds to tyrosine kinase receptors (TrkB) that activate phospho-extracellular signal regulated kinase (pERK). In other systems, pERK

has been shown to phosphorylate glutamatergic NMDARs in postsynaptic neurons and increase sensitivity to presynaptic glutamate release. (B) Physiologically, this increased sensitivity manifests as enhanced phrenic nerve activity and increased ventilation (primarily increased tidal volume); adapted from (225) with permission.

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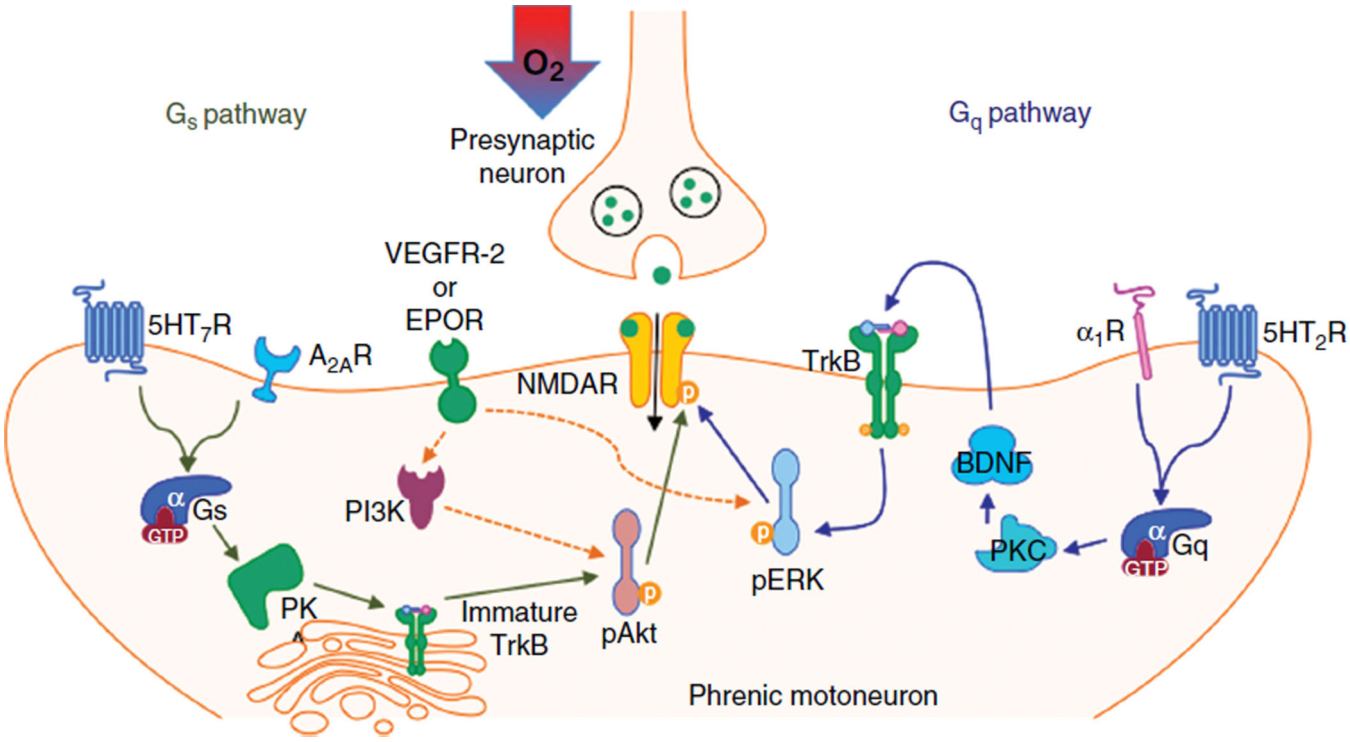

Figure 14.

New model for phrenic LTF with multiple molecular pathways. The G_q pathway (blue arrows) proceeds as described in Figure 13, but can also be activated by α1-adrenergic receptors (α_1Rs). The G_s pathway (green arrows) can be induced by the activation of adenosine type 2A receptors ($A_{2A}R$) or 5-HT type 7 receptors (5-HT_7R), which are coupled to G_s proteins. G_s signaling activates protein kinase A (PKA), which stimulates immature TrkB to modulate phospho-protein kinase B (pAkt). In other systems, this phosphorylates glutamatergic NMDARs and increases sensitivity to pre-synaptic glutamate release. Recently, additional pathways (dashed arrows) have been described wherein vascular endothelial growth factor receptor-2 (VEGFR-2) or erythropoietin receptor (EPOR) induce LTF via phosphoinositide 3-kinase (PI3K) and pAkt, and perhaps pERK.

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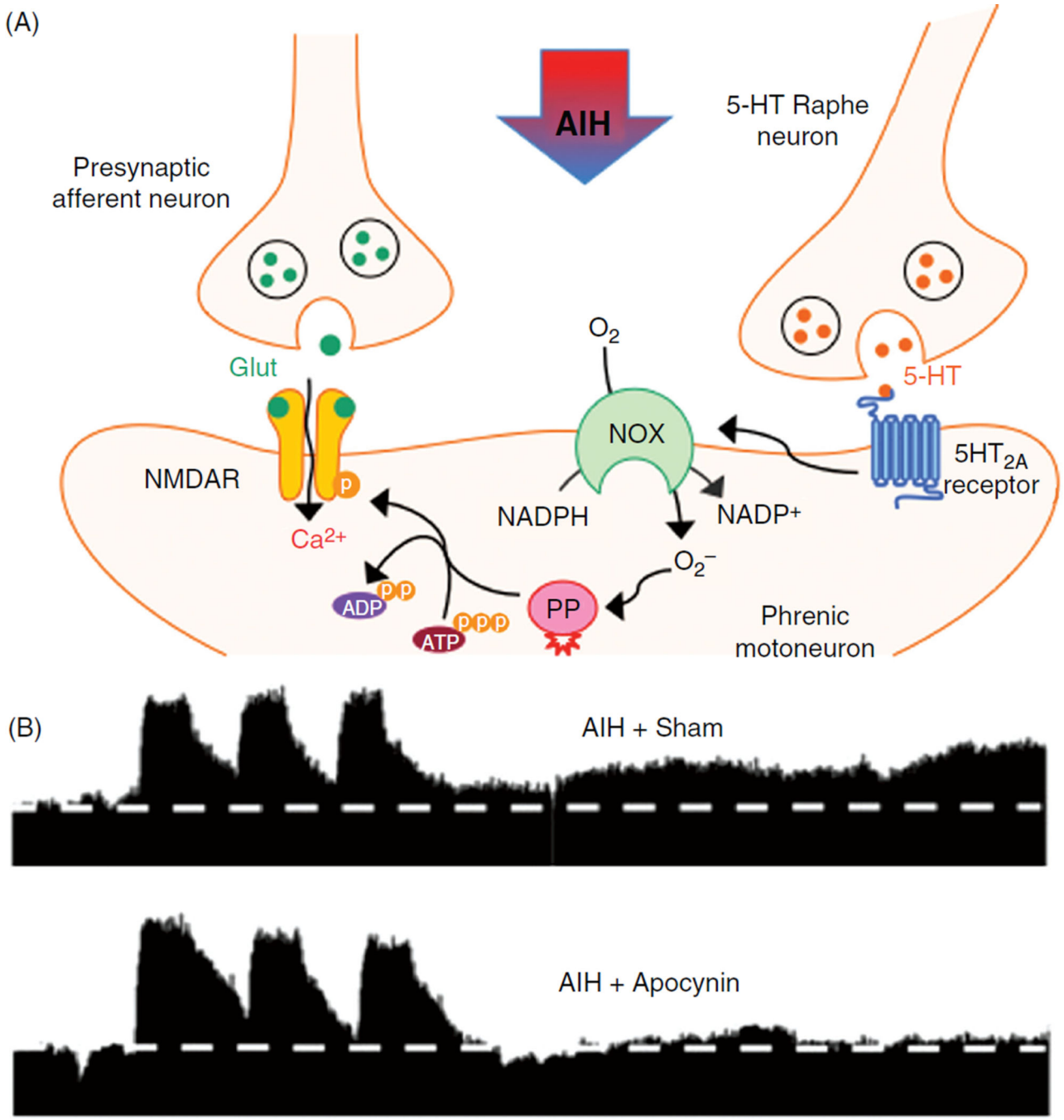

Figure 15.

Putative mechanism for ROS in phrenic LTF. Recent evidence supports a critical role for ROS produced from membrane-bound NOX in the manifestation of LTF following AIH. (A) Activation of 5-HT type 2 receptors in the post-synaptic cell induces NOX activity and generation of O_2^-. O_2^- acts to induce LTF, presumably via protein-phosphatase (PP)-mediated phosphorylation of NMDARs. (B) Application of the NOX inhibitor apocynin or

ROS scavengers (not shown) abolish phrenic LTF following AIH; adapted from (225) with permission.

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